Determination of Total Phenolic Content and Antioxidant Activity of *Commiphora mollis (Oliv.) Engl.* Resin

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Research Article

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Abstract

In this study, total phenolic contents (TPC) and antioxidant activity of Commiphora mollis (Oliv.) Engl. (Burseraceae) resin was investigated. The resin was extracted using petroleum ether, chloroform, and methanol and yielded 27.46 ± 0.48, 46.56 ± 0.42, and 53.00 ± 1.39% extractable solids, respectively. The Folin-Ciocalteu redox (F-CR) assay was optimized considering relevant parameters such as reaction time, maximum wavelength, and dilution effect before the determination of TPC. The amount of antioxidant necessary to decrease by 50%, the initial concentration of DPPH (EC_{50}) was determined at 60 min. The kinetics of the reaction was analyzed taking into account the pseudo-first-order model. For the F-CR assay, the optimum conditions for the maximum absorbance and analysis time were 760 nm and 30 min, respectively. Under these conditions, the method was valid and the instrumental response was linear and sensitive. Relatively, higher TPC was obtained in diluted samples (500 µg/mL): 168.27 ± 3.44, 137.43 ± 1.32, and 136.16 ± 0.42 mgGAE/g from methanol, chloroform, and petroleum ether extracts, respectively. By using different concentrations of the test sample, exhaustive reduction of phenolics and/or antioxidant substrates was achieved. The obtained DPPH radical scavenging capacity index (EC_{50}) was 44.72 ±0.48 for ascorbic acid, and 295.03 ± 3.55, 342.75 ±9.72 and 353.69 ± 7.30 µg/mL for methanol, chloroform, and petroleum ether extracts, respectively. The methanol extracts showed better kinetic behavior (k_2 values: 115.08 to 53.28 $M^{-1}s^{-1}$ and steady-state time < 30 min), which was closer to the standard L-ascorbic acid (k_2 values; 190 to 109 $M^{-1}s^{-1}$), than other extracts. Results of the current study showed that polar solvent, methanol resulted in better F-CR reducing capacity and DPPH radical scavenging activity than nonpolar solvents which displayed similar effects ($p > 0.05$). This could have resulted from different antioxidants extracted from C. mollis resin. In conclusion, C. mollis resin could be a useful source of antioxidants. For better understanding, further study accompanied by isolation of pure compounds is recommended.

1. Introduction

Free radical reactions are one of the major problems in the health and food industry. It causes many dreadful diseases including cancer [1] and oxidative rancidity of foods as well [2]. Synthetic and natural antioxidants are used routinely in medicine and foods to protect against oxidation damage [3–5]. Studies have indicated possible adverse effects and potential toxicities of excessive consumption of synthetic antioxidants [6]. Therefore, reduction of synthetic additives or their replacement by plant-based natural alternatives is recommended.

A variety of plant materials are natural sources of antioxidants that arise from their phytochemicals such as alkaloids, flavonoids, phenolics, and terpenoids [1]. More importantly, the popular use of plants in folk medicines to treat oxidation-related diseases might be used as a guide in searching for new antioxidant sources. Almost all of the plants from the genus Commiphora (Burseraceae family), produce aromatic resinous exudates (myrrh) which have long been used as a traditional medicine for the treatment of inflammation, arthritis, tumors, obesity, skin infections, and gastrointestinal diseases [7]. Commiphora

mollis (Oliv.) Engl. (Burseraceae) resin is traditionally used as a medicine to treat skin inflammation, gastrointestinal disorder, and wound especially in domestic animals in the Borana Oromo Society, south-eastern Ethiopia. Studies on myrrh indicated that the crude extracts and isolated pure metabolites of the plant have pharmacological effects including antioxidant [8–13], anti-inflammatory [8] anti-proliferative [11], cytotoxic [9, 14], and anti-hyperglycemic [10] activities. Phytochemicals such as sesquiterpenoids, diterpenes, triterpenes, ferulates, and sterols are responsible for antioxidant activity, lignans for the cytotoxicity, and steroids for antiproliferative, anti-inflammatory, hypolipidemic, and antidiabetic activities. Regarding C. mollis, antioxidant compounds such as flavonoids were isolated from its heartwood extracts [15] whereas curcumin was isolated from leaf extracts [16].

In vitro, antioxidant activity investigation is often used for preliminary testing of plants as a possible source of natural antioxidants. Factors such as extraction solvents and assay mechanisms used for testing antioxidant activity affect the reliability of results. Both polar and nonpolar organic solvents have been used to prepare crude extracts of resins from the bark of various Commiphora trees [8, 11, 12]. Different assay mechanisms including hydrogen atom transfer (HAT), electron transfer (ET), reducing power, and metal chelation [17, 18] are used for antioxidant testing. Although conditions may vary from one assay method to another, correlation analysis of the results obtained by different methods might be used as a validation tool.

Among various methods, the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay and Folin-Ciocalteu redox (F-CR) method are often used for in vitro analysis of the antioxidant potential and total phenolic contents of medicinal plants, respectively. They are opted because they are simple, rapid, and produce precise results [18]. Antioxidant assay DPPH involves ET and a HAT from the antioxidants to the DPPH free radical. The reaction is accompanied by fade of DPPH color, which is measured at 517 nm and the resulting de-colorization indicates an antioxidant activity. Antioxidant activity assay by DPPH is often reported as EC50, which indicates the efficient concentration of the antioxidant required to reduce the initial DPPH concentration by 50%. This is often measured at a defined period of reaction or a steady state. Moreover, kinetic analysis is important to study the reactivity of antioxidants towards free radical scavenging [19, 20].

The F-CR assay is a well-known method for determination of TPC [21]. The F-CR assay involves the reduction of the F-C reagent with phenolic compounds in an alkaline medium and the formation of a blue-colored complex with the maximum absorption measured at 765 nm. The absorbance is directly proportional to the TPC concentration which is reported as Gallic acid equivalent. To obtain reliable and precise data, various conditions such as absorption wavelength (λ_{max}), reaction time for color development, and volume ratio of alkali and F-C reagent are required to be studied [22, 23].

The literature survey showed that resins produced by various Commiphora plants exhibit antioxidant activity. However, this information is not available for C. mollis resin. Therefore, the present study aimed to determine the antioxidant activity and TPC of different extracts of C. mollis resin using DPPH radical scavenging and F-CR methods, respectively.

2. Materials And Methods

2.1. Chemicals

In this study, reagents grade solvents such as petroleum ether, chloroform, and methanol supplied by Loba Chemie Pvt, Ltd (Mumbai, India), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 85% obtaied from Alpha Chemika (Mumbai, India), 2 N Folin-Ciocalteau Phenol (F-CP)Reagent, 2 N, purchased from Sisco Research Laboratories Pvt. Ltd. (Navi Mumbai, Maharashtra, India), Sodium carbonate anhydrous, 99.5%, was purchased from Blulux Laboaratoty Pvt (Faridabad, Haryana, India), L-Ascorbic acid, 99% and gallic acid, 99% purchased from Nice Chemicals Pvt (Ernakulum, Kerala, India) were the major chemicals used during the experimental work.

2.2. Instrument

Double-beam UV/Vis spectrophotometer (SPECORD 200 PLUS, Analytik Jena, Germany) was used for quantitative analysis.

2.3. Sample Collection

The resin of C. mollis was collected from Gorile, Das district, Borana Zone, Oromia Regional State, Ethiopia in February 2020. The collected samples were shade dried and double-sealed with a plastic bag and transported to the Department of Chemistry Laboratory, Jimma University, for further treatment and analysis.

2.4. Preparation of Resin Extracts

The pulverized resin, 15 g was transferred to amber flasks and then, 200 mL of different polarity solvents including methanol, chloroform, and petroleum ether were separately added. The content was shaken for 15 min on the shaker and then stored in the dark at room temperature. After 24 h, the infusions were filtered with Whatman No. 1 filter paper. The obtained residue was again extracted using the same procedure with an equal volume of the same solvents. Finally, the filtrates were combined and evaporated to one-quarter of their volume using a rotary evaporator at 40°C. The obtained extracts were allowed to dry at room temperature and the extraction yield was calculated and reported as a percent of dry extract per weight of the dry resin [12].

2.5. Total Phenolic Contents

The F-CR method, reported by Ainsworth and Gillespie [27] and Adusei et. al., [29] was used with slight modification for the determination of the TPC in the different extracts of the resin. During the experiments, the reagents and sample solutions were prepared as follows: The FCP reagent was diluted at 1:10 with distilled water just before the experiment. Sodium carbonate (7.5% w/v) was also prepared in distilled water. Stock solutions of each dry extract of C. mollis resin (1 mg/mL) and standard compound, gallic acid, (0.5 mg/mL) were prepared in methanol (95% vol/vol).

2.5.1. Determination of maximum absorption wavelength and time

To investigate the λmax and time of the reaction gallic acid (0.05 and 0.1 mg/mL) and C. mollis resin extracts (0.2 and 0.4 mg/mL) were prepared from the stock solutions. Following this procedure, 0.5 mL of a solution of sample, standard or blank (methanol 95%,v/v) were transferred to screw cup tubes, and then, after the addition of 2 mL F-CR the content was mixed with a vortex mixer for a few seconds. After 3 min but before 8 min, 4 mL of Na2CO3 was added into the tube and mixed well. The sample was then transferred to a quartz cuvette and its absorbance was recorded in the wavelength range of 400 to 900 nm from 5 to 60 min at 5 min intervals. The λmax was determined from the obtained spectra and recorded absorbance. Using the obtained λ_{max}, the optimum reaction time was determined from the reaction kinetics, which is a plot of absorbance versus reaction times [25].

2.6.2. Determination of TPC

For the determination of TPC of C. mollis resin extracts, three independent samples of each extract (500 and 1000 µg/ml) were used. Gallic acid calibration solutions, containing 0, 25, 50, 75, 100, 125, and 150 µg/ml were prepared in duplicates. Then, the reaction mixtures were prepared as the procedure earlier stated and the absorbance was recorded at previously determined λ_{max} (760 nm) and time of incubation (30 min). All measurements were performed in triplicates. The average absorbance of the two-calibration series was used for the construction of the calibration curve. The coefficient of determinations (R^2) of the calibration curve was used to evaluate the linearity range of the curve. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated as 3.3σ/s and $10\sigma/_S$, respectively, where σ is the standard deviation of the response, and S is the slope of the calibration curve [26]. The concentration of gallic acid in each extract was calculated from the regression equation using their absorbance. Finally, these results were converted to the TPCas milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g) using Eq. 1 [27].

$$C = C_1 \times \frac{V}{m} \quad (1)$$

where C is TPC in mg/g, in GAE (gallic acid equivalent), C_1 is the concentration of gallic acid established from the calibration curve in mg/mL, V is the volume of the extract in mL, and m is the weight of the dry plant extract in g.

2.7. DPPH Radical Scavenging Activity

The antioxidant activity of the extract was determined by slightly modifying the earlier reported DPPH assay method [19].. A stock solution of DPPH (101.44 µM or 400 µg/mL) was prepared by dissolving 0.01 g of DPPH in 25 mL methanol. Then, 10, 20, 30, 40, 50, and 60 µM of DPPH solutions were prepared from a stock solution. Each of these solutions was transferred to a cuvette and their absorbance was measured over a wavelength range of 400–600 nm against the blank (methanol). The spectra and

absorbance data were used to find the λ_{max} and establish the linear relationship between absorbance and concentration of the standards.

2.7.1. Determination of EC_{50}

The stock solutions of each of the C. mollis resin extracts (1000 µg/mL) and L-ascorbic acid (800 µg/mL) were separately prepared in methanol. Then, a series of solutions: 400, 350, 300, 250, 200, 150, 100, and 50 µg/mL were prepared in triplicates by diluting the stock solution. Afterward, 1 ml of each of these solutions was mixed with 2 mL of 40 µg/mL DPPH and shaken vigorously. The control sample was also prepared by mixing 1 mL of methanol with 2 mL of 40 µg/mL DPPH. The mixture was then incubated in dark for 60 min at room temperature. Eventually, the absorbance of the mixture was measured at λ_{max} (517 nm). The exact concentration of DPPH in the reaction medium was calculated using the calibration curve equation and the remaining percent of DPPH was calculated by Eq. 2:

$$\%\ \text{of remaining DPPH} = \frac{A_s}{A_0} \times 100 \quad (2)$$

where A_0 is the absorbance of the DPPH solution without antioxidants and A_s is the absorbance of the sample[28].

The obtained % of DPPH was plotted against the concentration of antioxidants (C. mollis resin extracts and ascorbic acid) to obtain the amount of antioxidant necessary to decrease the initial DPPH concentration by 50% (EC_{50}). The measurements were carried out in triplicate, results were expressed as mean ± standard deviation.

2.7.2. Kinetics Analysis

Kinetics of the DPPH radical scavenging activity of C. mollis extracts and standard was determined by the previously reported method [20] with modification. Accordingly, an aliquot (1 mL) of a solution containing different concentrations of sample or standard (250, 100, and 50 µg/mL) was mixed with 2 mL of 101.44 µM DPPH solution. Similarly, a control sample (DPPH) was prepared by mixing 1 ml of methanol with 2 mL of 101.44 µM DPPH. Then, absorbance was measured at 517 nm 2 min after the addition of DPPH. The absorbance measurement was continued by 2 min intervals in the first 15 min and then by 5 min intervals until the reaction approaches its steady state.

The kinetics of the DPPH radical scavenging reaction depends on the concentrations of both reactants. So, the rate of reaction was defined as

$$-\frac{d[DPPH]}{dt} = k_1[DPPH] = k_2[DPPH][Antioxidan\ t] \quad (3)$$

Where k_1 and k_2 represent the pseudo-first-order and second-order rate constants, respectively.

In all cases, the initial concentration of antioxidants in the medium was always lower than that of DPPH. This condition was fulfilled when some DPPH concentrations remained in the medium at the end of the reaction. Thus, the reaction is pseudo-first-order to antioxidant groups in the tested samples which completely disappeared from the reaction medium according to Eq. 4.

$$A = A_0 e^{-K_1 t} \quad (4)$$

where A is the scavenged concentration of DPPH at any time t, A_0 is the span of the reaction (the total amount of DPPH scavenged at steady state), k_1 is the apparent first-order rate constant, which describes the velocity of the disappearance of antioxidants from the reaction medium.

Finally, k_2 was determined from the initial concentration of DPPH according to Eq. 4. In addition, time, t, was used to compare the reactivity of tested samples. All the kinetic parameters were determined by fitting the obtained data using the Levenberg–Marquardt method implemented in Graph pad prism version 8.0.2 software [29].

2.8. Statistical Analysis

All measurements were conducted in triplicates and the final results were reported as mean and its standard deviation (i.e., mean ± SD). Statistical analysis was performed using Graph Pad Prism version 8.0.2. Analysis of variance (ANOVA) with Tukey's multiple comparison test ($P < 0.05$) was performed to compare the mean values of the results.

3. Results And Discussion

3.1. Extraction Yield

The extraction step is crucial to isolate active compounds from plant materials and reduce interferents in the investigation of pharmacological and phytoconstituents of the plant [30]. Methanol, chloroform, and petroleum ether extracts of C. mollis resin were prepared under the same conditions. The results showed that methanol yielded the highest extractable solids than other solvents. The yield of extractable solids was 53.00 ± 1.39, 46.56 ± 0.42, and 27.46 ± 0.48 for methanol, chloroform, and petroleum ether extracts, respectively, showing the dependence of the isolated solid with the polarity of extraction solvents. The polarity of the extraction solvents plays a vital role in solvent extraction as plant components can either be polar or non-polar [30].

3.2. Total phenolic contents

A group of compounds with similar chemical structures may show the same chemical interactions with specific reagents during the reaction. The F–CR assay is a reaction based on an electron transfer (ET) from phenolic compounds and other oxidation substrates to the F-CR, phosphomolybdic/phosphotungstic acid complexes ($H_3PW_{12}O_{40}/H_3PMo_{12}O_{40}$), resulting in blue complexes (possibly $(PMoW_{11}O_{40})^{4-}$) which induce absorption maximum at about 765 nm [23]. The

deviation from the λ_{max} can result from oxyreduction responses of F-CR [25] and the complex structural differences of polyphenols (antioxidants) occurring in plants [31]. This deviation could be capable of compromising the experimental responses by underestimating the quantitative data. Therefore, the absorption spectrum of the extracts from each solvent was compared with that of the reference compound, i.e., gallic acid. It was observed that the extracts of C. mollis resin and the gallic acid, have shown a similar absorption spectrum (Fig. 1).

As can be seen from Fig. 1, for Gallic acid and C. mollis resin extracts, λ_{max} lies between 700 and 800 nm. Table 1, shows the absorbance values of Gallic acid and C. mollis resin extracts, recorded at 5 nm wavelength difference from 740 nm to 770 nm. The obtained results showed that there was no significant difference (≤ 0.65) in the value of absorbance in the studied wavelength range. Depending on the result, wavelength, 760 nm, was chosen. The obtained results were similar to the one given in the standard method [22] and other reported findings in the literature [25, 31, 32].

Table 1 The absorbance of solutions of gallic acid and C. mollis extracts after 30 min of reaction with FC-R in a wavelength range of 740–770 nm.

	Conc. (µg/mL)	Absorbance at the wavelength (nm) of						
		740	**745**	**750**	**755**	**760**	**765**	**770**
Gallic acid	50	0.374	0.376	0.377	0.377	0.377	0.376	0.374
	100	0.874	0.876	0.878	0.879	0.880	0.880	0.880
Chloroform extracts	200	0.302	0.302	0.302	0.302	0.300	0.299	0.297
	400	0.460	0.461	0.461	0.461	0.461	0.460	0.459
Methanol extracts	200	0.325	0.326	0.325	0.324	0.323	0.322	0.319
	400	0.482	0.483	0.483	0.483	0.483	0.482	0.481
Petroleum ether extracts	200	0.262	0.262	0.262	0.262	0.261	0.260	0.259
	400	0.415	0.416	0.416	0.416	0.415	0.415	0.414

The optimal time for color development was determined using the selected wavelength, 760 nm. The reaction kinetics showed that the absorbance increased during the initial 30 minutes, then, stabilized and gradually decreased after a while (Fig. 2). For Gallic acid, the absorbance remained stable from 30 to 60 min (coefficient of variation, CV $\leq$ 0.25%). However, for C. mollis resin extracts, the stability was achieved from 30 to 45 min (CV $\leq$ 0.65%).

Determination of TPC at steady-state increases the precision of analytical results. Conditions such as high temperature and alkali level, speed up color development, and its fading [22, 23]. Thus, reliable results can be obtained by studying reaction kinetics. Results of the current study demonstrated that 30 min of incubation time is needed before measurement of absorbance. The obtained findings were in

agreement with previous reports on optimization of the F-CR method for the determination of TPC from plant extracts [25, 31].

To determine the TPC in the resin extracts, a calibration curve was constructed using Gallic acid calibration standards (0 to 150, µg/mL). The obtained calibration curve (y = 0.0082x + 0.0108), had coefficient of determination, R^2 = 0.9997, which demonstrates excellent linearity of the curve in the selected concentration range. The LOD and LOQ of the method were 2.41 and 7.29 µg/mL, respectively. The TPC of C. mollis resin extracted by different solvents was calculated using the calibration curve equation and reported as mg GAE/g dry mass of the extract (Fig. 3).

For each extract, two concentration levels: 500 and 1000 µg/mL (in methanol) were used for the analysis. Independent of the extracts, 500 µg/mL provided the higher amount of TPC, i.e. the TPC was 168.27 ± 3.44, 137.43 ± 1.32, and 136.16 ± 0.42 GAE/g for methanol, chloroform, and petroleum ether extracts, respectively. At 1000, 112.44 ± 3.33, 73.91 ± 3.87, and, 71.94 ± 2.51 mg GAE/g were obtained for methanol, chloroform, and petroleum ether extracts, respectively. Apart from the position of λ_{max} and optimal reaction time for color development, an excess of F-C reagent is recommended for the exhaustive reduction of phenolic antioxidants in the sample [22]. The obtained lower TPC in more concentrated samples could be due to an insufficient amount of the F-CR in the reaction medium. By varying concentrations of the extracts, enhanced responses could be observed.

TPC is one of the important parameters of total antioxidant capacity (TAC) and is widely used for the evaluation of the antioxidant properties of plants [21]. Since plants contain a diversity of phenolics and antioxidant substrates with different structures, molecular sizes, and polarities, the nature of extraction solvents can greatly affect their results [33]. In the present study, polar solvent (methanol) showed the highest TPC than non-polar solvents (petroleum ether and chloroform). Besides, the TPC of petroleum ether and chloroform was not significantly different (P > 0.05). In previous studies, methanol was also chosen for the extraction of phenolic contents from different plant samples [34–36]. This indicates better solubility of these compounds in polar solvents. Generally, the findings demonstrated that C. mollis resin contains a high amount of TPC, Such high TPC in plants has a correlation with different pharmacological effects [21].

3.3. DPPH Radical Scavenging Activity

The DPPH radical scavenging assay measures the extent to which the antioxidants can decrease the concentration of the DPPH radical in the reaction medium. This is measured spectrophotometrically by monitoring the decrease of absorbance at a characteristic wavelength in the presence of the test sample [17]. Figure 4 **(a)** shows the spectra of different concentrations of DPPH solution. The maximum absorbance was obtained at 517 nm. Moreover, Fig. 4 **(b)** shows the linear relationship between the DPPH radical concentration and the measured absorbance at 517 nm.

DPPH is one of the free radicals widely used for testing the preliminary antioxidant activity of plant extracts. The loss of absorbance of DPPH solution is proportional to the antioxidant concentration [19]. In

this study, a DPPH radical scavenging capacity increased with increasing concentrations of extracts. Methanol extract of C. mollis resin, displayed the highest DPPH radical scavenging activity at most of the tested concentrations levels (Fig. 5). For instance, when 400 µg/mL extract of each solvent: methanol, chloroform, and petroleum ether were reacted with DPPH 59.43 ± 0.37%, 55.95 ± 1.41%, and 56.52 ± 0.90% of the initial concentration of DPPH were scavenged, respectively. Similarly, when an equal concentration of the ascorbic acid was used 96.39 ± 0.03% of the initial concentration of DPPH was scavenged.

The effective concentration that causes a decrease in the initial DPPH concentration by 50% is defined as EC_{50}. The substance with lower EC_{50} is considered to exhibit significant antioxidant activity [19]. The EC_{50} values of C. mollis resin extracts were 295.03 ± 3.55, 342.75 ± 9.72, and 353.69 ± 7.30 µg/mL for methanol, chloroform, and petroleum ether extracts, respectively. These results indicated that methanol extract has the highest antioxidant activity than chloroform and petroleum ether extracts, but has lower antioxidant activity compared to the ascorbic acid (EC_{50} = 44.72 ± 0.48 µg/mL). Besides, statistical analysis showed that EC_{50} values of chloroform and petroleum ether extracts were not significantly different ($P > 0.05$). These outcomes demonstrated that polar solvent (methanol) is more effective for the extraction of antioxidants than non-polar solvents (chloroform and petroleum ether). Moreover, a similar pattern was also observed for TPC. That means methanol extract contained the highest amount of TPC compared to the other two solvents. Previous studies also demonstrated a positive relationship between the DPPH and F-C assays [34–37].

Compared to aromatic exudates of other Commiphora species, methanol extract of C. mollis exhibited greater DPPH inhibition effects. For instances, C. myrrh resin extracts y various solvents demonstrated different DPPH inhibition effects (methanol extract, EC_{50} = 320 µg/mL; ethyl acetate extract, EC_{50} = 930 µg/mL, essential oil, EC_{50} = 11330 µg/mL) [12]. Hexane extract of C. erythrea resin exhibited $EC_{50} > 3000$ µg/mL [8].

3.4. Kinetics Analysis

The kinetics of the DPPH radical scavenging reaction of standard (L-ascorbic acid) and C. mollis resin extracts was studied as a function of time. During the entire time of the experiment, the absorbance of the control sample was monitored to check the stability of the DPPH radical in the reaction medium. Figure 6 illustrates the DPPH radical scavenging profile for the tested samples. The differences in the manner of loss of DPPH with time can be marked by the difference in the shapes of reaction curves.

In the DPPH assay, the radical ($DPPH^{\bullet}$) is reduced through hydrogen atom transfer, (HAT) or electron transfer, (ET), or a combination of the two mechanisms. The HAT reaction is a direct abstraction of H-atom by the free radical, $DPPH^{\bullet}$ from the antioxidant RH, according to Eq. 5 [17]. This reaction depends on the hydrogen bond dissociation enthalpy (BDE) of the compound.

$$RH + DPPH^{\bullet} \rightarrow R^{\bullet} + DPPHH \quad (5)$$

On the other hand, ET reaction involves transfer of electron from ionic intermediate to DPPH•. The following reactions (Equations 6–8) shows the ET mechanism in an H-bond-accepting solvent (S) [17].

$RH + S \rightarrow R^- + SH$ (6)

$$R^- + SH + DPPH^\bullet \rightarrow R^\bullet + DPPH^- + SH \quad (7)$$

$DPPH^- + SH \rightarrow DPPHH + S$ (8)

In polar solvents such as methanol, often used in this assay, the ET reaction (**6–8**) follows a sequential proton-loss electron transfer (SPLET) [17]. ET reaction is dependent on the de-protonation ability and ionization potential (IP) of the antioxidants [38].

The kinetics of the reaction also depends on the relative concentrations of DPPH and antioxidants [28]. In this study, fixed, but an excess concentration of DPPH was subjected to react with different concentrations of extracts of each solvent. The concentration of unreacted DPPH at the steady-state, reaction half-life and steady-state time, and rate constants are summarized in **Table 2.** The amount of DPPH removed from each medium was proportional to the concentration of the extract (antioxidants).

Table 2 Kinetic data of DPPH radical scavenging activity of ascorbic acid and C. mollis extracts at different concentrations.

Sample	Concentration (µg/mL)	Half-life, $t_{1/2}$ (min)	Steady-state time(min)	$k_2(M^{-1}s^{-1})$	Remaining [DPPH] at steady state
Methanol extract	250	0.988	16.53	115.08	56.98
	100	1.308	21.94	87.13	73.4
	50	2.132	28.04	53.28	84.4
Chloroform extract	250	4.572	63.49	24.51	57.77
	100	6.401	94.58	17.94	71.88
	50	7.633	129.58	14.96	84.61
Petroleum ether extract	250	4.846	66.97	23.39	55.30
	100	6.653	96.34	17.04	74.92
	50	7.664	112.93	14.68	86.50
Ascorbic acid	250	0.603	10.02	189.18	6.43
	100	0.695	13.15	162.41	13.06
	50	1.040	15.82	109.80	17.05

The half-life ($t_{1/2}$) indicates the time it takes for antioxidants to reach half of their total radical scavenging activity. This value was less than 3 min for methanol extracts while it ranges from 4 to 8 min

for chloroform and petroleum ether extracts. The steady-state reaction time also indicated maximum radical scavenging activity of methanol extracts, i.e., the reaction was completed within 16–30 min depending on the concentration of test samples. For the other two extracts, it took more than 1 h to reach the plateau indicating their slow radical scavenging potentials (**Table 2**). For methanol extracts the time constants were closer to that of the standard antioxidant, L-ascorbic acid.

Compounds with maximum rate constant, (k_2) are considered to be efficient radical scavenging agents [20]. As can be seen in **Table 2**, the values of k_2 were the highest for ascorbic acid showing its superior antiradical efficiency. Regarding, C. mollis resin extracts, methanol extracts have maximum k_2 which indicates its better reactivity toward DPPH radical than chloroform and petroleum ether extracts. For these extracts, the observed kinetic profiles also showed that the reaction was fast during the initial minutes and slowly declined at a longer time (Fig. 6).

Rate constants refer to the ability of electron-or-hydrogen atom donation of antioxidant molecules in the tested samples towards DPPH. Generally, the reactivity and mechanism of radical scavenging depend on the solvent polarity and H-bond strength, concentration, and other structural features of antioxidants such as the presence of oxygen, an aromatic ring, or conjugated double bonds [39, 40]. The ET reaction could be slower than the HAT due to the time needed for the reaction to have competed and solvent stabilization [38]. Moreover, plant extracts may constitute a mixture of antioxidants.

In previous studies, the antioxidant capacity of terpenoid groups such as sesquiterpenoids, triterpenoids, furanosesquiterpenoids, and sterols, was demonstrated to be responsible for the DPPH radical scavenging effect of resins obtained from Commiphora trees. Demonstration on a kinetic model of DPPH radical scavenging activity of common terpenoids [39], which were also detected in Commiphora trees resins [7–9, 12] showed that monoterpenes with conjugated double bonds such as myrcene and γ-terpinene exhibited rapid kinetic behavior due to electron delocalization capacity on their conjugated double bonds (π bond). Terpenes without conjugated double bonds displayed slower DPPH radical scavenging behavior [39].

Among antioxidants, phenolics were found in stem barks and leaves of C. mollis [15, 16]. Moreover, a report on C. wightii resin indicated ferulates to be responsible for its DPPH radical scavenging activity [13]. According to Villaño et al [20], phenolic antioxidants can exert rapid initial depletion of DPPH due to a fast abstraction of H-atom. The followed slow decay was ascribed to the termination step which could be slower as the radical-radical interaction is sterically hindered. Since plant extracts could constitute a mixture of antioxidants with differing reactivity, different kinetic behavior is possible.

4. Conclusion

In this study, the TPC and antioxidant activity of C. mollis resin methanol, chloroform, and petroleum ether extracted were investigated. Methanol extract showed the highest extraction yield TPC and DPPH radical scavenging activity. The TPC was determined after optimization of the F-C redox assay. A

wavelength of 760 nm and 30 to 40 min of the interaction of the extracts with the F-CR gave adequate results. The resin exhibited significant TPC with the highest value obtained from methanol extract. The TPC of chloroform and petroleum ether extracts was not statistically different. Regardless of the used extraction solvents, maximum TPC was obtained from the less concentrated sample. This ensured the use of enough amount of F-CR for the exhaustive reduction of phenolic antioxidants in the test samples. Moreover, the free radical scavenging activity of C. mollis resin extracts was determined by the DPPH method. For each tested sample, the E_{50} value was determined at 60 min reaction time with the DPPH. Methanol extract provided significantly better results than other extracts (EC_{50} = 295.03 ± 3.55 µg/mL). Moreover, the radical scavenging activity of the extracts was applied to determine their kinetic behavior. The methanol extract showed fast kinetic action compared to the chloroform and petroleum ether extract. This could probably be due to the different kinetic behavior of antioxidants present in C. mollis resin.

The antioxidant activity showed a positive correlation with TPC. The highest extraction yield, reducing properties, and kinetics of free radical scavenging activity observed for methanol extracts may be attributed to the high affinity of antioxidant compounds in C. mollis resin towards more polar solvents as compared to the nonpolar ones. For a better understanding of the antioxidant activity of C. mollis resin, further in vitro and in vivo studies supported by isolation and elucidation of individual component compounds could be important.

Abbreviations

% [DPPH]r: Percent of DPPH concentration remained, ANOVA: Analysis of variance, CV: Coefficient of variation, DPPH: 2,2-Diphenyl-1-picrylhydrazyl, EC_{50}: Amount of antioxidant necessary to decrease the initial DPPH concentration by 50%, ET: Electron transfer, F-C: Folin-Ciocalteu, HAT: Hydrogen atom transfer, LOD: Limit of detection , LOQ: Limit of quantitation, mg GAE/g: Milligrams of gallic acid equivalent per gram, SPLET: Sequential proton-loss electron transfer, TAC: Total antioxidant capacity, TPC: Total phenolic content, UV-Vis: Ultraviolet-visibe.

Declarations

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Authors’ contributions

All the authors made significant contributions to the manuscript and agree to its publication. GJM and AG designed the research topic; AG and NA supervised the experimental works and contributed in reviewing draft of the manuscript. GJM carried out the experimental works and wrote the manuscript.

Availability of data and materials

The datasets generated and/or analysed during the current study are included in the manuscript. Moreover, the datasets used to generate figures and results are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Permissions were obtained to collect *Commiphora mollis* resin sample from the public land in Gorile district, Borana Zone, Oromia regional state, Ethiopia. All methods were carried out in accordance with relevant guidelines and regulations. All authors declare that they have ethics approval and consent to participate.

Consent for publication

All authors consent to the publication.

Competing interests

The authors declare that they have no competing interests.

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Figures

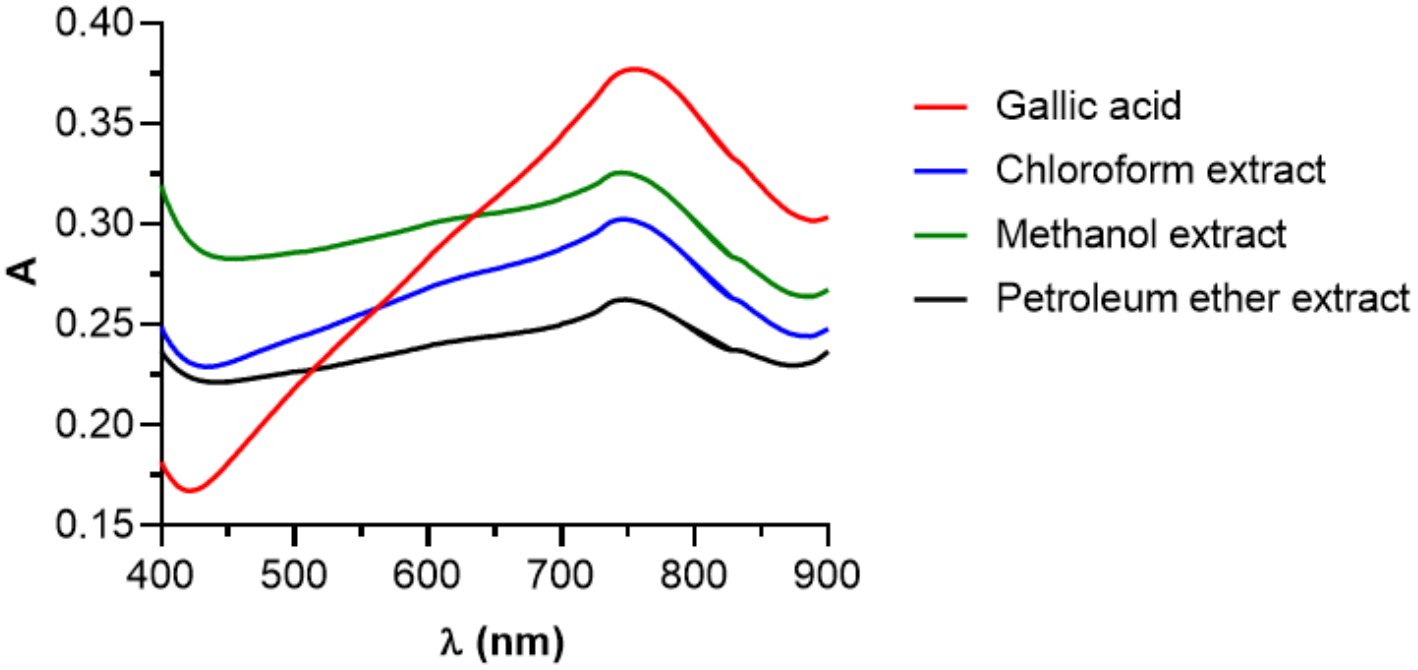

Figure 1

Absorption spectrum (400 to 900 nm) of the product of the reaction of F-CR with gallic acid (50 µg/ml) and *C. mollis* resin extracts (200 µg/ml)

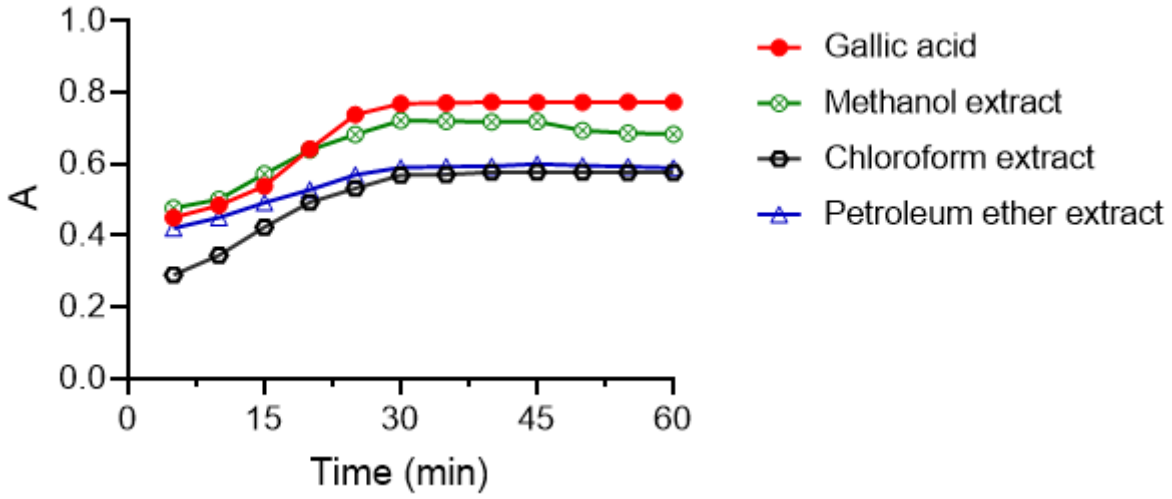

Figure 2

Change in absorbance, with a reaction time of F-CR with Gallic acid and *C. mollis* resin extracts.

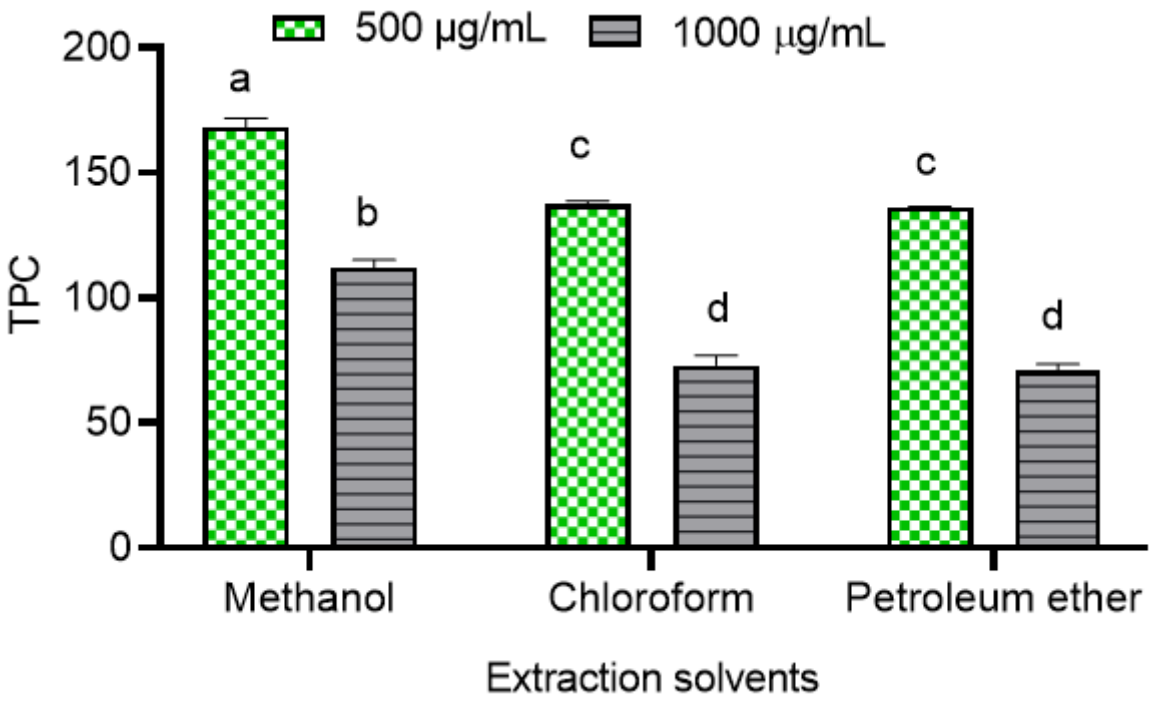

Figure 3

TPC of *C. mollis* resin extracted using the different solvents

[a, b, c, d] Values followed by different letters are significantly different (ANOVA with Tukey's test, n (instrumental × sample replicate) = 9, $p < 0.05$).

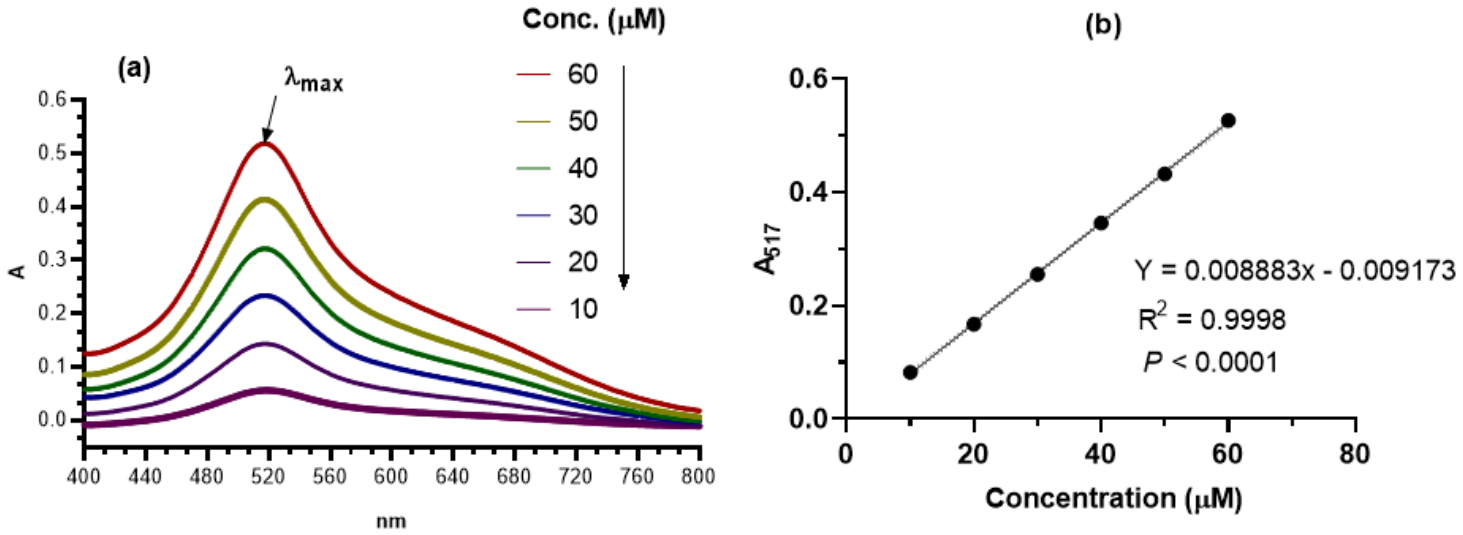

Figure 4

(a) Absorption spectra (400 to 800 nm) for solutions containing 10, 20, 30, 40, 50, and 60 μM DPPH and (b) linear relationship between concentration and absorbance measured at 517 nm.

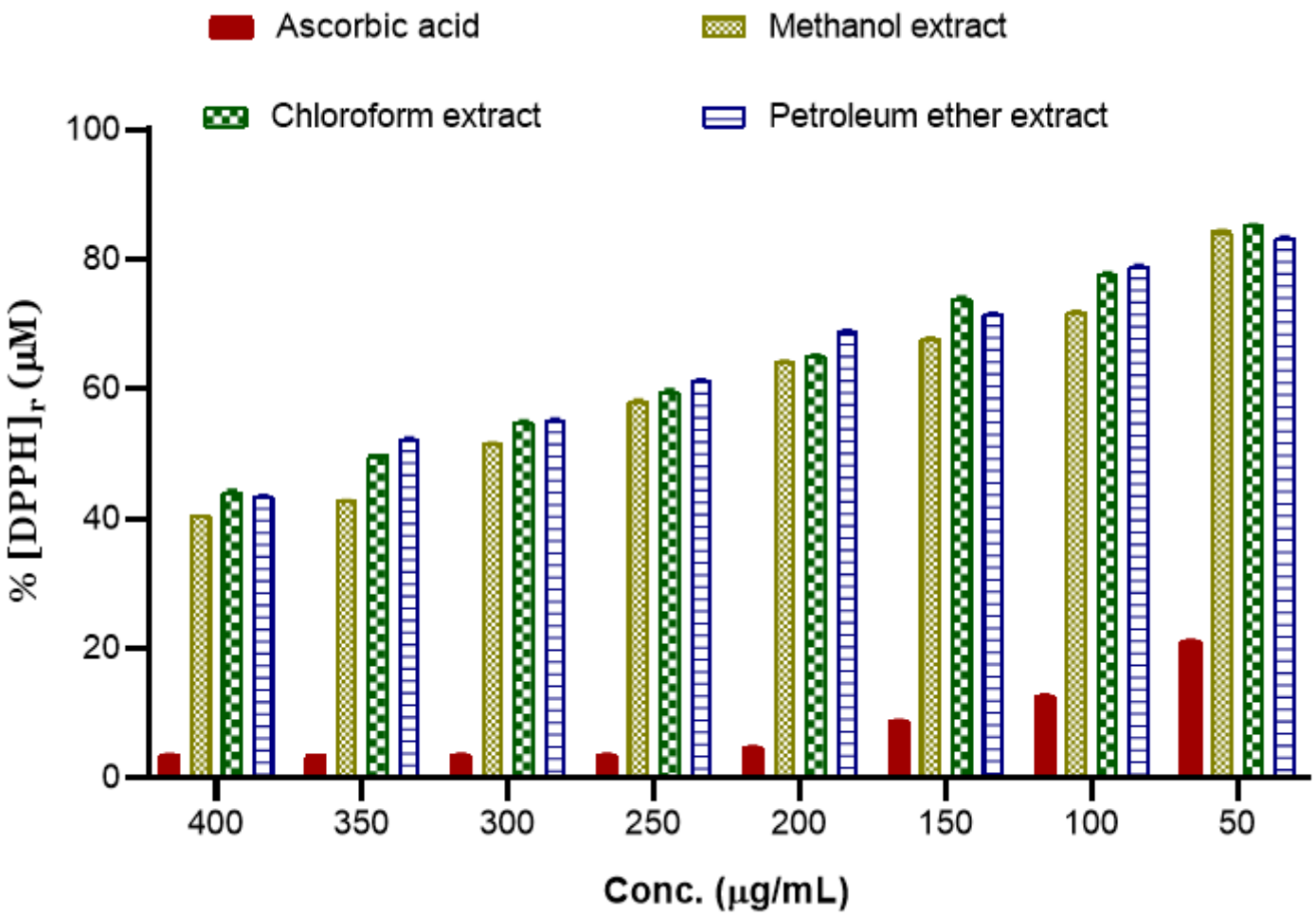

Figure 5

DPPH radical scavenging effects of *C. mollis* resin extracts and ascorbic acid. Results were expressed as mean ± standard deviation of three means (n = 3).

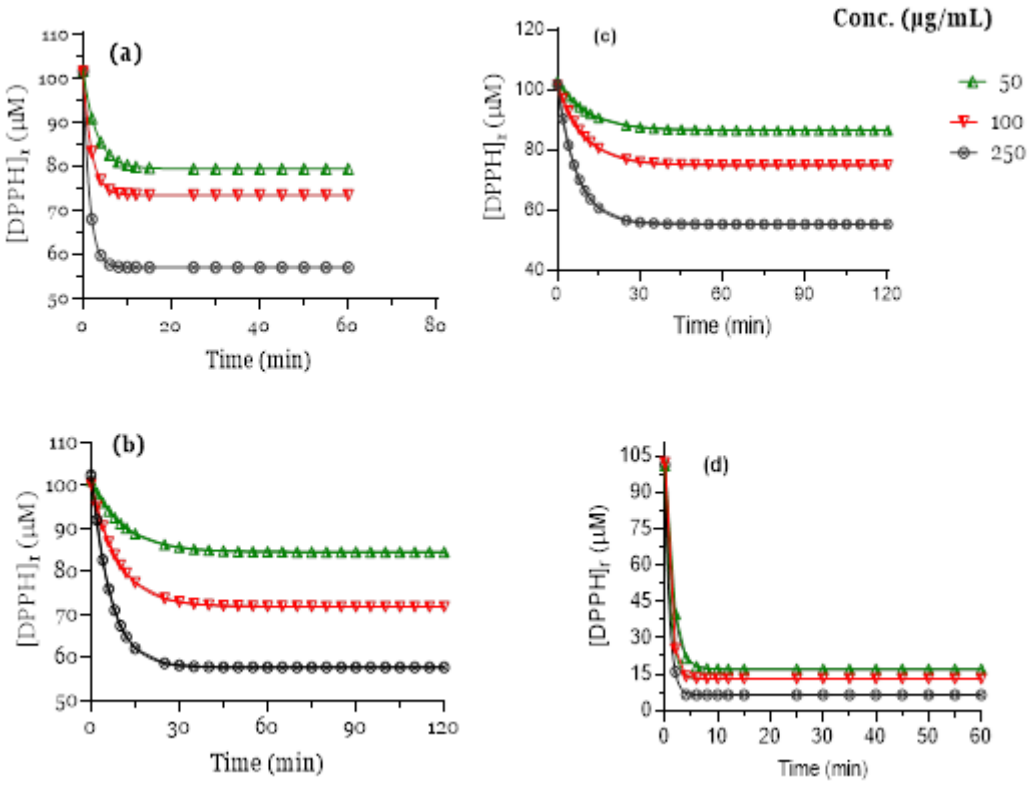

Figure 6

Kinetics analysis of DPPH radical scavenging reaction of C. mollis resin extracts ((a) methanol extract, (b) chloroform extract, (c) petroleum ether extract, and (d) L-ascorbic acid. The graphs were plotted as the remaining concentration of DPPH, ($[DPPH]_r$) versus time.

Supplementary Files

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- graphicalabstract.png