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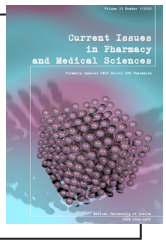

Phytochemical and antioxidant activities of methanolic extract of *Lawsonia inermis* L. Bark

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ABSTRACT

Many diseases are associated with oxidative stress are caused by free radicals. Current research has been directed towards finding naturally occurring antioxidants of plant origin. The aim of the present study was to evaluate the *in vitro* antioxidant activities of methanolic extract of *Lawsonia inermis* L. bark (MELIB). The present study was carried out for determination of qualitative, quantitative phytochemical and *in vitro* antioxidant activity for scavenging of free radical DPPH, superoxide radical scavenging and lipid peroxidation. The bark extract is a rich source of secondary metabolites like flavonoids at a rutin equivalent 73.43±0.26%, polyphenol at a gallic acid equivalent 84.70±0.43%, tannins at a tannic acid equivalent 88.75±0.14% and terpenoids at a linalool equivalent 68.13±0.31. MELIB showed free radicals scavenging capacity by way of the DPPH method (6.93±0.51 to 62.63±0.10% inhibition), the superoxide radical scavenging method (1.82±0.41 to 57.11±0.18% inhibition) and the ferric chloride induced lipid peroxidation method (9.87±0.33 to 80.32±0.82% inhibition). The results obtained in the present study indicate that MELIB can be a potential source of natural antioxidants due to the presence of flavonoids, polyphenols, tannins and terpenoids.

INTRODUCTION

Antioxidants are compounds capable to either delay or inhibit the oxidation processes that occur under the influence of reactive oxygen species [1]. Therefore, plants have attained the status of being natural sources of new and potent antioxidant agents that can be used for the stabilization of polymeric products, of petrochemicals, foodstuffs, cosmetics and pharmaceuticals [2].

Antioxidants are involved in the defense mechanism of the organism against the pathologies associated with the attack of free radicals. Endogenous antioxidants are enzymes, like superoxide dismutase, catalase or nonenzymatic compounds such as uric acid, albumin and metallothioneins. When endogenous factors cannot ensure the control and protection of the organism against the reactive oxygen species, the need arises for exogenous antioxidants as nutritional supplements, which contain as active principle as an antioxidant compound. Amongst the most important exogenous antioxidants, vitamin E, vitamin C, β-carotene, flavonoids and minerals Selenium (Se) are well known [3].

Exogenous antioxidants can be derived from natural sources like flavonoids, polyphenols and tannins, and some mineral compounds, etc. [4]. There is an increasing interest in exploring the administration of antioxidants, particularly those intended to prevent the presumed deleterious effects of free radicals in the human body [5]. In the recent era, antioxidants have attracted significant attention as prophylaxis and for free radical therapy, as well as oxidative stress-induced cancer, diabetes and neurological degenerating disease [6-8].

Primary antioxidants are the chain breaking antioxidants that react with lipid radicals and convert them into more stable products. Primary antioxidants are mainly polyphenols in structure and include minerals like selenium, zinc and manganese, as well as antioxidant vitamins like vitamin A, B and E and phytochemicals which include flavonoids, catechin, carotenoids, β-carotene, lycopene, black pepper, cumin and their derivatives [9].

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Secondary antioxidants are polyphenols compounds that perform the function of capturing free radicals and stopping chain reactions. The compounds include Butylated hydroxy anisole (BHA), butylated hydroxyl toluene (BHT) and propyl gallate (PG) [10].

Lawsonia inermis L. is commonly known as henna or mehandi, and belongs to the family *Lythraceae* which is grown in dry tropical and subtropical zones, including North Africa, India, Sri Lanka and the Middle East. *Lawsonia inermis* L. attains a height of 2.4-5 m. It is cultivated as a hedge plant throughout India and as a commercial crop in certain states of India. The bark is unarmed and grayish brown when young, but the branches of older trees are spine tipped [11,12]. It has been widely used over the centuries for medical and cosmetic purposes all over world. Mehndi was reported to contain various phytoconstituents such as chlorogenic acid, ferulic acid, isoferulic acid, gallic acid, o-coumaric acid, m-coumaric acid and myricetin [13]. The bark is known to contain napthoquinone, isoplumbagin, triterpenoids-Hennadiol, aliphatics (3- methylnonacosan-1-ol) [14]. Mehandi is applied for the cure of renal lithiases, jaundice, for wound healing and preventing skin inflammation [15]. The bark is traditionally used in the treatment of jaundice and for the enlargement of the spleen, renal calculus, leprosy and obstinate skin diseases [16].

The aim of this study was to determine the phytochemical screening, as well as the quantitative estimation of polyphenols, flavonoids, tannins and terpenoids; along with in vitro antioxidant activities of methanolic extract Lawsonia inermis L. bark (MELIB) thereof. Results from this work will enlighten the medical aspects of this herb.

MATERIALS AND METHODS

Chemicals

5% v/v acetic acid, 26.6 mg $AlCl_3 \cdot 6H_2O$, 80 mg CH_3COONa, 10mg rutin, 5 mL Folin-Ciocalteu's reagent, 7.5% w/v sodium carbonate solution, 10mg gallic acid, 50 mg Tannic acid 0.5 mL tungstophosphoric acid, 50% sodium carbonate, 95% v/v methanol, 20mg linalool, 1.5 mL chloroform, 2 mg 2, 2-diphenyl-1-picrylhydrazyl, 5 mg Nitro blue tetrazolium, 3 mg EDTA, 6 mg hydroxylamine hydrochloride, 0.15 M KCl, 10mg ferric chloride, 0.25 N HCl, 15% trichloroacetic acid (TCA), 0.38% thiobarbituric acid (TBA), 0.05% butylated hydroxyl toluene (BHT).

Collection and Authentication of the Plant Material

Lawsonia inermis L. Bark was collected from the Botanical garden of Anand Agriculture University, Anand, Gujarat, India. The plant was authenticated by Dr. Dilipbhai B. Patel, Botanist, Professor and Head, Department of Genetics and Plant Breeding, B. A. College of Agriculture, Anand Agriculture University, Anand, Gujarat, India. (Herbarium Specimen#: SPCP/02/2014).

Preparation of Plant Extract

Fresh bark of *Lawsonia inermis* L. were collected and dried for one week and pulverized into a coarse powder with the help of a grinder. About 500 g of powdered material was taken in a clean, flat bottomed glass container and soaked in 3000 mL of methanol. The container with its contents was sealed and kept for a period of 7 days at room temperature with occasional shaking and stirring. The whole mixture was filtered through a piece of clean, white muslin cloth. The extract was concentrated to dryness by rotary evaporator and stored in a sealed glass jar [17].

Phytochemical Screening

Herein, the existence of different primary and secondary metabolites was determined. For this purpose, detection tests for alkaloids, tannins, phenols, saponin, flavonoids, steroids and glycosides were carried out by following the standard methods [18].

Determination of Total Flavonoid content

In undertaking this, 0.2 g of MELIB was extracted in 2 mL of extraction medium (70% v/v methanol, 5% v/v acetic acid and 25% v/v distilled water) at room temperature for 60 min. The resulting solution was filtered through Whatman No 4 and filtrate volume adjusted to 10 mL. The samples were prepared by mixing together 5 mL of extract, 1 mL of distilled water and 2.5 mL of $AlCl_3$ solution (26.6 mg $AlCl_3 \cdot 6H_2O$ and 80 mg CH_3COONa dissolved in 20 mL distilled water). A blank sample was prepared by replacing $AlCl_3$ solution with distilled water. The absorbance of test samples and blank sample were measured immediately at 430 nm. Total flavonoid content, was expressed as µg rutin per mL of MELIB [19,20].

Determination of Total Polyphenolic content

Total polyphenol content (TPC) was determined by applying the spectrophotometry method, using gallic acid as standard, according to the method described by the International Organization for Standardization (ISO) [21]. Briefly, 1 mL of the diluted sample extract was transferred in duplicate to separate tubes containing 5 mL of a 1/10 dilution of Folin-Ciocalteu's reagent in water [22]. Subsequently, 4 mL of a sodium carbonate solution (7.5% w/v) was added. The tubes were then allowed to stand at room temperature for 60 min before absorbance at 765 nm was measured against water. The total polyphenol content was expressed as µg/L of gallic acid equivalents [23,24].

Determination of Total Tannin content

Tannic acid was used as a standard for the calibration curve; the stock solution was prepared by dissolving 50 mg into 50 mL water (1000 µg/mL). From that solution, 1 mL was taken and diluted with reagent to produce a different concentration (10-80 µg/mL). Test solutions from MELIB, were prepared by dissolving 4 mg into 10 mL water (400 µg/mL) then 1 mL was taken and diluted with reagent. 1 mL from each solution of standard and sample was subsequently treated with 0.5 mL tungstophosphoric acid in 10 mL volumetric flask. After this, the volume was made up to 10 mL with 50% sodium carbonate. After 3 min, the absorbance at a wavelength of 750 nm was read. The total tannin content is expressed as µg/L of tannic acid equivalents. [25].

Determination of Total Terpenoids content

The total terpenoids content of the MELIB was determined based on an assay described by Ghorai *et al*. [26]; Linalool was used as the standard for estimation. For the qualitative analysis of terpenoids in the extract, an aliquot of the reaction mixture obtained after the Salkowski test was transferred to a colorimetric cuvette. The absorbance was measured at 538 nm against blank, i.e., 95% v/v methanol. For the standard curve, 1.5 mL chloroform was added to 200 µL of linalool solution in methanol and serial dilutions were prepared in which total volume of 200 mL was made up by the addition of 95% v/v methanol. The total terpenoids content was expressed as µg/L of linalool equivalents.

In-Vitro Antioxidant Determination

DPPH (2, 2-diphenyl-1-picrylhydrazyl) method

The method is based on the reduction of a colored solution of DPPH in the presence of MELIB measured at 517 nm. The activity is expressed as percent of inhibition, which is the concentration of the test solution required to give a 50% decrease in absorbance compared to that of blank solution. To 150 µL of DPPH solution in methanol, different concentrations of ascorbic acid were added and the volumes were made up to 3 mL with methanol. DPPH diluted to 3 mL was taken as blank. A decrease in absorbance in the presence of ascorbic acid was noted down at 517 nm after 15 min., a linear graph of concentration *vs* absorbance was prepared and percent of inhibition values were calculated, MELIB solutions were treated in the similar manner [24].

Superoxide radical scavenging method

The method is based on generation of a super oxide radical (O^{2-}) by auto oxidation of hydroxylamine hydrochloride in the presence of NBT (Nitro blue tetrazolium), which is reduced to nitrite. Nitrite in the presence of EDTA gives a colour that can be measured at 560 nm. Aliquots of 0.1 to 1 mL of ascorbic acid solution were taken in a test tube, to which 1 mL of sodium carbonate, 0.4 mL of NBT and 0.2 mL of EDTA were added; a zero minute reading was taken at 560 nm. The reaction was initiated by the addition of 0.4 ml of hydroxylamine hydrochloride to the above solution. The reaction mixture was then incubated at 250°C for 5 min., the reduction of NBT was measured at 560 nm. A parallel control was also treated in the similar manner. The extract was treated in the similar manner, absorbance was recorded and percent of inhibition was calculated [27].

Ferric chloride induced lipid per oxidation method

Chicken liver samples were collected from an Anand slaughter-house and frozen in liquid nitrogen until required. From this, a chicken liver homogenate (20% w/v) was prepared according to the method described by Ahmad *et al.* [28]; Herein, 1 gm wet chicken liver in 0.15 M KCl was prepared using a tissue homogenizer under ice cold (0-4°C) conditions. The homogenate was centrifuged at 3000 rpm for 5 min and the clear supernatant was used for further study. Different concentrations of MELIB were taken in test tubes to which 1 mL of 0.15 M KCl buffer (pH 7.4) and 0.5 mL of cell free homogenate were added. Peroxidation was initiated by adding 100 mL of 1Mm (millimole) ferric chloride. The mixture was incubated for 30 min at 37°C. After incubation, the reaction was stopped by adding 2 mL of ice cold 0.25 N HCl containing 15% trichloroacetic acid (TCA), 0.38 % thiobarbituric acid (TBA), and 0.5% stock solution of 0.05% butylated hydroxyl toluene (BHT). The reaction mixture was heated for 60 min at 80°C. The sample was subsequently cooled and centrifuged at 3000 rpm for 15 min and absorbance of supernatant was measured at 532 nm. An identical experiment (control induced) was performed in absence of test compounds to determine the amount of lipid peroxidation obtained in the presence of inducing agents without any test compounds. A blank was performed with all the reagents except ferric chloride and test extracts [29].

Statistical Analysis

Statistical analysis was performed by statistical software Graph Pad Prism Version 8. A non linear regression correlation curve fit was done; percentage inhibitions of free radicals were analyzed. The data was expressed as the mean ± standard deviation, n=3.

RESULTS

Phytochemical Screening

MELIB revealed the presence of polyphenol compounds, steroids, terpenoids, flavonoids, alkaloids, carbohydrates, proteins, steroids, tannins and glycosides; results are shown in Table 1.

Table 1. Preliminary Phytochemical Screening of MELIB

Secondary Metabolites	MELIB
Alkaloids	+
Polyphenol	+
Flavonoids	+
Terpenoids	+
Steroids	+
Carbohydrates	+
Proteins	+
Tannin	+
Glycosides	+

Quantitative Phytochemical Screening

The presence of high quantities of phytochemicals, including secondary metabolites such as polyphenol, flavonoids, tannin and terpenoids; might contribute to the pharmacological activity possessed by certain plants [30]. Polyphenol, flavonoids, tannin and terpenoids are important secondary metabolites that exhibit medicinal properties such as antioxidant, anti-inflammatory, anticancer, antibacterial and antiviral activity [31]. These are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in human therapy, veterinary, agriculture, scientific research and countless other areas [32]. Knowledge of the chemical constituents of plants is desirable because such information will be value for the synthesis of complex chemical substances [33-35]. Mehndi has been

a traditional crude drug since time immemorial. Quantitative analysis of MELIB has demonstrated the presence of polyphenol (84.70±0.43%), flavonoids (73.43±0.26%), tannin (88.75±0.14%) and terpenoids (68.13±0.31%). The present study indicated particularly high concentrations of tannin (88.75%) during quantitative analysis. The four phytochemicals as established are shown in Table 2.

***Table* 2.** Quantitative Phytochemical Screening

Phytoconstituents	Equivalent (µg/mL)	Percentage (%)
Flavonoid	Rutin equivalent 29.37±0.24	73.43±0.26
Polyphenol	Gallic acid equivalent 31.41±0.18	84.70±0.43
Tannin	Tannic acid equivalent 35.05±0.35	88.75±0.14
Terpenoids	Linalool equivalent 26.38±0.45	68.13±0.31

Results are expressed in terms of mean ± SD (n=3)

In-Vitro Antioxidant Determination

DPPH (2, 2-diphenyl-1-picrylhydrazyl) method percent of inhibition

The DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extract [36]. DPPH radical scavenging activities of an extract depends not only on plant type, but also upon the extraction solvent. In general, DPPH scavenging activities increase with increasing polyphenol components, flavonoids, polyphenols and tannin. These secondary metabolites possess many hydroxyl groups, including o-dihydroxy groups that have very strong radical scavenging effect and antioxidant power. In the DPPH assay, the antioxidant was able to reduce the stable radical 2, 2-diphenyl-1-picrylhydrazyl to the yellow colored 1, 1-Diphenyl-2-picrylhydrazine. The molecule of 1, 1-Diphenyl-2-picrylhydrazine is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecules. The delocalization also gives rise to the deep violet colour, characterized by an absorption band in methanol solution centered at 517 nm [37]. In our work, a dose response curve of DPPH radical scavenging activity of MELIB was observed, when compared with standard ascorbic acid. This is shown in Figure 1. Antioxidant activity in the form of percent of inhibition values of different concentration of extracts were calculated and shown in Table 3. MELIB at the concentrations range from 0.25 µg/mL to 20.00 µg/mL inhibited 6.93±0.51 and 62.63±0.10 according to the DPPH method.

Table 3. DPPH (2, 2-diphenyl-1-picrylhydrazyl) method percent of inhibition

Conc. (µg/mL)	% Inhibition by Standard (Ascorbic Acid)	% Inhibition by MELIB
0.25	12.46±0.62	6.93±0.51
0.50	19.11±0.47	8.18±0.35
1.00	39.07±0.57	18.03±0.26
2.50	55.42±0.92	25.79±0.29
5.00	67.83±0.72	35.15±0.38
10.00	80.67±0.87	45.95±0.41
20.00	90.08±1.15	62.63±0.10

Results are expressed in terms of mean ± SD (n=3)

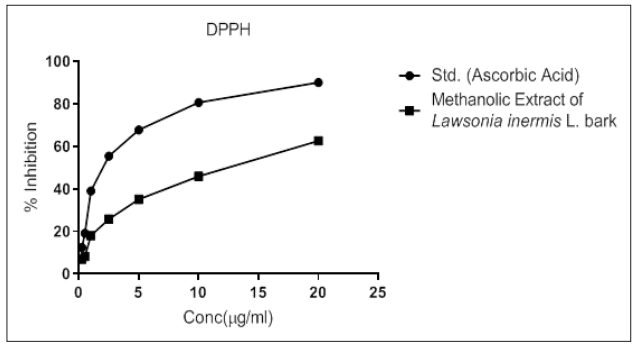

Figure 1. DPPH (2, 2-diphenyl-1-picrylhydrazyl) method

Superoxide radical scavenging method percent of inhibition

The generation of superoxide radicals can be measured by their ability to reduce NBT [38]. A decrease in absorbance at 560 nm with the MELIB and the reference compound ascorbic acid indicates their abilities to quench superoxide radicals within the reaction mixture. As shown in Figure 2 and Table 4, MELIB at concentrations ranging from 10 µg/mL to 100 µg/mL showed 1.82±0.41 and 57.11±0.18% of inhibition by way of application of the super oxide radical scavenging method.

Table 4. Superoxide radical scavenging method percent of inhibition

Conc. (µg/mL)	% Inhibition by Standard (Ascorbic Acid)	% Inhibition by MELIB
10	4.21±0.65	1.82±0.41
20	6.58±0.51	4.58±0.59
30	10.75±0.57	6.42±0.48
40	18.34±0.26	11.46±0.78
50	27.29±0.64	21.22±0.28
60	41.74±0.86	30.11±0.56
70	51.37±0.46	42.70±0.17
80	55.89±0.25	48.83±0.21
90	59.17±0.36	52.66±0.16
100	62.15±0.12	57.11±0.18

Results are expressed in terms of mean ± SD (n=3)

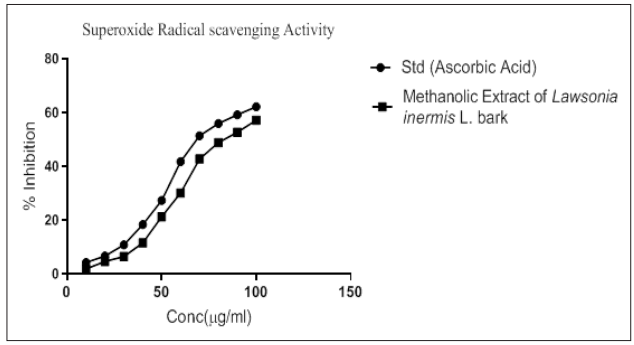

Figure 2. Super oxide radical scavenging method

Ferric chloride induced lipid per oxidation method percent of inhibition

MELIB indicated activity by inhibiting $FeCl_3$-induced lipid peroxidation in the liver homogenate (Figure 3 and Table 5). At higher concentration, MELIB indicated the highest inhibition of lipid peroxidation. MELIB at the

concentrations range from 3 to 1000 µg/mL inhibited 9.87±0.33% to 80.32±0.82 of lipid peroxidation.

Table 5. Ferric chloride induced lipid per oxidation method percent of inhibition

Conc. (µg/mL)	% Inhibition by Standard (Ascorbic Acid)	% Inhibition by MELIB
3	13.16±0.35	9.87±0.33
10	21.83±0.48	18.40±0.59
30	41.70±0.37	35.49±0.56
100	62.24±0.57	52.60±0.85
300	77.71±0.44	67.72±1.19
1000	91.62±0.36	80.32±0.82

Results are expressed in terms of mean ± SD (n=3)

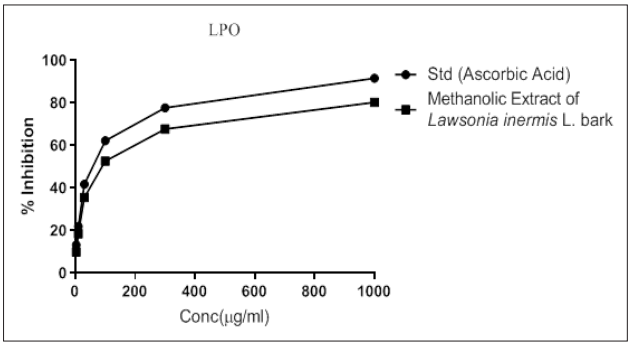

Figure 3. Ferric chloride induced lipid per oxidation method

From the preliminary results of phytochemical screening, as estimated by three methods DPPH (2, 2-diphenyl-1-picrylhydrazyl), super oxide radical scavenging and ferric chloride induced lipid per oxidation, MELIB was found to contain flavonoids, polyphenols, tannins and terpenoids compounds that are known to provide antioxidant activity [39].

DISCUSSION

The medicinal value of plants has assumed an important dimension in the past few decades. Plants produce a very diverse group of secondary metabolites such as polyphenols, flavonoids, terpenoids and tannin with antioxidant potential. Antioxidants block the action of free radicals. These have been implicated in the pathogenesis of many diseases and in the aging process [40-42]. In living systems, free radicals are constantly generated in the various biological processes that are necessary for the body. They have a role in implicating the cell-signaling mechanism. This shows that free radicals are necessary, but at the same time harmful for the body [43]. Hence they can cause extensive damage to tissues and to biomolecules – leading to various disease conditions, especially degenerative diseases [44]. Many synthetic drugs protect against oxidative damage, but they have adverse side effects. An alternative solution to the problem is to consume natural antioxidants from food supplements and traditional medicines [45,46]. Antioxidants have been demonstrated to play important roles in ROS (Reactive oxygen species) scavenging via several mechanisms, thereby reducing the adverse outcomes of ROS-induced injury. It is therefore not surprising that epidemiological studies have demonstrated that increased consumption of fruits and vegetables is associated with reduced risks of chronic diseases like cancers, likely due to their antioxidant rich contents, including polyphenols compounds [47]. Recently, many natural antioxidants have been isolated from different plant materials [48,49].

In the present study, the chemical composition and some anti-oxidant indices of MELIB were evaluated by way of DPPH, superoxide radical scavenging activity and lipid peroxidation.

Different flavonoid structures and their substitutions have influence on the phenoxyl radical stability, thereby affecting the antioxidant properties of the flavonoids. Flavonoids are also known to have potential activity against oxidative stress [50]. In our study, quantitative determination of total flavonoid contents in MELIB was performed using aluminum chloride in a colorimetric method. The results indicated a rutin equivalent of 29.37±0.24 µg/mL.

We also found the total polyphenols content of MELIB to be 31.41±0.18 µg/mL gallic acid equivalents. This suggests that MELIB is rich in polyphenol compounds. The higher value of total polyphenols in MELIB observed due to applying the Folin-Ciocalteu method could, however, be related to interference of sugars and protein. As can be recalled, polyphenols have been associated with high antioxidant potentials and increased capacity for scavenging free radicals [51,52]. The implication of such an effect is that the fewer radicals observed due to the scavenging ability of polyphenols from *Lawsonia inermis* L. bark indicate that MELIB could protect against development of oxidative stress-related diseases caused by excess free radicals.

Tannins are a group of polyphenols compounds that are widely present in plants and possess various antioxidant and biological activities. Therefore, tannins are a major research subject in developing natural alternatives to manufactured drugs [53]. The tannin content in MELIB were determined using tungstophosphoric acid in a colorimetric method. The results were a tannic acid equivalent of 35.05±0.35 µg/mL.

MELIB as assessed for total terpenoids content indicated a linalool equivalent of 26.38±0.45 µg/mL. This work was based on an assay described by Ghorai *et al*. [26]. Studies in recent decades have demonstrated that terpenes exert antioxidant, anti-inflammatory effects by inhibiting various pro-inflammatory pathways especially by scavenging free radicals. Terpenes have been used to relieve bronchitis, chronic obstructive pulmonary disease, skin inflammation, and osteoarthritis [54-59].

The DPPH method was evidently introduced nearly 50 years ago and it is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant capacity [60]. The parameter percent of free radical inhibition is used for the interpretation of the results from the DPPH method and is defined as the concentration of substrate that causes 50 % loss of the DPPH activity. Figure 1 and Table 3 showed the DPPH radical scavenging activity of MELIB, indicating a remarkable scavenging activity. DPPH is a stable free radical that would reduce in the presence of hydrogen donating antioxidants [61]. MELIB possess rich secondary metabolite (polyphenols, flavonoids, terpenoids and tannins) thus the higher radical scavenging activity may be attributed to higher amounts of hydrogen donating phenolic antioxidants.

The superoxide radicals scavenging activity assay was based on the capacity of the antioxidant compound to inhibit formazan formation by scavenging the superoxide radicals generated in the NBT system [62]. Figure 2 and Table 4 shows the superoxide radical (O_2.) scavenging activity of the extract as measured by the in vitro NBT system. Superoxide radicals are known to be very harmful to cellular components, being precursors of more reactive oxygen species [44]. MELIB was found to be a significant scavenger of superoxide radicals generated in the in vitro NBT system. The extract inhibited the formation of blue formazan and the percent inhibition was proportional to the concentration.

To determine whether the plant extracts possess antioxidant activity in vitro, lipid peroxidation was induced by $FeCl_3$.Fe^{+2}. This caused lipid peroxidation that may be associated with catalyzing one-electron transfer reactions effects that produce reactive oxygen species. Fe^{+2} also generates peroxyl and alkoxyl radicals via lipid peroxides decomposition, which favors lipid oxidation production [63]. However, the secondary metabolite present in MELIB showed a dose-dependent decrease in the TBARS (Thiobarbituric acid reactive substances) level in chicken liver homogenate tissues. Here, results are expressed in terms of percent of free radical inhibition and are shown in Figure 3 and Table 5. Phytochemicals thus can protect cells by preventing/decreasing the production of reactive oxygen species or by scavenging/inhibiting the free radicals that are generated in the body [64].

CONCLUSION

On the basis of the results obtained in the present study, it is concluded that MELIB, contains large amounts of flavonoids, polyphenols, tannin and terpenoids compounds. These exhibit high antioxidant and free radical scavenging activities. The *in vitro* assays indicate that this plant extract is a significant source of natural antioxidant and preparations might be helpful in preventing the progress of various oxidative stresses and free oxygen radical-induced diseases. Indeed, herbal drugs containing free radical scavengers are gaining importance in treating many diseases. Overall, MELIB may be useful as an antioxidant and free radical scavenging agent and thus help in treatment of many diseases mediated by reactive oxygen species.

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Nil.

CONFLICT OF INTERESTS

Authors of this work have no conflicts of interest.

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