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Research Article

Evaluation of Toxicity and Antioxidant Activity of Hydro-Ethanol Leave Extract of *Mentha piperita*

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ABSTRACT

Mentha piperita has numerous pharmacological, cosmetic and alimental applications due to its ability to produce terpene and terpenoid compounds. Antioxidants are considered as important bioactive compound on account of many health benefits along with their vital role in delaying oxidative rancidity of numerous food. The study was to investigate acute toxicity evaluation and in vitro antioxidant activity of hydro-ethanol leave extracts of *M. piperita*. The acute toxicity was evaluate in rat according to (OECD). Animals were daily observed for 24 hours after treatment for mortality. The antioxidant activity of the extract was evaluated based on the method such as DPPH radical scavenging activity and reducing power method. The results showed that mortality was not observed in phase one but was observed in phase two. The LD_{50} by oral route in rat was less than 5000 mg/kg body weight. The percentage of antioxidant activity of leaf extracts of *M. piperita* was assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay, reducing power (RP) assay, ferric reducing/antioxidant power assay, and nitric oxide (NO) radical scavenging assay. The analysis revealed that NO scavenging activity is higher than DPPH with extract. NO and DPPH exhibited remarkable antioxidant activity. This indicates that the hydro-ethanol leave extract of *M. piperita* has a good antioxidant activity.

Keyword: *Mentha piperita*; peppermint; acute toxicity; in vitro antioxidant; free radical

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INTRODUCTION

In different parts of the world, herbs were utilized for medicines, food, and many other purposes. In various countries, research is being done to discover the potential applications of medicinal plants in favor of human beings. Mentha is a member of the Lamiaceae family, and their plants generally contain flowers with prominent liplike lower petals. Oil extracted from Mentha has cosmetic, pharmaceutical, and perfumery applications. Sometimes, it is also used for culinary purposes for food and flavors [Hassan *et al*., 2015]. LD_{50} is the first step in the assessment and evaluation of the toxic characteristics of a substance. The human body has a complex system of natural enzymatic and non-enzymatic antioxidant defenses which counteract the harmful effects of free radicals and other oxidants. Antioxidants are compounds that can delay or inhibit the oxidative damage of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions [Amarowicz *et al*., 2004]. Antioxidant is a chemical that prevents the oxidation of other chemicals. They protect the key cell components by neutralizing the damaging effects of free radicals, which are natural by- products of cell metabolism [Ames *et al.,* 1993; Shenoy and Shirwaikar, 2002].Antioxidants may be of great benefit in improving the quality of life by preventing

or postponing the onset of degenerative diseases [Nur Alam *et al.,* 2012]. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [Nabavi *et al*., 2008]. Free radicals such as oxygen radical are chemical species that possess an unpaired electron in the outer (valance) shell of the molecule. This is the reason, why the free radicals are highly reactive and can react with proteins, lipids, carbohydrates and DNA [Patil*et al.,* 2003]. Free radicals are responsible for causing a large number of diseases. Protection against free radicals can be enhanced by ample intake of dietary antioxidants. Substantial evidence indicates that foods containing antioxidants and possibly in particular the antioxidant nutrients may be of major importance in disease prevention [Nur Alam *et al.,* 2012]. There is, however, a growing consensus among scientists that a combination of antioxidants, rather than single entities, may be more effective over the long term. Oxidant species such as hydrogen peroxide (H_2O_2) and superoxide (O_2^-) are produced following phagocytosis of the pathogen as part of their machinery to respond to harmful insults [Hamad *et al*., 2014]. High Nitric oxide (NO) increased levels of prooxidant species and may lead to damage and poor perfusion of vital organs of the host, contributing to multiple organ failure [Bandoniene *et al*., 2002].

MATERIALS AND METHODS

Sample Collection and Preparation

The leaves of *M. piperita w*ere collected from central market Katsina, Nigeria for Preparation of the Crude Hydro-ethanol extract. The collected leaves were washed then dried under forced air circulation at 45° C. The dried leaves were triturated, and the resulting powder stored in an air tight container. For preparation of hydro-ethanol (solvent) solution used for extraction, 200 g of the powdered leaves sample of *M. piperita* was weighed using an analytical balance, then soaked in 50% ethanol in ratio 1:1 of the sample and ethanol for extraction for a period of 48hours. After 2 days the mixture were filtered through cellulose filter paper (Whatman) and evaporated to dryness under reduced pressure using oven at 40° C. The residual solvent were removed in a vacuum centrifuge at 40° C to yield the extracts.

Experimental Animals

Seventeen (17) both sexes Wistar albino rats weighing 100-150geach, were purchased from Animal house of the Faculty of Biological Science, Ahmadu Bello University Zaria. The animals were housed in well ventilated room, three rats in a single cage and allowed a free access to food and water ad libitum. The animals were allowed to acclimatize for two week prior to the commencement of the treatment. A standard procedure was observed in line with the Good Laboratory Practice (GLP) Regulations of the WHO (2008).

Toxicity Studies

Phase I: After acclimatization, Nine (9) Swiss albino rats were randomly divided into 3 groups of 3 animals each. Groups 1, 2 and 3 were orally administered with 50, 100 and 1000 mg/kg body weight of the hydro-ethanol leave extracts of *M. piperita* [Lorke, 1983], respectively. The animals were observed frequently from the day of treatment for any changes up to 14 days.

Phase II: Eight (8) Swiss albino rats were divided into 4 groups of 2 animal each. The extract was orally administered at a dose of 1500, 2500, 3500 and 5000mg/kg body weight. Mortality was monitored over a period of 24 hours. The lethal dose (LD_{50}) was determined as the geometric mean of the dose that resulted in mortality [Lorke, 1983]. The animals were observed for two weeks.

In vitro Antioxidant Studies

Test for 2, 2-Diphenyl–2 Picrylhydrazyl (DPPH) Free Radical Scavenging Assay

The free radical scavenging ability of hydro-ethanol leave extract of *M. piperita* against DPPH (2, 2-diphenyl–2 picrylhydrazyl) free radical was evaluated using a slightly modified method as described by (Tuba*et al*., 2008). A 0.3mM solution of DPPH was prepared and 500 µL of the DPPH solution was added to 1 mL of hydro-ethanol leave extract of *M. piperita* at various concentrations (100–1000 µg/mL).

Test for Ferric cyanide (Fe^{3+} reducing/ antioxidant power (FRAP)) Assay

The total reducing power of hydro-ethanol leave extract of *M. piperita* was determined using the FRAP method of Oyaizu (1986), with slight modifications. To perform this assay, 1 mL of the extract (100–1000 µg/mL) was incubated with 1 mL

of sodium phosphate buffer (0.2 M, pH 6.6) and 1% potassium ferricyanide at 50°C for 30 minutes. Thereafter, 1 mL of 10% trichloroacetic acid was used to acidify the reaction mixtures. After the acidification, 1 mL of the sample was mixed with 1 mL of distilled water and 200 µL of 0.1% $FeCl_3$. The absorbance of the resulting solution was read using a spectrophotometer at 700 nm.

Nitric oxide (NO) radical scavenging Assay

This assay is based on the ability of aqueous solution of sodium nitroprusside at physiological pH to spontaneously produce nitric oxide (NO), which could interact with oxygen to generate nitrite ions that can be measured using Griess reagent. All agents that can scavenge NO compete with oxygen, resulting in decreased NO generation (Kurian*et al.,* 2010). The assay was carried out by incubating 500 µL of 10 mM sodium nitroprusside in sodium phosphate buffer (pH 7.4) and 500 µL of different concentrations of the extract (15-240 µg/mL) at 37°C for 2 hours. Thereafter, 500 µL of Griess reagent was transferred to the reaction mixture. Diazotization of nitrite with sulphanilamide produce a chromophore that can be measured at 546 nm. The percentage inhibition of NO generated was measured by comparing with the absorbance value of a control (10 mM sodium nitroprusside in phosphate buffer). The scavenging activities of the sample in the case of DPPH, and nitric oxide radicals scavenging assays were calculated using the following formula.

Scavenging activity % = (1− As / Ac) x 100

Where, As **=** is the absorbance in the presence of the sample and Ac **=** is the absorbance of the control.

Reducing Power Method (RP)

This method is based on the principle of increase in the absorbance of the reaction mixtures. Increase in the absorbance indicates an increase in the antioxidant activity. In this method, antioxidant compound forms a colored complex with potassium ferricyanide, trichloroacetic acid and ferric chloride, which is measured at 700 nm. Increase in absorbance of the reaction mixture indicates the reducing power of the samples (Jayaprakash *et al.,* 2001). In the method described by Oyaizu (1986) 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of $K_3Fe(CN)_6$ (1% w/v) are added to 1.0 mL of sample dissolved in distilled water. The resulting mixture is incubated at 50°C for 20 min, followed by the addition of 2.5 mL of Trichloroacetic acid (10% w/v).

RESULT

Toxicity Studies in Rats (LD_{50})

Phase I and II

Oral administration of hydro-ethanol leave extracts of *M. Piperita* shows the $LD_{50} \leq 5000$mg/kg body weight in rats. No mortality in phase 1 but the second phase lead to the mortality of 2 rats from the group administered 5000mg/kg, one died at the instant and another at 18 minutes after administration of the extract. Some clinical signs of toxicity such as initial excitement, restlessness, difficulty in breathing, loss of appetite, general weakness and depression were revealed within the two weeks of the experiment.

DPPH Scavenging Activity: The result shows that the different concentration against the absorbance, the concentration is at 100 (µ g/ml) up to1000 (µ g/ml), as the concentration increased the activity also increased.

Ferric reducing/antioxidant power assay

The result shows that the different concentration against ferric cyanide(fe^{3+}) reducing/ antioxidant power assay, As the reducing power assay increased the concentration also increased from 100-1000 (µ g/ml).

Nitric Oxide Scavenging Activity

This result shows that the concentration against the absorbance, the concentration is at 100-1000 (µ g/ml) the differences was showed and do not follows the common rate by the increases the concentration and activity which is punctuating.

Table 1. Toxicity of hydro-ethanol leave extracts of *M. piperita* administered orally to rats

Experiment	Number of Animals	Dose (mg/kg)	Mortality after 24 hours	Mortality after two weeks
Phase 1	3	50	0/3	0/3
	3	100	0/3	0/3
	3	1000	0/3	0/3
Control	3	0	0	0/3
Phase 2	2	1500	0/2	0/2
	2	2500	0/2	0/2
	2	3500	0/2	0/2
	2	5000	2/2	-

Table 2. The antioxidant activity of the hydro-ethanol leave extract of *M. piperita*

Concentration of extract (µg/mL)	DPPH Scavenging Activity (%)	Fe^{3+}reducing antioxidant power (%)	NO radical scavenging (%)
100	16.9 ±3.21*	12.9±1.22*	25.1±3.74
300	20.8±2.33*	16.0±1.05*	33.8±1.16
500	29.4±4.45*	18.9±2.38*	41.2±3.56
750	37.6±3.91*	25.2±4.33*	49.6±5.16
1000	46.9±5.21*	32.6±4.32*	57.3±5.23

Data are xpressed as mean ± SD (n=3); *Dose-dependent increase from 100 to 1000 (µg/mL). DPPH: 2,2-diphenyl-1-picrylhydrazyl, NO: Nitric oxide

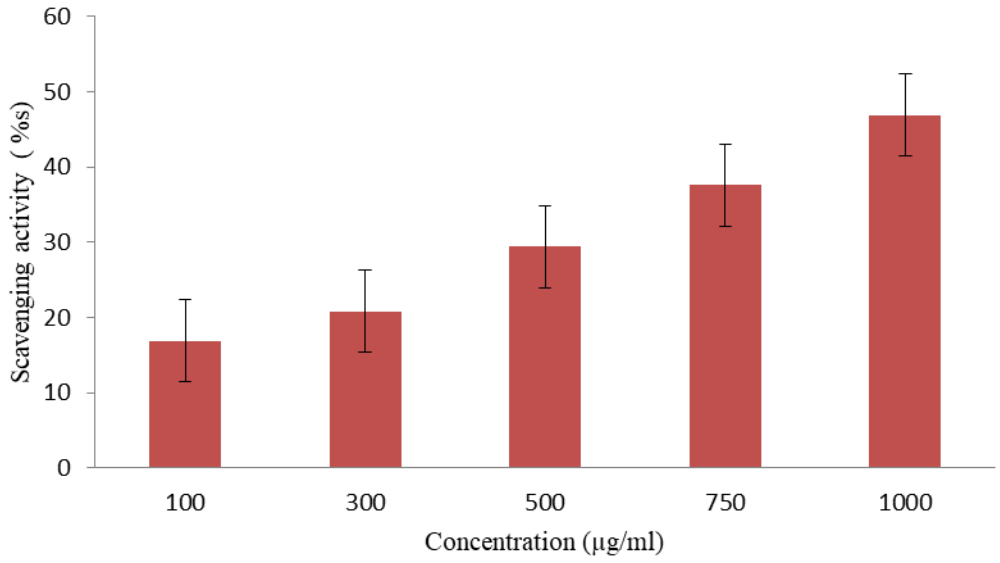

Figure 1. Scavenging activity (%) of the hydro-ethanol leave extracts of *M. piperita* against 2,2-diphenyl-2-picrylhydrazyl DPPH)

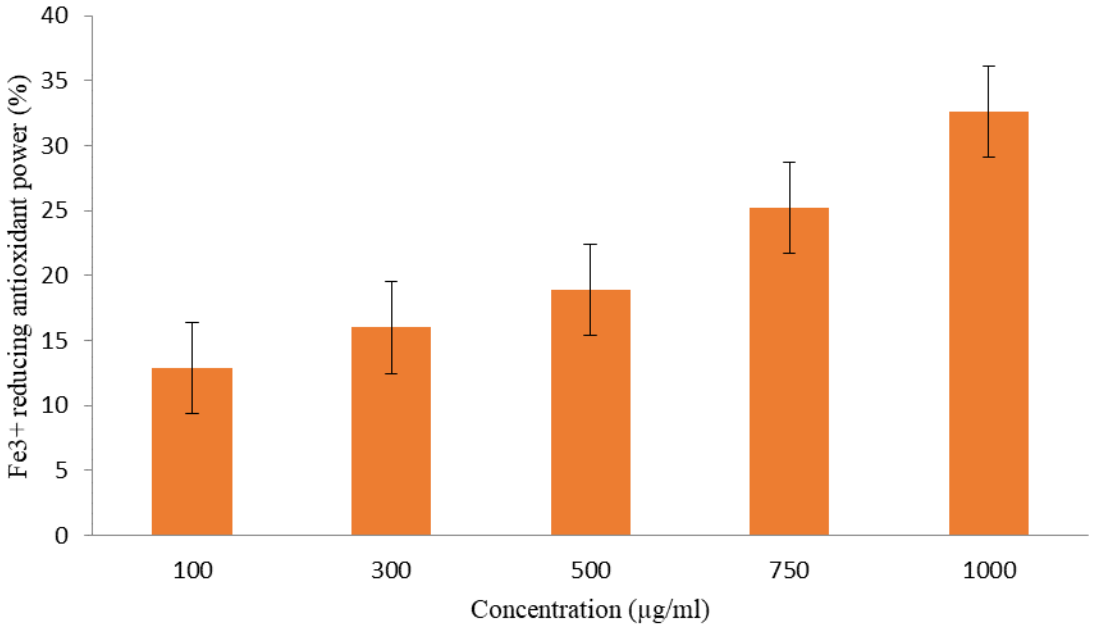

Figure 2. Scavenging activity (%) of the hydro-ethanol leave extracts of *M. piperita* against Ferric Cyanide (Fe^{3+})

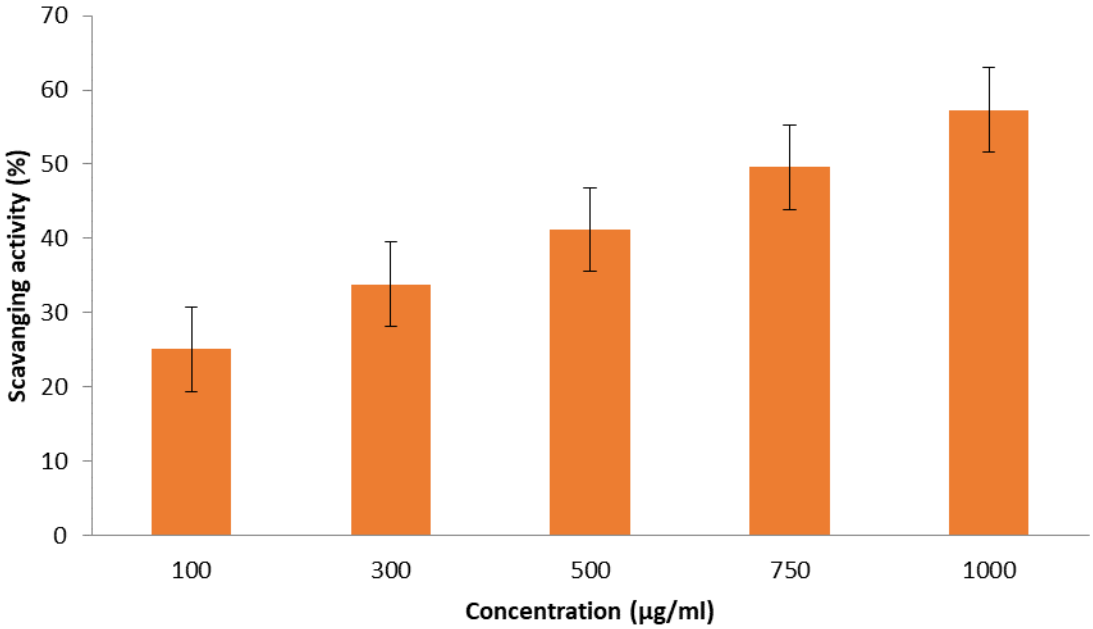

Figure 3. Scavenging activity (%) of the hydro-ethanol leave extracts of *M. piperita* against Nitric Oxide (NO)

DISCUSSION

The results of acute toxicity of hydro-ethanol leave extracts of *M. piperita* (HELEMP), showed LD_{50} greater than 5000mg/kg body weight in rats and therefore, these extracts are considered to be relatively safe as suggested by Lorke (1983). This is more so when no mortality was recorded in phase 1 but rather in phase II at higher dose of 5000mg/kg body weight (Table 1) at the time of the studies. According to the OECD (2001) guidelines, substances with an LD_{50} value of greater than 5000mg/kg through the oral route are regarded as being safe. The antioxidant studies revealed that *M. piperita* leave extract with DPPH and NO showed good antioxidant activity. From Table 2, at the highest

concentration of the extracts, the scavenging activity of NO (57.3%), DPPH (46.9%) and Fe^{3+} (32.6%) reducing antioxidant power. The DPPH is a stable free radical, due to the delocalization of the spare electron on the whole molecule. Reducing power (RP) assay with extract possess moderate antioxidant activity. Ferric reducing/antioxidant power assay (FRAP) assay combined with extract showed the poor antioxidant activity. The FRAP relies on the reduction by the antioxidants, of the complex ferric ion. Therefore, the absorbance diminution depends linearly on the antioxidant concentration. Ascorbic acid is used as standard antioxidants. This assay allows comparison of the reactivity of powerful antioxidants such as BHT and ascorbic acid with those present in extracts against DPPH radical.The DPPH radical scavenging activity of thymol rich essential oil from *M. piperita* [Liu *et al*., 1997]. The radical scavenging activity of the essential oil from *M. piperita* was the highest (%) at concentration of 1000µg/mL [Manzool *et al.,* 2013]. The radical scavenging activity could be credited to the presence of its main total phenolic contents, especially thymol and their recognized impact on oil lipid oxidation [Mahmoudi *et al*., 2009; Said *et al.,* 2022]. The antioxidant activity may be due to the inhibition of the formation of radicals or scavenging of the formed radical [Chesson and Collins, 1997].The herbal remedies have become more popular in the treatment of minor ailments [Bakht *et al.,* 2013; Pieri *et al.,* 2014].

CONCLUSION

The LD_{50} value of the extract is ≤ 5000 mg/kg, which is relatively toxic at high dose. Low doses should be cautiously used. This study indicate that the hydro-ethanol leave extracts of *M. piperita* has a good antioxidant properties as evaluated by NO, DPPH radical scavenging activity and FRAP.

CONFLICTS OF INTEREST

Authors declared that there is no conflict of interest

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