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### RESEARCH ARTICLE

## EVALUATION OF HYDROGEN PEROXIDE SCAVENGING ACTIVITY OF METANOLIC EXTRACTS OF MORINGA (M. STENOPETALA) LEAVES.

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Moringaceae stenopetala, soxhlet extraction, methanol, H<sub>2</sub>O<sub>2</sub>

#### Abstract

**Background:-** The aim of this research is to determine hydrogen peroxide scavenging of Moringa stenopetala methanol extracts of leaves. Moringaceae leaves are used in traditional medicine in the treatment of chronic heart failure, high blood pressure, and others.

**Methods and Materials:-** The methanol extracts were prepared from powdered moringa stenopetala leaves. An antioxidant activity was measured hydrogen peroxide scavenging assays with UV-Vis spectrophotometer.

**Results:-** the scavenging activity values on hydrogen peroxide 20 µg/mL of the extracts of moringa stenopetala leaves 84.188 ± 0.386 % increased than that of 40 µg/mL and 60 µg/mL were 59.96 ± 0.122 % and 46.995 ± 0.088 % respectively. Whereas decreased scavenging activity was exhibited at concentration of 60 µg/mL. The maximum scavenging activity was exhibited for all concentration at 10 minutes.

**Conclusion:-** the methanolic extract Moringa stenopetala leaves were a good scavenging activity by hydrogen peroxide when butylated hydroxytoluene as reference compound. Moringa stenopetala leaves can be used as a possibly for pharmaceutical applications

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#### Background:-

An 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 (Badarinath et al., 2010 and Milan et al., 2010). In the organism, hydrogen peroxide is generated by activated phagocytes used to kill several bacterial and fungal strains, additionally under physiological conditions by peroxisomes and oxidative enzymes. An increase in the rate of production or a decrease in the rate of removal disrupts this balance and increases the levels of reactive oxygen species and free radicals. This indicates harmful effects of free radicals and other oxidants (M. Rama et al. 2011 and Serhat et al., 2012). Free radicals are responsible for causing a large number of diseases including cancer, cardiovascular disease, neural disorders, alzheimer's disease, mild cognitive impairment, parkinson's disease, alcohol induced liver disease, ulcerative colitis, aging and atherosclerosis (Nur et al., 2013, Nooman et al., 2008 and Aurelia, M. P., and Gheorghe, P. N., 2011). The plants have a natural antioxidants scavenge harmful free radicals from our body (Ranju et al., 2009). In 2002 World Health Organization report, medicinal plants would be the best source to obtain a variety of drugs. In developed countries about 80% of plants are used in traditional medicine (Adamu et al., 2004).

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Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly, and inside the cell, H<sub>2</sub>O<sub>2</sub> probably reacts with Fe<sup>2+</sup>, and possibly Cu<sup>2+</sup> ions to form hydroxyl radical which may be the origin of many of its toxic effects. Thus, the removing of H<sub>2</sub>O<sub>2</sub> is very important for antioxidant defense in cell or food systems (Mahesh et al., 2010, Ranju et al., 2011 and Serhat et al., 2012).

Moringa plant family contains several phytochemicals and some of which are high interest because of their medicinal value (Mahesh et al., 2010). Moringaceae stenopetala is one of a species moringaceae, which have been found in Ethiopia. *M. stenopetala* is an important food and medicinal (heart failure, high blood pressure, and arrhythmia) plants that has been used for most southwestern Ethiopians, they called Aleco and/or Sheferaw. It is cultivated as a crop plant (Daljit et al., 2013). To quantify the antioxidant properties are important to human life. Therefore, Moringaceae stenopetala plants have been investigated for better understanding their medicinal properties.

Hence, the aim of this study was undertaken to evaluate the methanol leaves extract of moringa stenopetala for its antioxidant activity by using hydrogen peroxide scavenging method.

## Methods and Materials:-

### Sampling and Extraction:-

The fresh mature leaves of moringa stenopetala were collected from in wolaita Sodo, southern Ethiopia, March 2014. The plant was authenticated by senior expert Botanists, Wolaita Sodo University, Ethiopia. The moringa stenopetala leaves was dried under shade and crushed to coarse powder and the powdered moringa stenopetala leaves was taken for soxhlet extraction using methanol as solvent. The extraction was done 20 g of dried powdered material was extracted with 150 mL of methanol for three hours. The obtained extracts were filtered over Whatman No. 1 paper and the filtrate was collected and the fraction after filtration was dried under reduced pressure to get the crude dried fraction. The yield of dried fraction of methanol extract of methanolic leaves extract of moringa stenopetala was collected for further analysis (Vibha et al., 2012, Ranju et al., 2011, M. Rama, P. and K. Vasantha, 2011, C. Senthil et al., 2010 and Serhat et al., 2012).

### Hydrogen Peroxide Scavenging Capacity:-

The moringa stenopetala posse's potent antioxidant activity when compared with reference compound butylated hydroxytoluene (BHT). Hydrogen peroxide indirectly may enter into the human body through environmental contact. The ability of moringa stenopetala leaves extracts to scavenge hydrogen peroxide can be estimated according to the method of Ruch et al. (1989) stated by Nur et al. (2013), Keser et al. (2012), M. Rama et al. (2011), Ilhami et al. (2004) and etc. (Nur et al., 2013, M. Rama, P. and K. Vasantha, 2011, Serhat et al., 2012 and Ilhami et al., 2004).

$$\% \text{ Scavenged } [H_2O_2] = [(AC - AS)/AC] \times 100$$

Where AC is the absorbance of the control and AS is the absorbance in the presence of the sample of *M. stenopetala* extracts.

A solution of hydrogen peroxide (UNI-CHEM, 43 mM) is prepared in phosphate buffer (SAMIR TECH CHEM, 0.1 M, pH 7.4). The absorbance's of hydrogen peroxide is determined by 230 nm using a Model 752 UV-Vis spectrophotometer. Extracts (20, 40 and 60 µg/mL) in distilled water is added to hydrogen peroxide and absorbances at 230 nm were determined five times in 10 min interval against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for background subtraction. The values of percentage scavenging capacity were calculated for various concentrations of the extract. Tests were conceded out in triplicate.

### Statistical analysis:-

The results of these investigations are means and standard deviation of three measurements. The different concentrations of extracts have significantly different efficiencies and times variation is significantly greater than the variation due to the random error of measurement were tested by two-way ANOVA. A p value of 0.01 was taken to be significant.

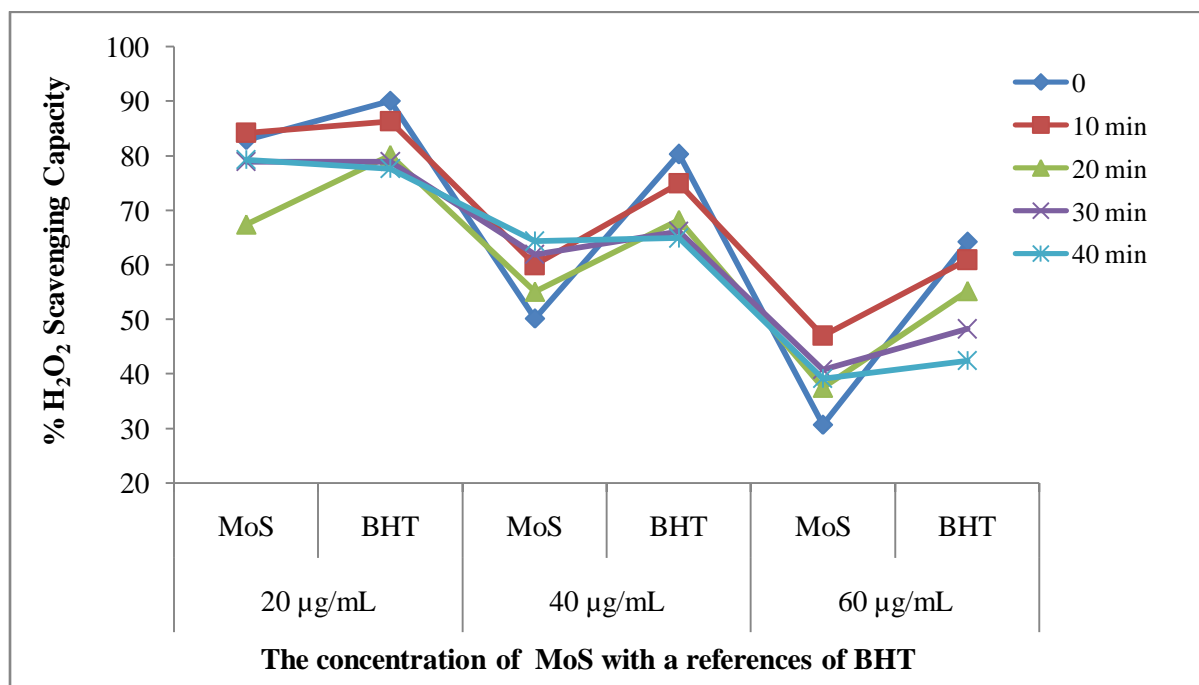
## Results and Discussion

The results of this study, Scavenging capacity of hydrogen peroxide in moringa stenopetala different concentration of  $H_2O_2$  containing extract was also evaluated in a series of the 10 min period of the assay and BHT as reference compound was comparable shown below table 1 and figure 1. 20  $\mu\text{g/mL}$  methanol extracted moringa stenopetala exhibited 67.341-84.574% scavenging activity on hydrogen peroxide. On the other hand, using the same moringa stenopetala, 40  $\mu\text{g/mL}$  and 60  $\mu\text{g/mL}$  exhibited 50.50-64.336% and 30.412-47.083% hydrogen peroxide scavenging activity respectively. Scavenging activity of hydrogen peroxide in moringa stenopetala and BHT as reference compound was not remarkably different and shown to be 90.245 % to 42.5 %.

**Table 1:-** hydrogen peroxide-scavenging activity of M. stenopetala leaves (20-60  $\mu\text{g/mL}$ )

Time minutes	Concentration in $\mu\text{g/mL}$					
	20		40		60	
	MoS	BHT	MoS	BHT	MoS	BHT
0	82.927 $\pm$ 0.028	90.021 $\pm$ 0.244	50.124 $\pm$ .074	80.251 $\pm$ 0.244	30.66 $\pm$ 0.148	64.25 $\pm$ 0.104
10	84.188 $\pm$ 0.386	86.321 $\pm$ 0.841	59.96 $\pm$ 0.122	74.897 $\pm$ 0.215	46.995 $\pm$ 0.088	60.895 $\pm$ 0.112
20	67.391 $\pm$ 0.050	80.021 $\pm$ 0.094	55.134 $\pm$ 0.166	68.214 $\pm$ 0.371	37.535 $\pm$ 0.053	55.234 $\pm$ 0.086
30	78.933 $\pm$ 0.074	78.952 $\pm$ 0.132	61.86 $\pm$ 0.083	66.135 $\pm$ 0.098	40.805 $\pm$ 0.077	48.216 $\pm$ 0.064
40	79.282 $\pm$ 0.070	77.658 $\pm$ 0.044	64.298 $\pm$ 0.038	64.89 $\pm$ 0.109	39.173 $\pm$ 0.024	42.398 $\pm$ 0.106

All the values are means of three independent determinations,  $n \pm \text{Sd}$ .



**Figure 1:-** Hydrogen peroxide scavenging activity of moringa stenopetala

Results show that the scavenging activity values on hydrogen peroxide 20  $\mu\text{g/mL}$  of the extracts of moringa stenopetala leaves increased than that of 40  $\mu\text{g/mL}$  and 60  $\mu\text{g/mL}$ . Whereas decreased scavenging activity was exhibited at concentration of 60  $\mu\text{g/mL}$ . The maximum scavenging activity was exhibited for all concentration at 10 minutes. Therefore moringa stenopetala is biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate.

Moringa stenopetala has the antioxidant components and the highest  $H_2O_2$  scavenging activity than Crataegus monogyna leaves, flowers and fruits in Turkey stated by Serhat et al., 2012. The two-way analysis of variances shows that, hydrogen peroxide scavenging activity was not a statistically significance between the concentration methanolic extract of M. stenopetala leaves whereas in the time interval was a statistically significance at 99 % confidence interval.

**Conclusions:-**

As a conclusion, the methanolic extract *Moringa stenopetala* leaves hydrogen peroxide scavenging and powerful total antioxidant activities when compared to and BHT as reference compound. The scavenging activity values on hydrogen peroxide 20 µg/mL of the extracts of *moringa stenopetala* leaves increased. The results of this study show that the methanolic extract *Moringa stenopetala* leaves can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical applications.

**List of abbreviations:-**

BHT: butylated hydroxytoluene  
MoS: *Moringa stenopetala*  
Sd: Sstandard deviation).

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