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Mukhtar M
Department of Science Lab. Tech. Binyaminu Usman Polytechnic Hadejia, Jigawa State

Adamu HM
Department of chemistry Abubakar Tafawa Balewa University, Bauchi

Kolo AM
Department of chemistry Abubakar Tafawa Balewa University, Bauchi

Correspondence
Mukhtar M
Department of Science Lab. Tech.
Binyaminu Usman Polytechnic Hadejia, Jigawa State

Anti-Oxidant activity and acute toxicity study of the methanolic root extract of *Mitragyna inermis*

Mukhtar M, Adamu HM and Kolo AM

Abstract
The acetone, methanol and water extracts of *Mitragyna inermis* were investigated for their antioxidant activity and acute toxicity. The result show strong radical scavenging activity against DPPH for all the three extracts. The LD50 of 1264mg/kg was calculated for methanol extracts and the values was found to be within the practically slightly toxic range and therefore care should taken when using the plants in traditional medicine healing.

Keywords: Antioxidant effect, acute toxicity and *Mitragyna inermis*

1. Introduction
Traditional medicines have being used in various community of the world depending on the health challenges and the culture of the community. African traditional medicine provide a holistic treatment (Ali, 2008) [3]. The type of treatment varies and include the use of vegetable organs/leaves, bark, and root, etc. or product (latex, resin) whole or part of the animal (snail, borne) and mineral substance (alum kaolin) other are fasting and dieting hydrotherapy, treatment of burn, massage, faith healing and therapeutic occultism (Sofowora, 2008) [18]. It is estimated worldwide that there over 323,328 species of plants (Roven *et al.*, 2005) [16]. Most of these plants are now known to have medicinal effect (WHO, 1991) [21].
Mitragyna inermis is a shrub or tree; it grow up to 16 meters tall. The bole is up to 60cm in diameter with branches usually from down (Burkil, 1985) [7]. Found in wild mainly on heavy clay soil (Von, 1990) [2]. The root, bark and leaves are used for the treatment of various diseases (Konko *et al*, 2008 and Zongo *et al.*, 2009) [24].
Biological investigation has been carried out in *mitragyana inemis*, which revealed it antibacterial and anticonvulsant effect (Zongo *et al.*, 2009 [24], Mu'azu and Kaita 2008 [15]. and Asase *et al.*, 2008) [4].
This work is designed to investigate antioxidant activity and acute toxicity of the root extract of *Mitragyna inermis.*

2.0 Materials and Methods
2.1 Collection and preparation of sample
The plant was identified by the taxonomist at the herbarium of Ahmadu Bello University zaria, Nageria. The fresh root was collected from from Hadejia Nguru wet land area of jigawa state, it was washed in water and re-washed in distilled water, air dried and grounded to fine powdered. The powdered was sequencially extracted using n-hexane, ethyl acetate, acetone, methanol and water

2.2 Animals
Albino mice were used for toxicity study; the animals were purchased from the animal house of the Department of pharmacology, Aminu Kano Teaching Hospital, Bayero University Kano.

2.3 Antioxidant Assay
The antioxidant activity of the extracts and standards were measured on the basis of the radical scavenging effect of the stable 2, 2-diphenylpicryhydrazyl (DPPH) free radical activity method modified by Braca *et al.*, 2002 [6]. The working solutions of the of the extracts were prepared in methanol, Ascorbic acids was used as a standard in 0.01g/ml. 0.004% of DPPH was prepared in methanol and 2 ml of the sample was mixed with 2 ml of this solution and standard solution separately. These solution mixtures were kept in dark for 30 minutes and optical activity was measured at 517nm using spectrophotometer. Methanol (2ml) with DPPH

solution (0.004%, 2 ml) was used as blank. The optical density was recorded and the % inhibition was calculated using the formula given.

$$AA\% = \frac{A_b - A_s}{A_b} \times 100$$

Where AA% = Antioxidant activity (%)
A_b = Absorbance of the blank
A_s = Absorbance of the sample

2.4 Acute toxicity

The method of Lorke (1983) [12] with slight modification was used for the determination of LD50 as a means of evaluating the safety of the extracts in the future pharmacological trials. 9 albino mice were randomly divided in to 3 groups of thrice for each extract. The animal in groups 1, 2 and 3 received 10mg/kg, 100mg/kg and 1000mg/kg respectively, all the treatment where giving through intraperitoneal (IP). Lorke (1983) [12] recommended using single animal for the second phase of the LD50 determination, three animal where divided in to three group of one. Mice in group 1, 2 and 3 were giving the extract at 1600mg/kg, 2900mg/kg and 5000mg/kg respectively and observed for toxic signs and mortality.

3. Results

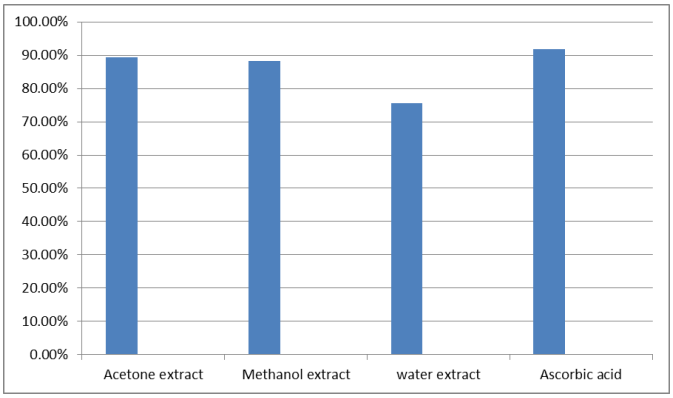

Fig 1: Antioxidant free radical scavenging activity of the root extracts of *Mitrigyna inermis* by DPPH method.

Table 1: Acute lethal effect of methanol extract of *Mitragyna inermis* root administered intraperitoneally (I.P) to the mice

Phase 1	
Doses (mg/kg)	**Mortality in Root**
10	-
100	-
1000	-
Phase 2	
1600	1
2,900	1
5000	1

LD50 = (100x1600)½
= 1264.9mg/kg

4. Discussion

The free radical scavenging ability of acetone, methanol and water in chart above show strong antioxidant activity in all the extracts, this radical scavenging activity in all the extracts were confirmed by the phytochemical constituents presence in the extracts. It is evident that constituents like tannins, sugar, protein, flavonoids, glycosides and saponin are present in the extract *Mitragyna inermis* (Uthman *et al.*, 2013) [19]. The flavonoids have been reported to posses antimicrobials, and antioxidants activities in various studies (Lin *et al.*, 2009 [10], Lopez, 2009 [11], Yoshidu *et al.*, 2009 [22], Amoral *et al.*, 2009) [1] the presence of alkaloids may also be responsible for an antioxidant activity (Maiza *et al.*, 2007) [13]. The presences of tannin in the extract may explain the antioxidant activity of the extracts as tannins are known to posses antioxidant properties (Zhang and Lin 2008) [23], The saponins have also shown to antioxidant activity (Gulcin *et al*., 2004) [8].

The toxicity study of methanol extract shows that no animal died within 24hours of treatment of mice with 10, 100, 1000mg/kg of the extract per body weight, but the major toxicity sign noticed for all the extract within the 24hrs were general weakness, erection of hairs and redness of eyes. However, for the second test (where 3 animals are divided in 3 group of once) and dosage of 1600, 2900 and 5000mg/kg were used intraperitoneally (I.P) all the animal died but similar toxicity sign were also observed before the death.

The *Mitragyna inermis* root methanol extract reveal the LD_{50} value of 1264.9mg/kg this values are slightly toxic as suggested by Matsumura (1975) [14], who classified chemicals base on their LD50 values, and point out that LD50 of 500-5000mg/kg as slightly toxic to the experimental model.

5. Conclusion

Methanol extract of *Mitragyna inermis* demonstrated some anti-oxidant properties that may be useful for treatments of various ailments. Care should be taken because of it slight toxic effect.

This study has demonstrated the need for Bioassay guided Isolation of pure compounds from *Mitragyna inermis* and the extracts need to be subjected to other disease causing microbes.

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