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# Antibacterial Potency of Combined Extracts of *Mitragyna inermis* (Linn) and *Monotes kerstingii* (Linn) on *Salmonella* Typhi and *Salmonella* Paratyphi A and Its *In vivo* Toxicity against Swiss Albino Mice

**[1]Zumbes, H.J., [1]Babalola, O.B.,[1]Nanle, C.D., [1]Katnap, R.S., [1]David, E.V., [1]Anejo-Okopi, A.J., [2]Dabo, D.A., [3]Gokir, J.D., [4]Nvau, J.B.**

[1]Department of Microbiology, Faculty of Natural Sciences, University of Jos, Jos, Nigeria
[2]Department of General health Sciences, College of Health Technology, Zawan, Nigeria
[3]Department of Community Health, College of Health Technology, Pankshin, Nigeria
[4]Department of Chemistry, Plateau State University, Bokkos, Plateau State
Corresponding Author: hoseazumbes@gmail.com

**Abstract**

The phytochemical screening, antibacterial and toxicological effect of the extracts of *Monotes kerstingii* (Linn) and *Mitragyna inermis* (wild) extracts were investigated. The plant extracts were prepared by cold maceration method using water and ethanol and screened against *Salmonella* Typhi and *Salmonella* Paratyphy A using the agar well diffusion method. The $LD_{50}$ of the extract was determined using Locke's method. Phytochemical analysis reveals the presence of Carbohydrates, Cardiac glycosides, Steroids, Anthraquinones, Resins, Saponins, Flavonoids Tannins and Alkaloids in both plant extracts. The combined ethanol and aqueous plant extracts shows activity against *S.* Typhi with diameter of zones of inhibition ranging from 14.00 mm and 15-24 mm respectively. The combined extracts were also active against *S.* Paratyphi A with diameter of zones of inhibition ranging from 10-24mm and 11-26 mm for ethanol and aqueous extracts respectively. Toxicity studies of the combined extracts revealed that the plant was well tolerated at 5000mg/kg body weight. These results suggest that the plant may be exploited for therapeutic purposes.

**Keywords:** *Monotes kerstingii, Mitragyna inermis*, Extracts, Antibacterial, Toxicity.

## INTRODUCTION

Plants are indispensable resources used by human being for different purposes. Medicinal plants have been an integral part of traditional herbal medicine since antiquity to date (Anthonia and Olumide, 2010). According to the World Health Organisation estimates, about 80% of the population in developing countries use traditional medicine to meet most of their primary health care needs (Samba *et al.*, 2015). It is estimated that out of the 250,000 species of higher plants around the world, only 17% have been scholarly investigated for medical potential (Mamedov, 2012).

Enteric fever is a major public health problem in the developing countries. It affects 16 million people worldwide with more than 600,000 deaths per annum (WHO, 2008). 80% of the cases of deaths due to poor standard of living are in Asia and the rest occur mostly in Africa and Latin America (Raveendran *et al.*, 2010). The emergence of multiple drug resistant strains of *Salmonella* Typhi has impaired the efficacies of antimicrobial agents that have been used for effective treatment of typhoid fever (Ang *et al.*, 2004)

*Salmonella* species have developed resistance to chloramphenicol, trimethoprim and ampicillin (Crump *et al.*, 2004). However, an alternative therapy to treat antibiotic resistant microorganisms is the use of plant extract and combination therapy in order to achieve bactericidal synergism (Chanda and Rakoliya, 2011). Although several synthetic drugs are presently in circulation, there is always an increasing demand for traditional herbal medicine by both developing and developed countries of the world (Ehiowemwenguan *et al.*, 2014).

The revival of interest in plant-derived drugs may be as a result of the widely held notion that antimicrobials of plant origins are not associated with side effects and have a great potential to heal many infectious diseases than synthetic drugs (Okwulehie and Ankawa, 2013). Several reports on the synergistic effects of antibiotics, plant extracts, or an antibiotic and plant extract have been documented (Gitig, 2013). Anthonia and Olumide (2010) investigated the *in-vitro* antibacterial potentials and synergistic effects of South-Western Nigerian plants used in folklore remedy for *Salmonella* Typhi infection. Synergistic effects was observed when mixtures of ethanolic extracts of Roesote bush, Tarbush and paddle Cactus were used against food-borne pathogenic bacteria (Rivera *et al.*, 2014).

The combination between Rhuscoriora extract and antibiotics could be useful in treating emerging drug resistant *Pseudomonas aeruginosa* (Adwan *et al*., 2010).

In Nigeria, various plant parts are used in the treatment of different kinds of infections with remarkable success. Among the enormous numbers of these plants are *Mitragyna inermis* (Linn) and *Monotes kerstingii* (Linn) (Wakirwa *et al*., 2013).

The genus *Mitragyna* belongs to the *Rubiaceae* family and widely distributed in swampy territory in the tropical and sub-tropical regions of Asia and Africa (Gong *et al*., 2012). *Mitragyna inermis* (Wild) "Giyayya" in Hausa is a low branching tree (up to 16m high) with a dense white crown. Wakirwa *et al* (2013) reported that the root, barks and leave of *Mitragyna inermis* are used for the treatment of constipation, anorexia and leprosy. Decoctions prepared from the roots, stem and leaves of the plant are reported to be used in Mali for the treatment of malaria and boils (Azaz *et al*., 2002). Anti- hypotensive, cardiothoracic and vasodilatory properties of the aqueous extract of *Mitragyna inermis* have been documented (Ouedraogo *et al*., 2014). Zongo et al. (2009) had reported the antibacterial properties of total Alkaloids extract of *Mitragyna inermis* shows moderate activity against 10 reference bacterial strains and 3 clinical isolates (*Bacillus cereus* LMG 13569, *Enterococcus faecalis* CIP 105150, *Shigella dysentereria* CIP 5451, *Staphylococcus aureus* ATCC 9244, *Proteus mirabilis* CIP 104588, *Staphylococcus aureus* ATCC 25293 and *Staphylococcus comorum* LMG 13567) and clinical strains (*Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pyoogenes*). They attributed the inhibitory effect of the plant to the presence of secondary metabolites in the plant. Similar study conducted by Tor-Anyii and Orokpo (2012) shows that the crude ethanol extract, water insoluble fraction residue, ethyl acetate fraction and n-butanol fraction inhibit the growth of *Staphylococcus aureus*, *P. mirabilis*, *S. pyogenes* and *S.* Typhi. They also attributed the inhibitory effect of the plant to the presence of bioactive components of the plant. Adeoum *et al*. (2012) reported 1600mg/kg body weight lethal dose ($LD_{50}$) indicating that the extract is slightly toxic to the test rats in their experiment.

*Monotes kerstingii* (Linn) also known as "Hansi" in Hausa is a tree or shrub with an open rounded crown that grow up to 16m tall. The bark surface is smooth to slightly fissured or scaly with rectangular scales, grey brown under the scales, inner dark brown to reddish brown, leaves alternate, simple and entire. *Monotes kerstingii* (Linn) usually flowers in the rainy season from June to October and the flowers are pollinated by insects (Valarmathy *et al*., 2010; Biu *et al*., 2009). There is a dart of literature reports with regard to the antibacterial effects of the plant but the bark and leaves of the plant has been reported to be used in traditional folklore medicine. Decoction of the bark and leafy twigs are taken to treat dysentery and diarrhea and jaundice (Yahaya *et al*., 2012). Leaf and root decoctions are applied to abscesses and fractures (Konkon *et al*., 2008). This study aimed to determine anti-typhoidal activities of the extracts of *M. inermis* and *M. kerstingii* extracts and as well their toxicity.

## MATERIALS AND METHODS

### Sample Collection and Processing

Fresh roots of *Mitragyna inermis* (Linn) and the flowers of *Monotes kerstingii* (Linn) were collected from Fanshiyanu Bauchi State, and Naraguta Village, Plateau State respectively in the month of September, 2015. The plants were identified at the Herbarium unit of the Federal College of Forestry Bauchi Road Jos, Nigeria, where voucher specimen was deposited. The roots of *Mitragyna inermis* (Linn) and flowers of *Monotes kerstingii* (Linn) were shade-dried for four weeks and further pulverized using a clean mortar and pestle and sieved using a mesh of 26µm pore size. The powdered plant materials were then stored in air tight plastic container.

### Test Organisms

Clinical isolates of *Salmonella* Typhi and *Salmonella* Paratyphi A were obtained from stock cultures in the bacteriological unit of Federal College of Veterinary and Medical Laboratory Technology, Vom, Jos, Plateau State, Nigeria. The test organisms were confirmed using standard microbiological procedures (Chessbrough, 2006). A twenty four hours (24hr) culture of the bacterial culture isolate were diluted with physiological saline solution and the turbidityadjusted by adding sterile physiological saline until a McFarland turbidity standard of 0.5 ($10^6$ CFU/ ml) were obtained (Cheesbrough, 2006).

### Extraction of *M. inermis* and *M. kerstingii* plant materials

One hundred and fifty grams (150g) of each powdered plant material was weighed using an analytical weighing balance (GR Series Semi-Micro Analytical balance) and soaked in 1500ml of 70% ethanol for ethanol extract. The aqueous extract was produced by soaking 150g of the powdered plant material in 1500ml of distilled for 24 hours.

This was filtered with the use of Whatman N0 1 filter paper pore size 11µm. Each filtrate was concentrated using a rotatory evaporator and dried in a desiccator. The dried extract was preserved in a refrigerator at a temperature 24°C prior to bacterial effect evaluation.

**Phytochemical Analysis**

Qualitative phytochemical analysis of the plant extracts of the plants were done using the methods used by Trease and Evans (1989).

**Preparation of Plant Extracts Concentrations**

Two grams (2g) of each aqueous and ethanol extracts was taken and the aqueous extract was dissolved in 10ml sterile distilled water, while the ethanol extracts was also dissolved in 20% 10ml of DiMethyl Sulphoxide (DMSO). Thus 200mg/ml of stock was obtained as a standard concentration of aqueous and ethanolic extracts respectively. From the stock concentration, different working concentrations (100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml) were prepared by doubling dilution of the prepared stock solution of 200mg/ml concentration (Aneja, 2005).

**Preparation of Combined ratios of the extracts**

Stock concentration of the combined extract was prepared in the ratio of 1:1 by taking 5ml each from the stock concentration of individual extracts and mixed properly with intermittent shaking for 1hr to ensure even mixtures. Thereafter, five varied extract concentrations 100mg/ml, 50mg/ml. 25mg/ml, 12.5mg/ml and 6.25mg/ml were prepared from the stock solution (200mg/ml) using 2-fold doubling dilution.

**Antibacterial Activity Assay of individual Plant Extracts**

The agar-well diffusion of Bauer (1996) was adopted. wells of 6 mm in diameter were made using a 6mm sterile corked borer on *Salmonella Shigella* Agar (SSA) which has already been seeded with the test organisms (*Salmonella* Typhi and *Salmonella* Paratyphi A) using the pour plate technique. Aliquot of 20µl from each concentration of extracts were added into each well using a micropipette on the seeded medium and allowed to stand on the bench for 1 h for proper diffusion and thereafter incubated at $37^0$C for 24 h. The experiment was done in duplicate. Gentamycin (5mg/ml) was used as a positive control while sterile distilled water were used negative control for both aqueous and ethanolic extracts.

**Combined effects of Plant extracts**

The combined effects of plant extracts was determined by agar well diffusion assay of by Bauer and Tihel (1996) as described above. Wells of 6 mm in diameter were made using a 6mm sterile corked borer on *Salmonella Shigella* Agar (SSA) which has already been seeded with the test organisms (*Salmonella* Typhi and *Salmonella* Paratyphi A) using the pour plate technique. Aliquot of 20µl from each combined extracts of varying concentration were added into each well using a micropipette and allowed to stand on the bench for 1 h for proper diffusion and thereafter incubated at $37^0$C for 24 h. The experiment was done in duplicate. Gentamycin (5mg/ml) was used as a positive control while sterile distilled water were used negative control for both aqueous and ethanolic extracts.

**Acute Toxicity Studies**

The $LD_{50}$ of the combined extracts was determined using the method of Lorke (1983) in consonance with Organization for Economic Cooperation and Development (OECD) guideline on animal acute toxicity testing (OECD, 2001). Twelve (12) swiss albino mice weighing 20-25g were used. In the first phase, nine (9) were fasted for 2hrs before the study. In this phase, mice were divided into three groups of three mice each and were treated with the combined extracts of *M. inermis* and *M. kerstingii* at different doses of 10, 100 and 1000 mg/kg (body weight) orally. They were observed for 24 h for signs of toxicity such as inaction, dizziness, loss of weight and mortality. In the second phase, three (3) mice were divided into three groups of 1 mice each and were treated with the extract doses of 1500, 2900, and 5000 mg/kg (body weight). The $LD_{50}$ was calculated using the formula below to determine any toxicity or mortality.

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

Where $D_0$ = Highest dose that gave no mortality,

$D_{100}$ = Lowest those that produce mortality (Locke, 1983).

**RESULTS**

Table 1 shows the result of the phytochemical analysis of the extracts of *Mitragyna inermis* and *Monotes kerstingii*. Carbohydrates, Flavonoids, Resins, Saponins, Tannins, Cardiac glycosides and Alkaloids were all present in both the aqueous and ethanolic extracts of the plants respectively. Steroids were absent in aqueous extract of *Mitragyna inermis* but present in ethanol extract while Anthraquinones were absent in the ethanolic extracts of both plants but present in the aqueous extracts.

**Table 1: Phytochemical results of *Mitragyna inermis* (Linn) and *Monetes kerstingii* (Linn)**

| Secondary metabolites | *Mitragyna inermis* | | *Monotes kerstingii* | |
|---|---|---|---|---|
| | EE | AE | EE | AE |
| Carbohydrates | + | + | + | + |
| Flavonoids | + | + | + | + |
| Resins | + | + | + | + |
| Saponins | + | + | + | + |
| Tannins | + | + | + | + |
| Cardiac glycosides | + | + | + | + |
| Steroids | + | - | + | + |
| Anthraquinones | - | + | - | + |
| Alkaloids | + | + | + | + |

**Key: EE = Ethanolic extracts, AE = Aqueous extracts, (+) = present, (-) = absent.**

Table 2 depicts the antibacterial activities of the root extract of *Mitragyna inermis*, the flower extract of *Monotes kerstingii* and the combined extracts against *Salmonella* Typhi. The combined ethanol and aqueous plant extracts showed stronger activity against *S.* Typhi with diameter of zones of inhibition ranging from 14.00 mm and 15-24mm respectively. The combined extracts were also active against *S.* Paratyphi A with diameter of zones of inhibition ranging from 10-24mm and 11-26mm for ethanol and aqueous extracts respectively. The results of acute toxicity testing reveals that the combined ethanol and aqueous extracts was not toxic even at a high dose of 5000mg/kg body weight. Neither death nor other signs of toxicity were observed (Table 3).

**Table 2: Antibacterial activities of the Root extract of *Mitragyna inermis* and the flower extracts of *Monontes kerstingii* and the Combined Plant Extract against *S.* Typhi.**

| Plants | Aqueous Extract | | | | | Control | | Ethanoic Extract | | | | | Control | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc (mg/ml) | 100 | 50 | 25 | 12 | 6.25 | +ve | -ve | 100 | 50 | 25 | 12.5 | 6.25 | +ve | -ve |
| *M. inermis* | 19 | 16 | 14 | 13 | 10 | 29 | 0.0 | 18 | 16 | 14 | 10 | 9 | 32 | 0.0 |
| *M.kertingii* | 22 | 20 | 18 | 16 | 15 | 28 | 0.0 | 20 | 19 | 16 | 14 | 13 | 29 | 0.0 |
| *Combined extract* | 24 | 22 | 19 | 17 | 15 | 31 | 0.0 | 25 | 23 | 18 | 17 | 14 | 30 | 0.0 |

Key: AA= Aqueous extract, EE= ethanolic extract, (+ve) = Positive control (Gentamycin) (-ve) = Negative control (Distilled water)

**Table 3: Antibacterial activities of *the* Root extract of *Mitragyna inermis* and the flower extracts of *Monontes kerstingii* and the Combined Plant Extract against *S.* Paratyphi A**

| Plants | Aqueous Extract | | | | | Control | | Ethanol Extract | | | | | Control | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc(mg/ml) | 100 | 50 | 25 | 12.5 | 6.25 | +ve | -ve | 100 | 50 | 25 | 12.5 | 6.25 | +ve | -ve |
| *M.inermis* | 22 | 19 | 17 | 14 | 13 | 28 | 0.0 | 20 | 18 | 16 | 13 | 11 | 29 | 0.0 |
| *M.kertingii* | 19 | 17 | 14 | 12 | 10 | 14 | 0.0 | 23 | 20 | 18 | 16 | 12 | 32 | 0.0 |
| *Combined extract* | 26 | 23 | 17 | 13 | 11 | 30 | 0.0 | 24 | 22 | 18 | 14 | 10 | 33 | 0.0 |

Key: AA= Aqueous extract, EE= ethanolic extract, (+ve) = Positive control (Gentamycin) (-ve) = Negative control (Distilled water)

**Table 4: Acute toxicity test of combined ethanolic extracts of *M. kerstingii* and *M. Inermis* on Swiss Albino Mice**

| Doses (mg/kg) | Result | Remark |
|---|---|---|
| | **PHASE 1** | |
| 10 | No signs of toxicity or mortality observed | 3/3 |
| 100 | No signs of toxicity or mortality observed | 3/3 |
| 1000 | No signs of toxicity or mortality observed | 3/3 |
| | **PHASE 2** | |
| 1500 | No signs of toxicity or mortality observed | 1/1 |
| 2500 | No signs of toxicity or mortality observed | 1/1 |
| 5000 | No signs of toxicity or mortality observed | 1/1 |

**Key:** 3/3, 1/1 = All survived

**Table 5: Acute toxicity test of the combined aqueous extracts of *M. kerstingii* and *M. inermis* Swiss Albino Mice**

| Doses (mg/kg) | Result | Remark |
|---|---|---|
| | **PHASE 1** | |
| 10 | No signs of toxicity or mortality observed | 3/3 |
| 100 | No signs of toxicity or mortality observed | 3/3 |
| 1000 | No signs of toxicity or mortality observed | 3/3 |
| | **PHASE 2** | |
| 1500 | No signs of toxicity or mortality observed | 1/1 |
| 2500 | No signs of toxicity or mortality observed | 1/1 |
| 5000 | No signs of toxicity or mortality observed | 1/1 |

**Key:** 3/3, 1/1 = All survived

## DISCUSSION

Plants have been the source of medicinal agents for thousands of years and impressive number of modern drugs has been isolated from natural sources, many based on their uses in traditional medicines (Chatterjee, *et al*., 2011). The phytochemical analysis of *Mitragyna inermis* and *Monetes kerstingii* revealed the presence of secondary metabolites such as Carbohydrates, Flavonoids, Resins, Saponins, Tannins, Cardiac glycosides and Alkaloids. These phytochemicals have been established to be frequently responsible for antimicrobial properties of most medicinal plants (Cowan, 1999; Aspidi *et al*., 2008). Zongo et al. (2009) had reported the presence alkaloids, tannins, Saponins and Cardiac glycosides in in the extract of *Mytragyna inermis*. The result also in agreement with the report of Tor-Anyii and Okpo (2012) who reported the presence of Saponins, Tannins, Carbohydrates in the extracts of *Mitragyna inermis*. The tannins mode of antimicrobial action may be related to their ability to inactivate microbial adhesions, enzymes and cell envelope transport proteins (Ya *et al*., 1998). Alkaloids intercalate into cell wall and/or DNA of organisms thereby causing structural instability and genetic modifications (Scalbert, 2001). The saponins destabilize the cytoplasmic and plasma membranes of microbial organisms (Just *et al*., 1998).

This study has shown that the combined extracts of *Mitragyna inermis* and *Monetes kerstingii* can be used in the treatment of infectious diseases. The combined extracts of these plants demonstrates varying activities in terms of their inhibitory effects on the test organisms.

Zongo et al. (2009) had reported the antibacterial properties of total Alkaloids extract of *Mitragyna inermis* shows moderate activity against 10 reference bacterial strains and 3 clinical isolates (*Baceillus cereus* LMG 13569, *Enterococcus faecalis* CIP 105150, *Shigella dysentereria* CIP 5451, *Staphylococcus aureus* ATCC 9244, *Proteus mirabilis* CIP 104588, *Staphylococcus aureus* ATCC 25293 and *Staphylococcus comorum* LMG 13567) and clinical strains (*Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pyogenes*). The finding of this study is also in agreement with the report of Tor-Anyii and Orokpo (2012), they reported that the crude ethanol extract, water insoluble fraction residue, ethyl acetate fraction and n-butanol fraction inhibit the growth of *Staphylococcus aureus*, *P. mirabilis*, *S. pyogenes* and *S.* Typhi respectively. They all attributed the antibacterial activities of these plants the presence of secondary metabolites.

There is a dart of literature reports with regard to the antibacterial effects of the extracts of Monetes kerstingii but the bark and leaves of the plant has been reported to be used in traditional folklore medicine. Decoction of the bark and leafy twigs are taken to treat dysentery and diarrhea and jaundice (Yahaya *et al.*, 2012). Leaf and root decoctions are applied to abscesses and fractures (Konkon *et al.*, 2008). Phytochemicals have been established to be frequently responsible for antimicrobial properties of most medicinal plants (Cowan, 1999; Aspidi *et al.*, 2008). Hence, the observed antimicrobial activity of the extracts against the test microorganisms may be attributed to the presence of the phytochemical components of the plants.

The result of acute toxicity of the combined extracts of *Mitragyna inermis* and *Monetes kerstingii* reveals that the plant exhibit no significant toxicity as no signs of toxicity such as dizziness, inactiveness, loss of appetite, loss of weight and even death was observed in both phases of the experiment. This however contradicts the report of Adeourn *et al*. (2012) who reported 1600mg/kg body weight lethal dose ($LD_{50}$) of *Mitragyna inermis* indicating that the extract is slightly toxic to the test rats in their experiment. According to Hodge and Sterner, (1949) and locke, (1983), compounds of slight toxicity will have $LD_{50}$ between 5000 and 15000mg/kg Thus, no $LD_{50}$ was established in this study. It can thus be said that the $LD_{50}$ of the extract is higher than 5000mg/kg body weight hence the extract is relatively non-toxic.

**CONCLUSION**

The phytochemical results indicate the presence of metabolites such as tannins, flavonoids, Carbohydrates, saponins and alkaloids which may be responsible for the activity and medicinal uses of the plant. The strong antibacterial activities demonstrated by this plant indicate that the plant can be safely used ethno-medically. This plant therefore present a potential novel and cheap source of potent antimicrobial agents which could justify it been claimed for ethno-medicinal uses.

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