

Effects of standardized stem bark extract of *Mangifera indica* L. in wistar rats with 2,4-dinitrophenylhydrazine-induced haemolytic anaemia

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ABSTRACT

Background: The aqueous decoction of the stem bark of *Mangifera indica* L. has been traditionally used for the treatment of various illnesses among them includes anaemia. **Aims:** The aim of this study was to investigate the anti-anaemic properties of standardized stem bark extract of *M. indica* in animals with 2,4-dinitrophenylhydrazine-induced haemolytic anaemia. **Methods and Material:** An *in vivo* animal model was used in this experiment. 2,4-dinitrophenylhydrazine was used to induce haemolysis and treatment was done with three different concentrations (25, 50, and 100 mg/kg b.wt) of the plant extract. Astifer[®] was used as a positive control. Haematological parameters such as PCV, HGb concentration, and TLC were performed and to ascertain the level of haemolysis. GC-MS was used to determine the presence of phytoconstituents within the crude extract. **Results:** PCV and HGb concentration increased significantly ($p < 0.001$) at a dose of 50 and 100 mg/kg b.wt respectively while no significant ($p > 0.05$) effect was observed at a dose of 25 mg/kg b.wt. TLC was decreased significantly ($p < 0.001$) at a dose 100 mg/kg b.wt while no significant ($p > 0.05$) effect was observed at a dose of 25 and 50 mg/kg b.wt respectively. GC-MS analysis revealed presence of 15 compounds viz: 2,2-Dimethoxybutane, N-Acetyl-Alpha-D-glucosamine, 1,2-Benzenediol, Phenol, 2,4-bis(1,1-dimethylethyl)-, Vitamin E, Pentadecanoic acid, 13-methyl-, methyl ester, 2-Ethylacridine, Benzofuran-6-ol-3-one, 2-(4ethoxycarbonyl)benzylidene-, 9-Octadecanoic acid, (E)-, 2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-, and Benzo[h]quinoline,2,4-dimethyl-. **Conclusion:** The results of our present finding suggest the significant anti-anaemic properties of standardized stem bark extract of *Mangifera indica* L. This finding highlights the potentials of the extract and *M. indica* in the treatment of haemolytic anaemia.

Key words: 2,4-dinitrophenylhydrazine, Anaemia, GC-MS analysis, Haemolysis and *Mangifera indica* L.

INTRODUCTION

Anaemia is a public health problem that affects populations in both rich and poor countries with major consequences

for human health as well as social and economic development. The primary cause of anaemia is attributed to iron deficiency. However, anaemia co-exists with a number of other causes, such as malaria, parasitic infection, nutritional deficiencies,¹ drug toxicity as well as genetic or acquired defect.² The prevalence of anaemia is widely common in pregnant women due to high demand from the developing foetus³ while in African countries; the main causes of anaemia in children are attributed to poor nutrition and malaria.⁴

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Mangifera indica L. (Anacardiaceae) grows in the tropical and subtropical regions⁵ and widely distributed around the regions in Nigeria. Standard aqueous stem bark extract of *M. indica* has gain popularity in Cuba as potent antioxidant drug under the brand name VIMANG®. Vimang® contains a definite mixture of components including polyphenols, triterpenes, phytosterols, fatty acids and microelements.⁶ Reports have shown that Vimang possesses several pharmacological activities such as the ability of the extract to reduce the formation of reactive oxygen species (ROS) in mice.⁷ It has also shown to prevent iron overload in serum as well as liver oxidative stress in rats.⁸ It also inhibits necrosis factor alpha (TNF α) and nitric oxide (NO) in endotoxic shock and microglia.⁹ A standardized stem bark extract of *M. indica* has also shown to provide significant better protection against TPA-induced oxidative damage when compared with other exogenous antioxidant Vitamin C, Vitamin E, mangiferin as well as β -carotene.¹⁰

The aqueous decoction of the stem bark of *Mangifera indica* L. have been traditionally used for the treatment of menorrhagia, scabies, diarrhea, syphilis, diabetes, cutaneous infections, anemia, malaria, fever, and dysentery.^{11,12} In the Northern region of Nigeria, the decoction of the stem bark extract is traditionally used for the treatment of wound healing and anemia.¹³ However, due to the facts that no scientific detailed information has been reported so far on the anti-anaemic properties of standardized stem bark extract of *Mangifera indica*, in view of this, we therefore investigate the anti-anaemic properties of the standardized stem bark extract of *M. indica* in animals with 2, 4-dinitrophenylhydrazine-induced haemolytic anaemia.

MATERIALS AND METHODS

Reagents

2,4-dinitrophenylhydrazine (2,4-DNPH) was obtained from BDH pool, England. All other chemicals and reagents were of analytical grade and where obtained from Sigma-Aldrich Company Ltd.

Plant material, preparation, and drugs

The stem bark of *Mangifera indica* L., Anacardiaceae, was collected on 24th June, 2013 at 11:30 am around the cultivated mango trees in the rural region of Dundaye area, Usmanu Danfodiyo University permanent site, Sokoto, Nigeria. The plant was identified by comparing with the existed voucher specimen (No. 1944) by Dr. Mshelia H.E at

the Herbarium section of the Biological Sciences, Ahmadu Bello University Zaria, Nigeria.

The test extract was prepared by decoction of stem bark extract of *Mangifera indica* L for 1hr, concentrated by evaporation at reduced temperature and spray dried to obtain a fine powder. The solid extract was dissolved in distilled water for pharmacological studies.⁸ Astifer® used as standard drug was supplied from Fidson Healthcare Plc.

Animals

Thirty Wister rats of either sex (230-250 g) were obtained from Usmanu Danfodiyo University, Sokoto. The animals were kept at the animal house of Faculty of Pharmaceutical sciences, Usmanu Danfodiyo University under controlled environment at $22\pm 2^{\circ}\text{C}$. A 12 hour light and 12 hour dark cycle was ensured during which they were allowed to acclimatize under optimum feeds and water access for a period of 2 weeks before the commencement of the experiment.

Experimental procedures

All procedures using animals in this investigation were followed in accordance with the ethical standard of the European Union Guidelines for Animals Experimentation (Dir86/609/EEC) and approved by the Institutional Animal Care Committee, Usmanu Danfodiyo University, Sokoto.

Animal grouping

The animals were randomly divided in to six groups of five animals each ($n=5$) as follows: group I serve as a normal control (distilled water only), group II serve as a negative control (2,4-DNPH only), group III, IV, and V serve as a test groups (2,4-DNPH and different concentrations of plant extract), and finally group VI serve as a positive control (2,4-DNPH and Astifer®).

2,4-dinitrophenylhydrazine-induced haemolytic anaemia

A modified method described by Berger¹⁴ was used in this investigation. The animals were randomly divided in to six groups of five animals each ($n=5$). One group received distilled water (1 ml/kg b.wt, p.o.) and the other five groups received 2,4-DNPH (20 mg/kg b.wt, p.o) once daily for 7 consecutive days. On the 8th day, their blood sample were collected by sinus puncture at the tail veins of each rat in to heparinised capillary tubes for haematological analysis. Rats with $\geq 30\%$ reduction in red blood cell count and

haemoglobin concentration were considered anaemic and used for this study.⁴

Drug treatment

The anaemic groups III, IV and V received test extract (25, 50, and 100 mg/kg b.wt, p.o.) and VI received standard heamatinic drug Astifer® (50 mg/kg b.wt, p.o.). All drugs were administered once daily for 14 consecutive days by oral feeding cannula.

Haematological assessment

The blood sample was collected by sinus puncture at the tail veins of each rat in to heparinized capillary tubes for determination of haematological parameters. Pack cells volume (PCV) was determined using microhaematocrit method,¹⁵ haemoglobin (HGb) concentration¹⁶ and total leucocytes count (TLC)¹⁷ were also determined.

GC-MS Analysis

The GC-MS analysis of the crude extract of *M. indica* was carried out on Agilent Technologies 6890N network GC system and Agilent Technologies 5973 network mass selective detector coupled with 7683B series injector. The model number of the column used was Agilent 122-5533 capillary column (DB-5 ms, 30 m × 0.25 mm × 1 μm). The carrier gas used was helium at a flow rate of 1.2 ml/min. the injection volume was 1 ul. The inlet temperature was maintained at 230°C. The oven temperature was programmed initially at 50°C for 5 min then the programmed to increase to 300 at a rate 10 ending with 25 min. Total run time was 45 min. The MS transfer line was maintained at a temperature of 250. The source of temperature was maintained at 230 and the MS quad at 150. The ionization mode used electron ionization mode at 70 Ev. Total ion count (TIC) was used to evaluate for compound identification and quantification. Data analysis and peak area measurement was carried out

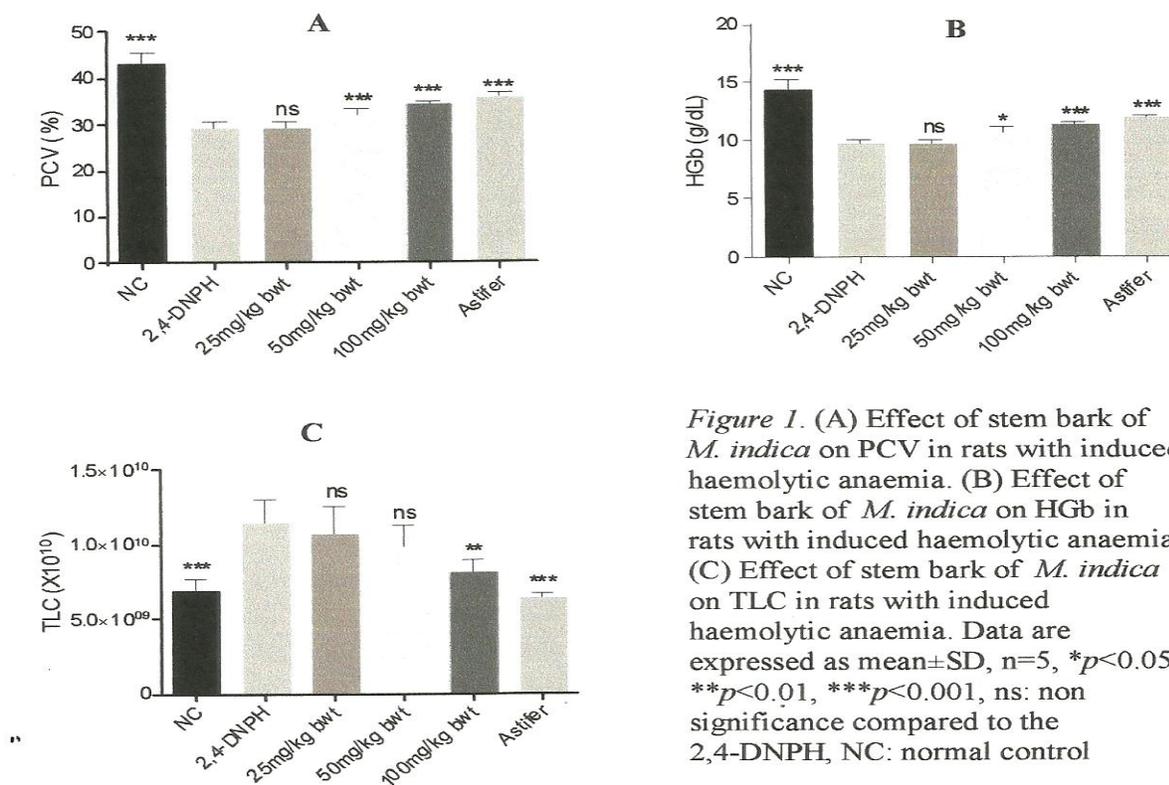


Figure 1. (A) Effect of stem bark of *M. indica* on PCV in rats with induced haemolytic anaemia. (B) Effect of stem bark of *M. indica* on HGb in rats with induced haemolytic anaemia. (C) Effect of stem bark of *M. indica* on TLC in rats with induced haemolytic anaemia. Data are expressed as mean±SD, n=5, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns: non significance compared to the 2,4-DNPH, NC: normal control

Figure 1: (A) Effect of stem bark of *M. indica* on PCV in rats with induced haemolytic anaemia. (B) Effect of stem bark of *M. indica* on HGb in rates with induced haemolytic anaemia. (C) Effect of stem bark of *M. indica* on TLC in rates with induced haemolytic anaemia.

Date are expressed as mean ± SD, n=5, * $p < 0.005$, ** $p < 0.001$, *** $p < 0.0001$, ns: non significance compared to the 2,4-DNPH, NC: normal control

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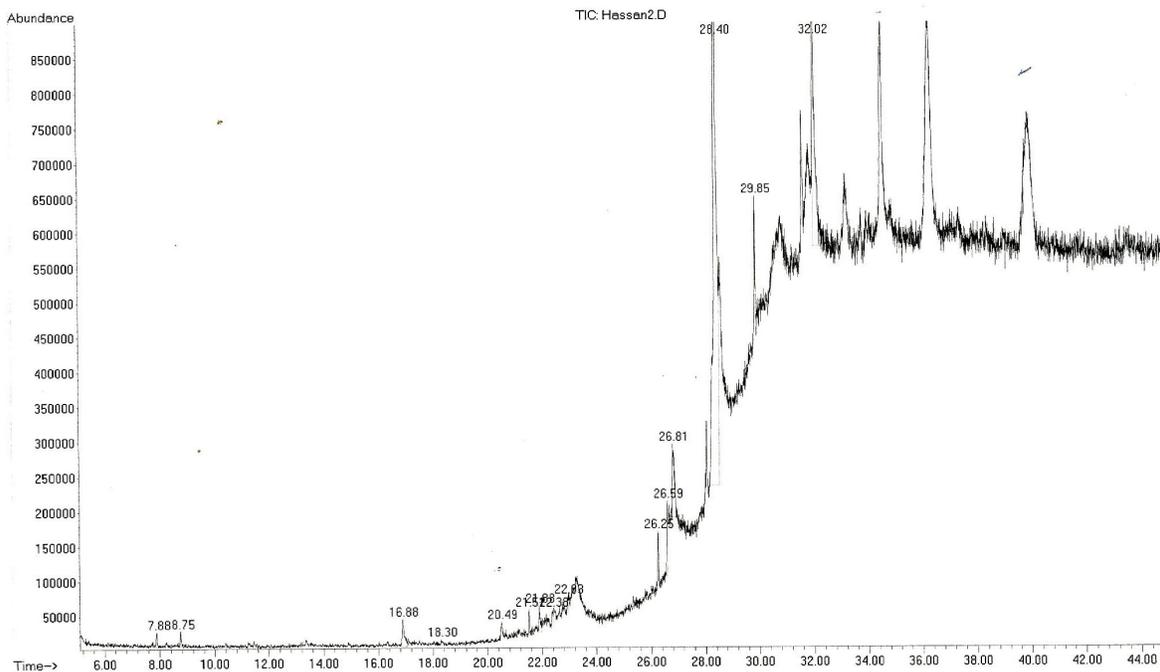


Figure 2: GC-MS chromatogram of crude stem bark of *Mangifera indica*

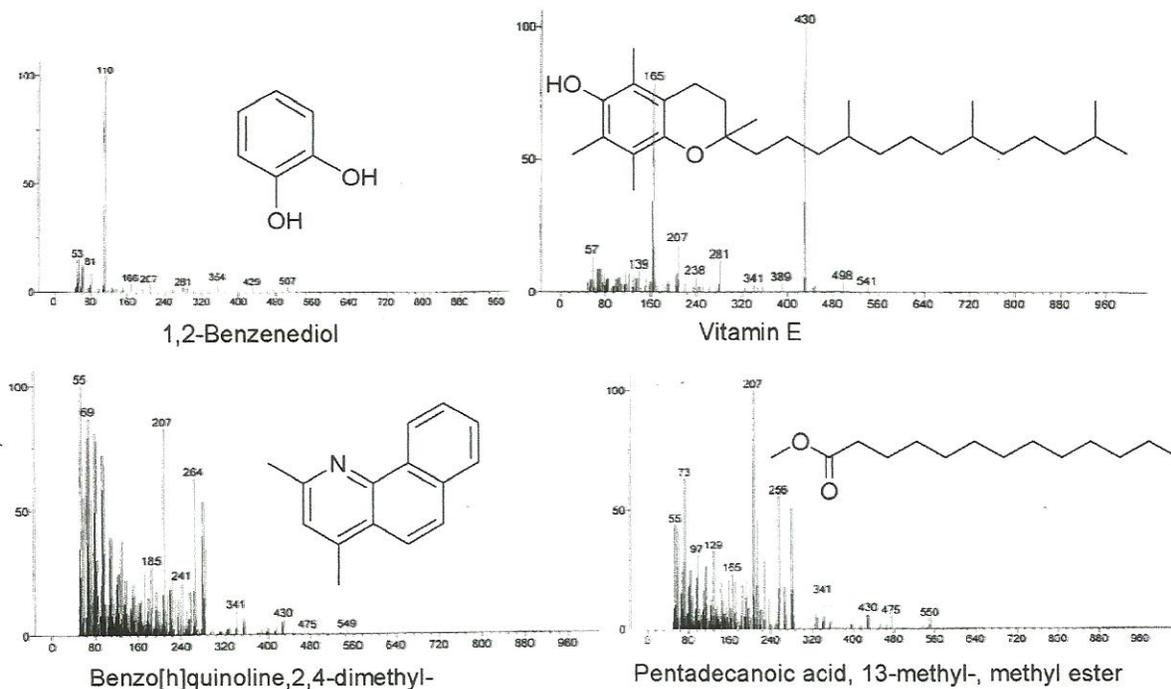


Figure 3: Mass spectrum and structure of compounds identified by GC-MS with in the crude extract of *M. indica*

Table 1: Compounds Separated from the crude stem bark extract of *Mangifera indica* L. using GC-MS analysis

RT	Peak Area%	Name of the compound	MF	MW
7.88	0.49	2,2-Dimethoxybutane	C ₆ H ₁₄ O ₂	118
8.75	0.41	N-Acetyl-Alpha-D-glucosamine	C ₈ H ₁₅ NO ₆	221
16.88	1.69	1,2-Benzendiol	C ₆ H ₆ O ₂	110
Not identified				
Not identified				
21.51	0.52	Phenol,2,4-bis(1,1-dimethylethyl)-	C ₁₄ H ₂₂ O	206
Not identified				
22.38	0.65	D, alpha-Tocopherol-	C ₂₉ H ₅₀ O ₂	430
22.93	0.33	Vitamin E	C ₂₉ H ₅₀ O ₂	430
26.25	1.19	Pentadecanoic acid,13-methyl-, methyl ester	C ₁₆ H ₃₂ O ₂	256
26.59	1.81	2-Ethylacridine	C ₁₅ H ₁₃ N	270
26.81	4.09	Benzofuran-ol-3-one, 2-(4ethoxycarbonyl)benzylidene-	C ₈ H ₁₄ O ₅	310
28.40	63.83	9-Octadecanoic acid, (E)-	C ₁₈ H ₃₄ O ₂	282
29.85	3.51	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-	C ₁₃ H ₂₂ O _{Si} ₂	250
32.02	19.14	Benzo[h]quinoline,2,4-dimethyl-	C ₁₅ H ₉ N	207

using Agilent chemstation software. The spectrum of the separated compound was compared with the database of the spectrum of known compound saved in the NIST02 reference spectra library, structural determination was compare to those spectral patterns to ChemSpider data base and those reported in the literature.

Statistical analysis

The results were expressed as means±SD using one-way ANOVA followed by Dunnett's test for multiple comparisons

RESULTS

The effect of crude stem bark extract of *M. indica* in rats with 2,4-DNPH induced anemia is shown in Figure 1. The result of the PCV was increased significantly ($p < 0.001$) at a dose of 50 and 100 mg/kg b.wt respectively when compare to the 2,4-DNPH induced anemic group. No significant ($p > 0.05$) effect was observed at a dose of 25 mg/kg b.wt. Hgb concentration significantly ($p < 0.001$) increased at a dose of 50 and 100 mg/kg b.wt respectively when compare to the 2,4-DNPH induced anemic group. No significant ($p > 0.05$) effect was observed at a dose of 25 mg/kg b.wt as well. TLC decreased significantly ($p < 0.001$) at a dose 100 mg/kg b.wt when compare to the 2,4-DNPH induced anemic group. No significant ($p > 0.05$) effect was observed at a dose of 25 and 50 mg/kg b.wt respectively.

The result of the GC-MS analysis leads to the separation of 15 peaks as shown in Figure 2, while the results of their corresponding compounds are shown in Table 1. Of

the 15 compounds, effort made to verify the structural identification of 3 compounds has proven unsuccessful while vitamin E was identified two times at different retention times. The most abundant compound within the crude extract of *Mangifera indica* is 9-Octadecanoic acid, (E)- with 63.83% followed by benzo[h]quinoline,2,4-dimethyl- with 19.14%, benzofuran-6-ol-3-one,2-(4ethoxycarbonyl)benzylidene- with 4.09% and 2,4,6-Cycloheptatrien-1-one,3,5-bis-trimethylsilyl- with 3.51%, while the least in abundance is Vitamin E with a total area percent of 0.33%. Few of the individual fragmentation of the compounds are shown in Figure 3.

DISCUSSION

Phenylhydrazine (PHZ) and its derivatives 2,4-DNPH induced hemolytic anemia is a validated methods used to determine the anti-hemolytic properties of drugs in experimental animals. PHZ induces hemolysis of RBCs by inducing the formation of toxic free radicals (peroxidation of lipids) that can attack cellular macromolecules like hemoglobin resulting in the oxidative damage within the RBCs and oxidative degradation of spectrin in the membrane skeleton resulting in their destruction.¹⁸ PHZ decreases Hgb level, RBC concentration, PCV, and impairs erythrocyte deformability.¹⁹ The apparent decrease in Hgb level, PCV, and increase in TLC in this study is an indication of haemolytic action induced by 2,4-DNPH. Administration of *Mangifera indica* at a dose of 50 and 100 mg/kg b.wt were able to significantly reverse the anaemia induced by 2,4-DNPH after 14 days of treatment. Meanwhile, at a dose of 100 mg/kg b.wt shows apparent anti-haemolytic effect almost as the same as standard

heamatinic agent Astifer®. Significant increased in TLC is only seen at a dose of 100 mg/kg b.wt.¹⁰ reported that a standardized stem bark extract of *M. indica* provide significant better protection against TPA-induced oxidative damage when compared with other exogenous antioxidant Vitamin C, Vitamin E, mangiferin as well as β -carotene. The present finding suggest effectiveness in our crude extract in proving protection against 2,4-DNPH-induced haemolytic anemia almost as the same as standard heamatinic agent Astifer®.

Of the twelve compounds identified from the GC-MS result, only few have been reported to have pharmacological activities. 2,2-dimethoxybutane has also been reported in *Xanthorrhoea johnsonii*,²⁰ *Clematis graveolens*,²¹ *Piper longum*,²² and *Petalostigma triloculare*.²³ The branched ether 2,2-dimethoxybutane have been reported to be toxic to microbial membrane.²⁴ 1,2-benzenediol is present in plant-derived products, such as vegetables, fruits, coffee, tea, wine, areca nut and cigarette smoke²⁵ among them include *Diospyros kaki*,²⁶ *Annona senegalensis*,²⁷ *Petalostigma triloculare*²³ among others. Report have shown that 1,2-benzenediol possess antimicrobials as well as antiplatelet^{25,26} activity. Phenol, 2,4-bis (1, 1-dimethylethyl)- has also been reported in *Scolopendra subspinipes* with strong antioxidant activity,²⁸ *Pereskia bleo* with anticancer activity,²⁹ and strain of *Pseudomonas monteilii* PsF84 with antifungal activity,³⁰ Additionally, vitamin E identified two times at different retention times in this study is a well known antioxidant compound used for the treatment of various illnesses while N-Acetyl-Alpha-D-glucosamine has been used as safe alternative therapies for osteoarthritis,³¹ Pentadecanoic acid, 13-methyl-, methyl ester is also found *Excoecaria agallocha* with antimicrobial activity.³² Other compounds detected with no apparent bioactivity reported so far include 2-Ethylacridine found in *Dryopteris cochleata*,³³ *Oldenlandia corymbosa*,³⁴ and *Sesamum indicum* while 9-Octadecanoic acid, (E)- and Benzo[h]quinoline,2,4-dimethyl- is also found in *Sesamum indicum*.³⁵ Another compound with no apparent biological activity reported so far is 2,4,6-Cycloheptatrien-1-one,3,5-bis-trimethylsilyl- found in *Garcinia kola*,³⁶ *Aporosa lindleyana*,³⁷ *Arthrocnemon glaucum*,³⁸ and *Jatropha tanjorensis*,³⁹ Benzofuran-6-ol-3-one, 2-(4ethoxycarbonyl)benzylidene- is only found in *Psidium guajava*,⁴⁰ Upon all the chemical composition detected in this study, it is difficult to attribute the bioactivity of a complex mixture; however, many polyphenolic compounds have been reported to possess antioxidant activity a good example is Phenol, 2,4-bis (1, 1-dimethylethyl)- detected in the present finding. Vitamin E also found in this study may also be

responsible for the biological activity along with other polyphenolic compounds which may exert their effect synergistically. Research has shown that a standardized stem bark extract of *M. indica* provide significant better protection against TPA-induced oxidative damage when compared with other exogenous antioxidant Vitamin C, Vitamin E, mangiferin as well as β -carotene.¹⁰ The “better protection” used by the author in reference to the standardized stem bark extract of *M. indica* might be due to the synergistic effect exerted by the apparent presence of phenolic acids, phenolic esters, flavan-3-ols components and in special, mangiferin.¹¹ However, mangiferin was not detected in our present study and its absence in the crude extract may be due to the fact that the plants are from different geographical location thereby affecting the secondary metabolites present within the plant material.

CONCLUSION

In conclusion, the results of our present finding suggest the significant anti-anaemic properties of standardized stem bark extract of *Mangifera indica* L in 2,4-dinitrophenylhydrazine-induced haemolytic anaemia in experimental rats. This finding highlights the potentials of standardized stem bark extract *M. indica* in the treatment of haemolytic anaemia. This finding supports the ethno and medicinal use of stem bark extract of *M. indica* for the treatment of anaemia. The anti-anaemic properties in this study may be as a result of the polyphenolic and other compounds found within the plant extract. However, do to the fact that we are unable to identify a single chemical entity that may be fully responsible for the bioactivity found within the extract; the anti-anaemic properties of the plant extract can be better understood if the biologically active compounds are isolated and characterized.

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CONFLICT OF INTEREST

All authors involved declared no conflict of interest.

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