GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Formulation and evaluation of herbal tablets containing root bark extract of *Terminalia Macroptera* Guill and Perr (Combretaceae) for antibacterial activity

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GSC Biological and Pharmaceutical Sciences, 2026, 34(03), 142-149

Publication history: Received on 07 February 2026; revised on 16 March 2026; accepted on 19 March 2026

Article DOI: https://doi.org/10.30574/gscbps.2026.34.3.0101

Abstract

The use of crude extracts of plants parts or phytochemicals of known antimicrobial properties could be of great significance in the therapeutic treatment of diseases. The study was aimed at formulating and evaluating tablets from the root bark extract of *Terminalia macroptera*. The root bark of T*erminalia macroptera* was extracted with acetone, and the median lethal dose of the extract was determined by Lorke method. The extract was made into granules with some excipients and the granules evaluated. The granules were formulated into tablets which were evaluated using standard methods and procedures. The extract yield was 18.80 %, and no mortality at 5000 mg/kg body weight. The granules had good flow properties and the formulated tablets passed hardness, uniformity of weight, diameter and thickness tests, and also USP disintegration test, and BP dissolution test for one of the batches.

Keywords: *Terminalia Microptera*; Crude Extract; Granules; Tablets; Evaluation

1. Introduction

The use of crude extracts of plants parts or phytochemicals of known antimicrobial properties had been of great significance in the therapeutic treatment of diseases. In recent years, a number of studies were conducted in various countries to prove such efficiency. Many plants have been used as antimicrobial due to the secondary metabolites they synthesize. Leaves and flowers of experimental plants have been used for treating many diseases in traditional medicines. The leaf of *Azadiracta indica* is commonly used against intestinal worms and some poisonous bites such as Arizona coral snake and Gila monster bite, and also in the treatment of inflammation and cholera [1]. The essential oil possesses anti-bacterial activity against *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, *E. coli*, *Bacillus subtilis* and *Salmonella typhimurium*. In Argentina, terpene compounds (eugenol, geraniol, thymol and carvacrol) derived from essential oils of native plants showed inhibitory effects on MRSA [1]. About 80 % population of developing countries use herbal medicines for primary healthcare. Herbal medicines have stood the test of time for their safety, cultural acceptability and lesser side effects. Improvements in analysis and quality control alongside advances in clinical research have confirmed their value in the prevention and treatment of diseases [2,3]. *Terminalia macroptera* parts and decoction have been used variously in the treatment of diseases among Nigerians and Africans, and also sold in the local markets for this purpose [2,5, 6]. Developing suitable dosage forms would increase the scope of use, acceptability and patient compliance. Formulation of *Terminalia macroptera* into pharmaceutical dosage forms other than the traditional mixtures would ensure reproducibility of product quality, accurate dosing of medicines, predictive therapeutic outcomes and easy compliance with usage directives. Developing and formulation of herbal medicines would enhance acceptability by all classes of people. The aim of this study was to formulate and evaluate the root bark extract tablets of *Terminalia macroptera* for its antimicrobial activity.

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2. Materials and Methods

The fresh root bark of *Terminalia macroptera* was collected from Ede Alaba in Idah Local Government of Kogi State and authenticated at the herbarium section of the Department of Biological Sciences, Kogi State University, Anyigba where the plant was deposited with a voucher number of 299.

2.1. Preparation of Plant Parts for Extraction

The root bark was air dried at room temperature for three (3) weeks, and thereafter pulverized using mortar and pestle and a mechanical blender into powdered material which weighed 150 g. A 130 g of powdered root bark was weighed and transferred into glass bottle and extracted with 1000 ml of acetone, shaking the bottle intermittently for 72 h and filtering with muslin bag and Whatman filter paper. The filtrate was evaporated using rotary evaporator (Model RE 100, England)). The material was further subjected to air drying in the laboratory. The dried material was stored in a bottle at room temperature until required for use

2.2. Acute toxicity study of acetone root bark extract

After obtaining approval from the Animal Ethics Committee of the Faculty of Pharmaceutical Sciences, University of Jos, the acute toxicity study was conducted in two phases, and twelve rats were selected. In the first phase, nine rats were randomly allocated into 3 groups of 3 each. Groups 1, 2 and 3 animals were given 10,100 and 1000 mg/kg body weight of the extract respectively in order to establish the range of doses producing any toxic effect. Each rat was given a single dose. In the second phase, specific doses (1600, 2900 and 5000 mg/kg body weight.) of the extract were administered to three rats (one rat per dose). The extract was dissolved in normal saline water and given via oral route. All animals were observed frequently on the day of treatment and surviving animals were monitored daily for 2 weeks for signs of acute toxicity [6, 7].

2.3. Formulation of granules

As shown in Table 1, the extract, lactose, disintegrant and microcrystalline cellulose were weighed and triturated appropriately. The polyvinylpyrrolidone was dissolved in small quantity of water and gradually added to the powder mix. The mixing went on until a damp coherent mass was formed. The wet mass was then screened through sieve No.10 (2000 µm) and dried at room temperature. After drying, it was further screened through sieve No 16 (1190µm) to obtain the granules.

Table 1 Unit Formula for Terminalia microtear Acetone Extract Tablets

Ingredient	**Concentration (mg)**
Terminalia microtear	100 (20 %)
Lactose	275 (55 %)
Maize starch	70 (14 %)
Microcrystalline cellulose	25 (5 %)
Polyvinyl pyrolidone	25 (5 %)
Mg. Stearate	5 (1 %)
Total	500 (100 %)

3. Evaluation of granules

3.1. Bulk density

A 25 g of the granules were weighed and carefully poured into a 100 ml measuring cylinder without tapping. The volume occupied by the granules was taken as the bulk volume. The bulk density is calculated by the formula:

$$\text{Bulk density} = \frac{\text{weight of granules}}{\text{bulk volume of granules}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad 1$$

3.2. Tapped density

The 25 g granules in the 100 ml measuring cylinder for the bulk density was tapped until there was no volume change. The volume was then read off as the tapped volume. The tapped density was calculated by the formula:

$$\text{Tapped density} = \frac{\text{weight of granules}}{\text{tapped volume of granules}} \quad \ldots\ldots 2$$

3.3. Hausner's Quotient

From the bulk and tapped density obtained above the Hausner's quotient was calculated using the formula:

$$\text{Hausner ratio} = \frac{Tapped\ density\ (\rho Bmax)}{Poured\ density\ (\rho Bmin)} \quad \ldots\ldots 3$$

3.4. Carr's Compressibility Index

From the bulk and tapped density calculated, the Carr's compressibility was calculated by the formula

$$\text{Carr's index (\%)} = \frac{Tapped\ density - Poured\ density}{Tapped\ density} \times 100 \quad \ldots\ldots 4$$

3.5. Flow rate

A 30 g quantity of the granules was carefully poured into the funnel while a finger was placed at the tip of the funnel and, when removed, was allowed to flow on a sheet of paper forming a heap of granules. The time it takes the granules to flow out completely through the orifice of the glass funnel was noted. This procedure was carried out in triplicate. The flow rate is calculated using the formula below:

$$\text{Flow rate} = \frac{\text{weight of granules}}{\text{average time}} \quad \ldots\ldots 5$$

3.6. Angle of repose

The angle of repose was performed using the fixed height method. A funnel was clamped with its tip 5cm above a flat surface. A 30 g quantity of the granules was carefully poured into the funnel while a finger was placed at the tip of the funnel orifice and, when removed, was allowed to flow on a sheet of paper forming a heap of granules. The base of the heap was marked and the height of the heap measured. The angle of repose was calculated from the height (h) and the radius of its base (r) using the formula, $\tan \theta = h/r$, and $\theta = \tan^{-1}$ [8].

4. Compression of tablet

The granules were blended with the magnesium stearate prior to compression. The blend was compressed using a single punch tableting machine (Erweka EP-1, Germany) set at 4.5 and 5.0 Metric tonnes compression pressure for the two batches [9].

4.1. Evaluation of tablets

The tablets produced were evaluated for uniformity of weight, diameter, thickness, hardness, friability, disintegration time, and dissolution rate.

4.2. Weight uniformity

The British Pharmacopoeia [10] method was adopted. Twenty tablets were randomly selected from each batch and weighed individually. The mean weight as well as standard deviation were calculated.

4.3. Uniformity of diameter

The diameters of the tablets were measured using digital vernier caliper. Twenty (20) tablets were randomly selected and used for the test [10]. The mean diameter and standard deviation were calculated.

4.4. Uniformity of thickness

The thickness of the tablets was measured using digital vernier caliper. Twenty (20) tablets were selected at random for the test [10]. The mean thickness and standard deviation were calculated.

4.5. Friability test

According to the United States Pharmacopoeia [11], tablets with a unit mass equal to or less than 650 mg, take a sample of whole tablets corresponding to 6.5 g. For tablets with a unit mass of more than 650 mg, take a sample of 10 whole tablets. Because the average weight of the tablets was less than 650 mg, twenty (20) tablets were randomly selected per batch instead, and weighed. The Roche Friabilator (Erweka, Germany) was used for the test. After the machine was loaded with the twenty (20) tablets, it was allowed to revolve 25 times per minute for four minutes. The tablets were removed, brushed and blown free from adhering dust and weighed. The experiment was carried out in triplicate. The percentage loss in weight which occurred was taken as a measure of friability.

4.6. Hardness test

A Monsanto tablet hardness tester (Copley Scientific Ltd, Nottingham, United Kingdom) was employed to determine the mechanical strength of the tablets. The average force required to crush the tablets from each batch was obtained.

4.7. Disintegration test

The BP [10] method was adopted using disintegration equipment (Eagle Scientific, England). The disintegration media used were 0.1 N HCl and distilled water. Six tablets were tested for each batch. Each tablet was placed on the screen of the disintegration apparatus immersed in 0.1 N HCl solution maintained at 37 ± 2^{o}C by the equipment. The time required for complete disintegration of each tablet was taken using a stop watch. The procedure was repeated for the other batches using distilled water.

4.8. Dissolution test

The rotating basket method was adopted [10]. A 0.1 N HCl solution was used as the dissolution medium. In vitro release of *T. macroptera* from the tablets was measured at 37 ± 1°C and 100 rpm in 900 ml of the medium. Samples (5 ml) were withdrawn at predetermined time intervals of 5, 15, 30, 45 and 60 min and analysed spectrophotometrically at 352 nm. An equal volume of fresh dissolution medium, maintained at the same temperature, was added after each withdrawal to maintain the volume. The test was repeated thrice.

A serial dilution using acetone and the extract was prepared. The absorbance of these concentrations was determined using a UV-Spectrophotometer with acetone serving as the blank.

5. Results and Discussion

The extract yield was 18.80%. The acute toxicity test (Table 2) of the acetone root bark extract of *T. macroptera* showed no animal death after 24 hours. The major sign of toxicity noticed was general weakness and loss of appetite. The sign became increasingly pronounced as the dose increased towards 5000 mg/kg. The signs were not noticed in 10 and 100 mg/kg doses respectively. The LD_{50} being greater than 5000 mg/kg body weight is considered to be safe [7]. In addition, there was no record of death among the treated rats within the two weeks of exposure. A similar case was reported by Yakubu et al., 2015 that the ethanolic root extract gave an acute toxicity value of more than 5000 mg/ kg body weight for treated rats after 24 h, and even after two weeks of exposure to the extract, none of the rats died in any of the groups.

Table 2 Acute Toxicity Result of *Terminalia macroptera* Administered Orally

Experiment	Dose mg/kgbw	No of rats after 24 hours	Treated rats after 24 hours
Phase-1	10	0/3	0/3
	100	0/3	0/3
	1000	0/3	0/3
Phase-2	1600	0/3	0/3
	2900	0/3	0/3
	5000	0/3	0/3

Granules Properties (Table 3) show that the bulk and tapped densities of the granules were 0.54 and 0.68 g/cm^3 respectively. They help to reveal the extent of the difference in size and shape of the granules. Much difference shows the degree of variability of these properties among the granules. Carr's compressibility Index and Hausner's ratio depend on the bulk and tapped densities. Carr's compressibility index and Hausner's ratio was 20.58% and 1.25 respectively. Carr's index of 16 - 20 and Hausner's ratio of 1.19 - 1.25 indicate fair flow [8]. The result also reveals that the angle of repose of the herbal granules was 35±0.2o. It was mentioned by Aulton [8] that the angle of repose of between 31 – 35^o indicates good flow. It is obvious from the above indicators that the herbal granules exhibit fair flow property (4.07±0.05 g/ Sec).

Table 3 Flow Properties of Granules of *T. macroptera* Acetone Extract

Property	Values
Bulk density (g/cm^3)	0.54
Tapped density (g/cm^3)	0.68
Compressibility (%)	19.56
Hausner's ratio	1.24
Angle of repose (^{0})	35±0.2
Flow rate (g/sec)	4.07±0.05

Evaluation of the tablets revealed the tablets' weight uniformity (Table 4). Weight uniformity test ensures that all tablets within a batch fall within reasonable limits of weight. It is an important in-process control measures. Weight uniformity of tablets is influenced by the flow properties of the granules, the fill weight of the die cavity and the particle size distribution of the granules. Weight uniformity in turn predicts uniformity of medicament content. The British Pharmacopoeia [10] specifies that for tablet mean weight greater than 250 mg, not more than 2 tablets should deviate from the mean weight by ±5 percent deviation and none should deviate by twice that percent deviation. Tablets compressed at 4.5 and 5.0 metric ton had average weight of 496±19 and 501±15 mg respectively, and gave an indication that they passed BP weight uniformity test.

Table 4 Post Compression Parameters for *T. macroptera* Tablets

S/No	Parameters	Compression Pressure (Metric ton)	
		4.5 (Batch II)	**5.0 (Batch I)**
1	Uniformity of weight (mg)	496.**0. ±**19	501.0± 15
2	Uniformity of diameter (mm)	12.3± 0.07	12.2±0.03
3	Uniformity of thickness (mm)	3.1±0.06	3.0±0.03
4	Hardness (KgF)	5.0±0.85	7.30±1.64
5	Friability (%)	0.96	0.56
6	Disintegration (min)	18.90±2.21	27.38±2.58

5.1 Uniformity of diameter/thickness

Not only is the tablet thickness important in reproducing tablets that are identical in appearance, but also to ensure that every production lot will be usable with the selected packaging components. If the tablets are thicker than specified, a given number may no longer be contained in a volume of a given sized bottle. Tablet thickness also becomes an important characteristic in counting tablets using filling equipment [12]. All the tablets produced conformed to the specification in the BP (± 5% deviation)

5.2 Hardness test

From the result (Table 4), the average hardness from the six tablets for batch I (5 MT) is 7.3kg and that of batch II (4.5 MT) is 5.1kg. Oral tablets hardness is between 4 and 10 kg. This indicates that the tablets passed the hardness test [9].

5.3 Friability test

Friability test is defined as the per cent of weight loss by tablets due to mechanical action during the test. This is a tablet property relating to hardness. According to the United States Pharmacopoeia [11], tablets with a unit mass equal to or less than 650 mg, take a sample of whole tablets corresponding to 6.5 g. For tablets with a unit mass of more than 650 mg, take a sample of 10 whole tablets. Loss in tablet weight should not be more than 1 %. The tablet batches, having a friability value of 0.96 and 0.56% for compressed tablets at 4.5 and 5.0 metric ton for batch I and batch II respectively passed the friability test and thus could withstand all normal handling stress.

5.4 Disintegration test

To be absorbed, a drug substance must be in solution, and the disintegration test is a measure only of the time required under a given set of condition for a group of tablets or capsules to disintegrate into particles. Generally, this test is a useful quality-assurance tool for conventional (non-sustained-release) dosage form [12]. The BP [10] specifies that for uncoated tablet, the time for disintegration should be ≤ 15 minutes. At the end of the 15 minutes, all the tablets are expected to disintegrate completely. If one or two tablets failed to disintegrate completely, the test is repeated on twelve tablets. The requirement is met if not less than sixteen (16) of the total of eighteen (18) tablets tested are disintegrated. Disintegration time for uncoated tablet according to USP [11] should not be greater than 30 min. The disintegration time for the two batch was 27 and 19 min for batch I and batch II respectively. This means that the two batches failed BP disintegration test but passed USP disintegration test.

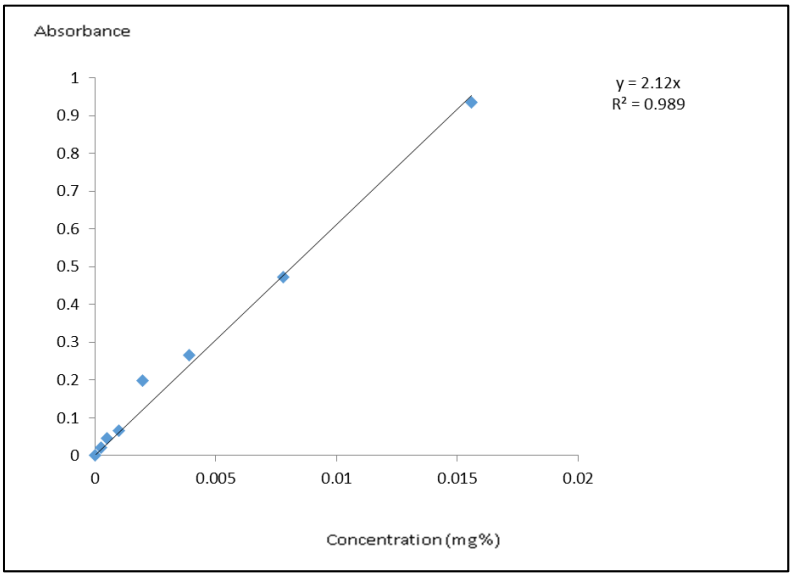

Figure 1 Beer's plot of *Terminalia macroptera* in 0.1N HCL at 352nm and 37± 1 ℃

6 Dissolution test

Dissolution testing (Figure 1 & 2), unlike disintegration, is the most important way to study under in vitro conditions, the release of a drug from a solid dosage form. It represents an important tool to assess factors that affect the bioavailability of the drug from a solid dosage form. The British Pharmacopoeia [10] specifies that uncoated tablets must release not less than 70% of its active content in 45 min to pass the dissolution test. From the dissolution profile, batch II tablets which was compressed at 4.5 metric ton complied with the BP dissolution time of 70% release at 45 min, because bath II released 70.6% of active drug at 45 min. but batch, I released 56% at 45 min, and did not pass the test for dissolution by BP. From Figure 2, batch II released approximately 82% while batch I was 71% after 60 min.

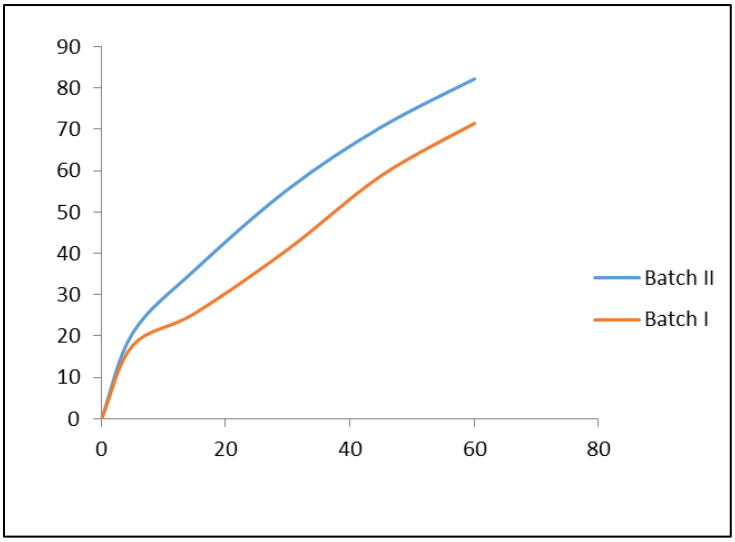

Figure 2 Dissolution profile of batches of *Terminalia macroptera* tablets at 0.1N HCl and 37± 1 ℃

7 Conclusion

The root bark acetone extract of *Terminalia macroptera* was successfully formulated into herbal tablets. The granules flow property was fair, and the formulated tablets compressed at 4.5 metric ton passed some official tests for diameter and thickness, weight uniformity, friability, disintegration and dissolution. The herbal tablets could be used as alternative for treatment of susceptible bacterial infections orally.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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