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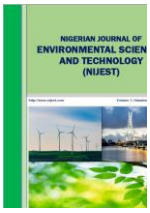

Phytochemical Screening, Antibacterial and Toxicological Effect on Extract of *Morinda lucida*, *Psidium guajava* and *Vitellaria paradoxa*

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ABSTRACT

The present study investigated the phytochemical, biochemical, hematological and antimicrobial properties of Vitellaria paradoxa, Psidium guajava and Morinda lucida. The oral acute toxicity study was conducted on mice with the limit test dose of 5000 mg/Kg body weight. The phytochemical, biochemical and hematological analysis were carried out using standard methods. The methanolic and aqueous extracts of V. paradoxa, P. guajava and M. lucida leaves were tested against selected microorganisms for the antimicrobial activities. The phytochemical results revealed the presence of tannin, flavonoids, steroids, cardiac glycoside, alkaloids, phenol and anthraquinone, alkaloids, saponin, phlobatanin and terpenoids in the three selected plants. The biochemical activity showed that the three plants have the high Aspartate aminotransferase (AST) with V. paradoxa ranging from 115.9U/L to 160.4U/L, M. lucida ranging from 189.3 U/L to 139.4 U/L and P. guajava ranging from 140.8 U/L to 174.6 U/L at low dose and high dose respectively . The low dose revealed high glucose. The hematological indices affect blood cell counts and hemoglobin levels, with potential implications for immune response, anemia prevention and overall blood health. The antimicrobial activity showed that only the extracts of P. guajava with high zone of inhibition, 24.50 ±0.50 on Salmonella typhi and Staphylococcus aureus and the antibiotics (levofloxacin) showed slightly higher zone of inhibition, 24.83 ±0.29 on S. typhi. The oral acute toxicity study suggests no serious or life-threatening side effects. The result demonstrates that P. guajava has a broad antimicrobial properties, therefore has a potential as a good therapeutic agent.

Keywords: *Hematology, Biochemical, Phytochemical, Antimicrobial, Medicinal plants*

1.0. Introduction

Medicinal plants are of great importance to the health of individual and communities. The medicinal values of some plants lie in some chemical substances that produce definite physiological actions in the human body. These chemical substances are generally known as phytochemicals (Nayak *et al*., 2015).

Phytochemicals are naturally occurring in the barks, leaves, vegetables and roots of medicinal plants which play a defensive role against major chronic diseases in both host metabolic or genetic dysfunctional disease and infectious disease, and found in grains, vegetables, fruits, and other plant products (Nayak *et al*., 2015) that have defense mechanism and protect them from various diseases (Esposito *et al*., 2016). Phytochemicals perform intermediary metabolic activities, and they function as primary metabolites such as fats and sugars found in all plants, while secondary metabolites are found in a smaller range of plants and provide specialized functions. Secondary metabolites and pigments, because of their healing effects in humans are processed into drugs such as inulin (dahlias plant), morphine and codeine (poppy plant), quinine (cinchona plant), and digoxin (foxglove plant) (Agunu *et al*., 2011). They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas. These secondary compounds include but not limited to terpenoid, alkaloids and phenolic compounds (Krishnaiah *et al.*, 2007). Drugs from the plants are easily available, less expensive, safe, and efficient and rarely have side effects. According to World Health

Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. About 80% of individuals from developed countries use traditional medicines, which has compounds derived from medicinal plants. However, such plants should be investigated to better understand their properties, safety, and efficiency (Arunkumar, 2009). The determination of the phytochemical properties of *Morinda lucida*, *Vitellaria parvadoxa* and *Psidium guajava* cannot be overlooked in the fight against microbial infections. These plants of medicinal importance have been proven to be very effective even where treatments with antibiotics failed (Oshim *et al*., 2016).

Toxicology is the important part of pharmacology which deals with the undesirable effect of phytocompounds on living organisms previous to the use as drug or chemical in clinical use (Aneela *et al*., 2011). Several studies are concentrated on toxicity analysis so as to determine the safeness of medicinal plants and their products. Toxicity analysis is essential, as some herbs consumed might have some toxic effects and many reports have been published for toxicity caused due to long term consumption of herbs. According to OECD guidelines, in order to ascertain the protection and effectiveness of a new drug, toxicological studies are extremely significant in animals like mice, rat, guinea pig, dog, rabbit, monkey etc. Toxicological studies aid to extend decision whether a new drug must be adopted for clinical use or not. OECD guidelines such as 401, 423 and 425 do not permit the use of drug clinically without its clinical trial as well as toxicity studies (Ecobichon Ansari, 2007). The aim of the study is to investigate the phytochemical, antibacterial and toxicological effect on extract of *Psidium guajava, Morinda lucida and Vitellaria paradoxa.*

2.0 Methodology

2.1. Study Site

The soxhlet extraction of the plants was carried out at Yaba College of Technology Central Laboratory. Phytochemical screening was done at the Biochemistry Laboratory of the National Institute of Medical Research (NIMR). The antimicrobial analysis and toxicity screening were carried out at the Microbiology Laboratory of the Lagos University Teaching Hospital (LUTH) Idi Araba.

X represents the area where samples were obtained for the study

2.2. *Medicinal Plant Collection*

The plants *Psidium guajava* and *Morinda lucida* were collected at Ikotun, Alimosho local government in Lagos while the *Vitellaria paradoxa* was collected at Badagry. The plants were identified by a Botanist Dr. Ani at Yaba College of Technology. They were placed in the big brown envelope and transferred to the laboratory where they were stored under shade at room temperature (25^{o}C). Taxonomy, identification, and authentication were done.

2.3. *Preparation of Plant*

The plants materials were washed separately and allowed to dry at room temperature in a clean, well ventilated room. The dried parts were then ground to fine powder using electric grinding machine. The samples were packed in translucent paper bags and stored at room temperature until use.

2.4. Extraction of Plant Materials

2.4.1. Aqueous Extraction

Extraction was carried out based on modification to the method previously described by (Awoyinka et al. 2007).

2.4.2. Methanol extraction

Three hundred and fifty gram (350g) of the grounded leaves were extracted by maceration using methanol as solvent (Parekh et al., 2005).

2.5. Evaluation of Phytochemical Constituents of Psidium guajava, Morinda lucida and Vitellaria paradoxa

2.5.1. Test for Tannis

It was carried out based on modified method previously described by (Sumathy et al. 2011).

2.5.2. Test for Saponins

The presence of saponins was determined as described by (Prashant et al. 2011).

2.5.3. Test for Alkaloids

The presence of alkaloid was confirmed as described by (Prashant et al. 2011).

2.5.4. Test for Glycosides

Using Modified Borntager's Test as described by (Evans 2009).

2.5.5. Test for flavonoids

It was carried out using the method of (Sumathy et al. 2011).

2.5.6. Test for Phenols

The presence of phenols was determined using Prashant et al. (2011) method.

2.5.7. Test for Terpenoids

The presence of terpenoids was determined using Nasrabadi et al. (2013) method.

2.5.8. Test for Anthraquinones

It was carried out based on the method previously described by (Sumanthy et al. 2011).

2.5.9. Detection of Steroids

It was carried out based on the method previously described by)Sumanthy et al. 2011).

2.5.10. Detection of Phlobatanni

Two milliliters of extract were boiled with 5cm3 of 1% aqueous hydrochloric acid. A red precipitate showed positive test.

2.6. Quantitative Phytochemicals Analysis

Preliminary quantitative phytochemicals screening for bioactive compounds was carried out by the methods (Evans and Trease, 2009).

2.7. *Sterility Test of the Plant Extracts*
Each of the extract (methanol and distilled water extract) was tested for sterility, after sterilization by inoculating 1 mL of each extract on sterile nutrient agar and incubated at 37°C for 24h. The plates were observed for growth. No growth in the extracts after incubation indicates that the extracts were sterile.

2.8. Antimicrobial Analysis

2.8.1. Sample Preparation
There were three working samples of the concentrated extracts, 300g/ml, 150mg/ml, and 75mg/ml which were achieved in weighing 3g of extract and dissolving in sterile distilled water as solvent and then double diluting to have other working volumes.

2.8.2. The Assay Organisms
The assay organisms were bacterial group and they include Standard and clinical strains *of Staphylococcus saprophyticus DSM18669, Bacillus subtilis ATCC 6633, Staphylococcus aureus ATCC 25923, Shigella flexneri ATCC 12022, Salmonella typhi ATCC 13311, Klebsiella pneumonia, Pseudomonas aeruginosa, Escherichia coli, Listeria monocytogenes,β-haemolytic Staphylococcus aureus* obtained from the Laboratory stock cultures of Microbiology Laboratory. So, they were all standard and clinical isolates that were primarily isolated on various diagnostic and selective media to suppress other contaminants. They were then subcultured onto Mueller Hinton Agar to remove the effects of indicators and suppressive chemical agents in primary isolation media. They were then subcultured into sterile nutrient broth for optical density adjustment. Incubation periods were 24 hours for all the bacteria at 37^{o}C.

2.9. Acute Toxicity Study
The oral acute toxicity study was conducted according to the procedures of (Ugwah *et al.* 2019).

2.10. Biochemical Parameters
Biochemical parameters was determined using the method of (Zaitsev *et al.* 2020).

2.11. Hematological Examination
Hematological test was conducted according to the procedures of (Ugwah *et al.* 2019).

3.0 Result and Discussion

3.1. Phytochemical Result
Table 1 shows the qualitative phytochemical screening of methanolic extracts of *Vitellaria paradoxa, Morinda lucida, Psidium guajava* leaf revealing the presence of tannin, flavonoids, steroids, cardiac glycoside, alkaloids, phenol and anthraquinone, alkaloids, saponin, phlobatanin and terpenoids. However, few of them were absent in the individual plants.

The presence of tannins, anthraquinone, phenols, steroids and cardiac glycosides in the leaves extract of the three plants was in line with the reports of Ekom and Tamokou (2018); Fodouop *et al.* (2017) and Hossain and Nagooru (2011) who carried out the qualitative and quantitative phytochemical composition of *Psidium guajava, Vitellaria paradoxa* and *Corydyline terminalis* respectively. In addition, the study of qualitative and quantitative phytochemical analysis of *V. paradoxa, M. lucida,* and *P. guajava* leaves extract reported by Irulandi *et al.,* (2016); Ekom and Tamokou (2018) and Falana *et al.,* (2016) agreed with the result obtained in this present study due to the presence of similar phytochemicals and close values of quantitative phytochemicals. The aforementioned authors reported absence of flavonoids also which agrees with this current study. Hence, it shows presence of alkaloids, flavonoids, steroids, saponins, glycosides, terpenoids and tannins. Differences in these reports could be due to environmental factors, time of collection and handling (Akerele *et al.,* 2011). The presence of these biologically active compounds suggest that the plant could serve as potential sources of drugs and their secondary metabolites could exert some biological activities when taken by animals (Bosha *et al.,* 2018).

Table 1: Qualitative Phytochemical Screening of Methanolic Extracts 0f Different Plants

Extract Phytochemical	*Vitellaria paradoxa*	*Morinda lucida*	*Psidium guajava,*
Tannin	+	+	+
Saponin	-	+	+
Phlobatannin	-	-	+
Flavonoids	+	-	+
Steroids	+	+	+
Terpenoids	-	+	+
Cardiac Glycosides	+	+	+
Alkaloids	+	+	-
Anthraquinone	+	+	+
Phenol	+	+	+

Indication of (+) signifies the presence of the phytochemical constituent in the methanolic extract and (-) signifies the absence of phytochemical constituent.

Table 2: Quantitative Analysis Result for Some Methanolic Plant Extracts

Plant Extracts	**Tannins%**	**Phenols%**	**Flavonoids%**
Vitellaria paradoxa leaf	4.79	30.16	-
Morinda lucida leaf	6.03	31.6	74.9
Psidium guajava leaf	5.62	7.81	22.8

The diversity and abundance of these phytochemicals in the plant extracts studied suggest a complex mechanism of antimicrobial action and support the traditional use of these plants in herbal medicine. Different plants exhibit varied phytochemical profiles. For instance, *Morinda lucida leaf* is found to be high in phenolic content and high in flavonoid content, suggesting potent antioxidant properties as shown in Table 2.

3.2. Biochemical Parameter

Table 3 presents the effects of the different leaves extracts at different concentrations (high dose, medium dose and low dose) on biochemical parameters such as AST, BIL-T, Creatinine, Urea, ALT, Glucose, Globulin, ALB, Total protein, cholesterol and ALP. The current findings revealed that *P. guajava* decreased ALT and ALP; *M. lucida* increased ALT but decreased ALP, lastly *V. paradoxa* increased ALT and ALP activities in laboratory mice.

This study presents the effects of the different leave extracts at different concentrations (high dose, medium dose and low dose) on biochemical parameters such as AST, BIL-T, Creatinine, Urea, ALT, Glucose, Globulin, ALB, Total protein, cholesterol and ALP. This current study agreed with studies reported by Oyibo *et al.* (2021); Kumar and Thakur, (2018) and Gholamine *et al.* (2019) on biochemical evaluation of wistar albino rats exposed to different concentrations of several medicinal plants. The authors reported similar values of the aforementioned biochemical parameters recorded in this current study. However, there is disagreement in the study reported by Ojo *et al.* (2013) that showed *Vitellaria paradoxa* leaf to be hepatoprotective against acetaminophen toxicity and ascertain of the biochemical evaluation.

The current findings revealed that *P. guajava* decreased ALT and ALP; *M. lucida* increased ALT but decreased ALP, lastly *V. paradoxa* increased ALT and ALP activities in rats. This finding corroborates reports in which *V. paradoxa* increased the activities of these enzymes in the blood of animals (Chattopady *et al.*, 2013; Gbadegesin *et al.*, 2014). According to Oyibo *et al.* (2021), the liver is a major organ responsible for regulating several processes in the body system (Joshi *et al.*, 2015). During liver damage conditions, enzymes like ALT and AST leak into the bloodstream (Dilruba *et al.*, 2017). This suggest that the biochemical

evaluation of the mice exposed to the methanolic leave extracts of the three plants at different concentrations revealed the most effect from the high dose concentrations.

Table 3: The Biochemical Parameter of Methanolic Extract on Laboratory Mice

S/N	AST U/L	BIL-T µMOL/L	CREAT µMOL	UREA MMOL/L	ALT U/L	GLU MMOL/L	ALB G/L	T.P G/L	CHOL MMOL/L	ALP U/L
PG(HD)	174.6	1.5	33.6	6.4	67.3	3.7	46.4	62.8	1.32	181.9
PG(MD)	128.0	0.98	32.6	8.1	85.0	4.9	41.0	71.6	2.32	181.3
PG(LD)	140.8	1.03	34.8	9.2	88.6	4.1	44.1	74.7	2.49	265.6
ML(HD)	139.4	1.02	34.3	8.8	87.4	3.8	45.4	73.7	2.42	269.4
ML(MD)	121.7	1.2	33.5	7.9	85.7	4.2	42.7	72.2	1.82	180.7
ML(LD)	189.3	1.1	30.6	7.2	82.6	3.4	37.8	75.3	1.67	291.7
VP(HD)	160.2	1.3	33.4	7.8	111.4	3.3	42.8	76.1	1.98	261.3
VP(MD)	155.7	0.5	30.7	6.7	99.8	4.8	38.5	72.8	1.91	243.7
VP(LD)	115.9	0.8	34.2	8.1	84.5	5.2	43.8	75.6	1.76	189.7

KEYS: PG – *Psidium guajava*; ML - *Morinda lucida*; VP - *Vitellaria paradoxa;* HD – High dose; MD – Medium dose; LD – Low dose

AST- Aspartate aminotransferase
ALT- Alanine aminotransferase
BIL-T- Total Bilirubin
GLU- Globulin
CREAT- Creatinine
CHOL- Cholesterol
T.P- Total protein
ALB- Albumin
ALP- Alkaline phosphatase

3.3. Haematological Activity

Table 4 details the impact of methanolic extracts of *Vitellaria paradoxa, Morinda lucida, Psidium guajava* on hematological parameters in mice, such as white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HB), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), granulocytes (GRAN), and lymphocytes (Lymph%). The data shows how the extracts affect blood cell counts and hemoglobin levels, with potential implications for immune response, anemia prevention and overall blood health. Differences in dose responses across the extracts suggest varying degrees of influence on the hematological systems, highlighting the need for careful consideration in therapeutic applications.

The result on the hematological parameters of the mice exposed to different concentrations of V. paradoxa in this study was in agreement with the findings of Chineke et al. (2016) and Coles (2016). The authors recorded similar haematological indices which support our findings and there by acknowledging the effectiveness of this study. The one of M. lucida and P. guajava agreed with findings of Meyer et al. (2012) and Udeh et al. (2011) respectively in terms of the values recorded and how their various extracts affected the mice. However, some findings showed contrary opinions which include the reports from Oyibo et al., 2021, Falana et al. (2016) and Ihedioha and Chineme, (2014). They reported various values that are different from this current findings and this could be due to different plants' extracts used and processed involved in administration of the extracts to the test animals.

Table 4: The Haematology Activities of Methanolic Extract on Laboratory Mice

S/N	Wbcx10^9/L	Rbcx10^{12}/L	HB g/dl	PCV %	Mcv fL	Mch lg	Mchcg /dL	GRAN %	Lymph %
PG(HD)	11.2	6.73	10.8	26.9	40.0	16.0	40.1	44.6	26.2
PG (MD)	2.0	9.00	13.6	42.1	46.8	15.1	32.3	36.5	26.7
PG (LD)	3.6	9.51	14.2	44.5	46.8	14.9	31.9	34.6	35.2
ML(HD)	7.8	8.32	13.6	39.1	47.1	16.3	34.7	43.5	24.9
ML(MD)	3.0	3.37	11.8	37.1	41.4	14.0	31.8	42.1	18.3
ML (LD)	4.3	3.64	16.3	41.3	43.6	15.5	31.6	25.4	49.1
VP(HD)	10.3	7.51	12.8	29.6	44.2	15.4	38.3	42.7	28.5
VP(MD)	7.6	8.72	14.0	34.2	45.6	16.2	31.7	45.8	18.6
VP (LD)	5.9	9.83	13.4	36.3	42.7	15.5	31.4	43.6	15.9
Control	7.7	9.66	14.7	45.9	47.6	15.2	32.0	45.3	13.7

KEYS:PG – *Psidium guajava*; ML - *Morinda lucida*; VP - *Vitellaria paradoxa;* HD – High dose; MD – Medium dose; LD – Low dose

Wbc- White blood cells count
Rbc- Red blood cells count
HB- Hemoglobulin count
Mcv- Mean corpuscular volume
Mch- Mean corpuscular hemoglobin
PCV- Packed cell volume
Mchc- Mean corpuscular hemoglobin concentration
GRAN- Granulocytes
LYMPH- Lymphocytes

3.4. Antimicrobial Activity of Methanolic Extract of the Leaves of Psidium guajava

Antibacterial Activities of the methanolic and aqueous leave extracts of V. paradoxa, Ps. guajava and M. lucida were recorded in the present result. The antibacterial activities of methanolic and aqueous extracts of the leaves of Vitellaria paradoxa, psidium guajava and Morinda lucida were tested against ten (10) selected assay organisms (bacteria isolates). However, it is worthy to note that among the methanolic and aqueous extracts of the three plants used, only P. guajava was active against the selected bacterial isolates. The other two plants which include V. paradoxa and M. lucida did not present any activity against the selected bacteria species as shown in Table 5, 6 and 7.

Table 5 revealed results of the inhibition zone diameter (mm) of methanolic leave extract of P. guajava against selected bacterial species at different concentrations which include 75mg/ml, 150mg/ml and 300mg/ml. The antibacterial activities were observed to be highest at (300mg/ml) concentration against both Staphylococcus aereus strain, the least antibacterial activity were observed against Pseudomonas aeruginosa while Klebsiella pneumonia and Staphylococcus saprophyticus were all observed to be resistance to the extract at all concentrations. This study further shows the results of the inhibition zone diameter (mm) of aqueous leave extract of P. guajava against selected bacterial species at different concentrations which include 25%(neat), 50%(neat) and 100%(neat). The antibacterial activities were observed to be highest at (100%) concentration against Shigella flexneri strain, the least antibacterial activity were observed against Escherichia coli and S. saprophyticus while E. coli was observed to be resistance to the extract at all concentrations.

These results are in tandem with previous studies done on ethanolic leaf extracts of P. guajava that showed in vitro antibacterial activities (Ayienda 2019; Biswas et al., 2013; Vieira et al., 2011). The current study is in agreement with Ayienda (2019) who reported the antibacterial activities of dichloromethane: methanolic leaf and stem bark extracts of psidium guajava linn against selected bacteria. In a study done in Brazil, Biswas et al. (2013) and Nascimento et al. (2010) reported antibacterial activities of Methanolic guava leaf extracts against Gram positive and Gram negative bacteria. They both have contrary opinion to the result observed in this current study. In a similar work, Vieira et al. (2011) reported that aqueous guava leaf extracts inhibited growth of S. aureus, S. typhi, and B. cereus while guava sprouts were effective against E. coli. In Nigeria, Itelima et al. (2017) documented the antibacterial effects of ethanolic leaf extract of P. guajava on E. coli, whereby E. coli exhibited the highest inhibition zone of (25mm), which compared favorably with that of oxtetracycline (30mm), a commercial antibiotic. This study presents similar opinion which revealed the resistivity of E. coli. However, Chanda and Kaneria (2011), in a study done in India, found that ethanolic leaf extracts of P. guajava were not active against the Gram negative bacteria, E. coli which was in contrary with our findings.

The methanolic stem bark extracts of P. guajava also demonstrated antibacterial activities on Bacillus subtilis ATCC 6633, Staphylococcus aureus ATCC 25923, B- Haemolytic staphylococcus aureus, Listeria monocytogenes, Salmonella typhi ATCC 13311, Shigella flexneri ATCC 12022, Escherichia coli and Pseudomonas aeruginosa with zones of inhibition ranging from 11.50±0.50mm to 24.50±0.50mm. In other studies, which were in agreement with our findings, Abdelrahim et al. (2012), Arima and Danno (2012) indicated that aqueous and Methanolic root extracts of Psidium guajava were found to possess in vitro antibacterial activities on E. coli, S. aureus and S. typhi.

The reference drug used in this study, Levofloxacin, belongs to the fluoroquinolone class, which are bactericidal antibiotics. Levofloxacin was more active than the methanolic and aqueous leaf extracts of P. guajava with zones ranging from 16.17±0.29 to 35.33±0.58mm. These results were within the required standard diameter of zone of inhibition for sensitive organisms for this antibiotic, which is 19-26mm as provided by CLSI (Ayienda, 2019). Moreover, other studies have shown that standard antibiotics have more antibacterial activities than the crude plant extracts. This can be attributed to the fact that levofloxacin a

standard antibiotic, consists of pure active compounds (Kohanski et al., 2010). The average zones of inhibition observed for the plant extracts against the studied pathogens ranged from 11.50±0.50mm to 24.50±0.50mm. These determined values show that the pathogens are either resistant and /or intermediate sensitive microbes in comparison to the standard antibiotic. The low values recorded for the plant extracts may be associated to the fact that the extract, being in crude form has a mixture of phytochemicals which may not work in synergy. Previous studies have, however, recorded bioactivities of crude extracts of medicinal plants within such ranges as those found in this study (Olukuya et al., 2013; Ilori et al., 2016; Akinyemi et al., 2015).

At a concentration of 75mg/ml, both extracts recorded relatively smaller inhibition zones of 11.50±0.50mm. This could be attributed to the possibility of a slow rate of diffusion of bioactive compounds through the medium (Kokwaro, 2009). Generally, inhibition of bacteria increases with corresponding increase in the volume of plant extracts as it contains more concentration of a particular phytochemicals or group of antibacterial compounds (Ayienda, 2019).

Table 5: Inhibition Zone Diameter (Mm) of Levofloxacin Standard on Selected Bacteria Species

ASSAY ORGANISM	**50µg/ml**	**25 µg/ml**	**12.5 µg/ml**	**6.25 µg/ml**
Staphylococcus	31.00	28.50	24.00	21.50
saprophyticus DSM 18669	31.00	28.50	24.00	22.00
	30.50	28.00	24.50	21.50
Average Readings	30.83±0.29	28.33±0.29	24.17±0.29	21.75±0.35
Bacillus subtilis ATCC	28.50	26.00	23.00	21.00
6633	29.00	26.00	23.00	21.00
	28.00	26.50	23.50	21.50
Average Readings	28.50±0.50	26.17±0.29	23.17±0.29	21.17
Staphylococcus aureus	35.00	30.00	23.50	20.00
ATCC 25923	35.50	30.00	23.00	20.00
	35.00	30.50	24.00	20.50
Average Readings	35.17±0.29	30.17±0.29	23.50±0.50	20.17±0.29
B- Haemolytic	35.00	30.00	26.00	22.00
staphylococcus aureus	35.00	30.00	26.00	21.00
	36.00	31.00	26.50	22.00
Average Readings	35.33±0.58	30.33±0.58	26.17±0.29	21.67±0.58
Listeria monocytogenes	25.00	22.00	19.00	16.00
	25.00	22.00	19.00	16.00
	26.00	22.00	19.50	16.50
Average Readings	25.33±0.58	22±0.00	19.17±0.29	16.17±0.29
Salmonella typhi	24.50	23.00	20.00	17.00
ATCC 13311	25.00	22.50	20.50	17.00
	25.00	22.00	20.00	16.00
Average Readings	24.83±0.29	22.50±0.50	20.17±0.29	16.67±0.58
Shigella flexneri ATCC	29.50	22.50	19.00	14.00
12022	29.00	23.00	19.00	13.50
	29.00	23.00	19.50	13.50
Average Readings	29.17±0.29	22.83±0.29	19.17±0.29	13.67±0.29
Klebsiella pneumonia	32.50	30.00	27.50	24.50
	33.00	31.00	27.00	25.00
	32.00	30.50	27.00	25.00
Average Readings	32.5±0.50	30.50±0.50	27.17±0.29	24.83
Escherichia coli	23.00	20.00	16.50	15.50
	25.50	19.00	17.00	15.00
	25.00	19.50	17.00	16.00
Average Readings	24.50±1.32	19.50±0.50	16.83±0.29	15.50±0.50
Pseudomonas aeruginosa	18.00	15.00	12.00	0.00
	18.00	15.50	13.00	
	19.00	16.00	12.50	
Average Readings	18.33±0.58	15.50±0.50	12.50±0.50	0.00

Table 6 evaluates the antibacterial activity of methanolic leaf extract of *Psidium guajava* against various bacterial isolates at different concentrations (300mg/ml, 150mg/ml, 75mg/ml). The results indicate that *P. guajava* extract exhibits varying degrees of antibacterial efficacy, with some bacteria showing resistance at all tested concentrations, highlighting the selective antimicrobial potential of the extract.

Table 6: Inhibition Zone Diameter (Mm) of Methanolic Leave Extract of *P. guajava* on Selected Bacteria Isolates

Assay Organism	**300mg/ml**	**150mg/ml**	**75mg/ml**	**Solvent Control**
Staphylococcus saprophyticus DSM 18669	0.00	0.00	0.00	0.00
Average Readings	0.00	0.00	0.00	0.00
Bacillus subtilis ATCC 6633	17.00	13.00	12.00	0.00
	18.00	13.50	11.50	
	17.00	13.50	11.00	
Average Readings	17.33±0.58	13.33±0.29	11.50±0.50	0.00
Staphylococcus aureus ATCC 25923	24.50	22.00	19.00	0.00
	25.00	21.00	19.00	
	24.00	22.00	18.50	
Average Readings	24.50±0.50	21.67±0.58	18.83±0.29	0.00
B- Haemolytic staphylococcus aureus	18.00	16.00	14.00	0.00
	18.50	16.00	14.50	
	19.00	16.50	14.00	
Average Readings	18.50±0.50	16.17±0.29	14.17±0.29	0.00
Listeria monocytogenes	17.50	14.00	12.00	0.00
	18.00	13.50	11.50	
	17.00	13.50	11.00	
Average Readings	17.50±0.50	13.67±0.29	11.50±0.50	0.00
Salmonella typhi ATCC 13311	25.00	21.00	19.00	0.00
	24.00	21.50	19.50	
	24.50	21.00	19.50	
Average Readings	24.50±0.50	21.17±0.29	19.33±0.29	0.00
Shigellaflexneri ATCC 12022	17.50	15.00	13.00	0.00
	16.50	15.00	13.00	
	17.00	14.50	13.50	
Average Readings	17.00±0.50	14.83±0.29	13.17±0.29	0.00
Klebsiella pneumonia	0.00	0.00	0.00	0.00
Average Readings	0.00	0.00	0.00	0.00
Escherichia coli	18.00	16.00	0.00	0.00
	18.50	16.00		
	19.00	16.50		
Average Readings	18.50±0.50	16.17±0.29	0.00	0.00
Pseudomonas aeruginosa	15.00	0.00	0.00	0.00
	15.00			
	15.50			
Average Readings	15.17±0.29	0.00	0.00	0.00

3.5. Antimicrobial Activity of Aqueous Extract of the Leaves of Psidium guajava

Table 7 displays the antibacterial activity of aqueous *P.* guajava leaf extracts. It measures the antibacterial effect of aqueous guava leaf extract at different dilutions (100%, 50%, 25%) against selected bacteria, compared to a solvent control. The data demonstrate the extract's ability to inhibit bacterial growth, with effectiveness generally decreasing at lower concentrations, underscoring the aqueous extract's potential as a natural antibacterial agent.

Table 7: Inhibition Zone Diameter (Mm) of Aqueous Guava Leaf Extract on Bacteria

ASSAY ORGANISM	100% (NEAT)	50 % (NEAT)	25% (NEAT)	SOLVENT CONTROL
Staphylococcus saprophyticus DSM 18669	12.00 12.00 13.00	0.00	0.00	0.00
Average Readings	12.33±0.58	0.00	0.00	0.00
Bacillus subtilis ATCC 6633	13.00 13.00 13.50	0.00	0.00	0.00
Average Readings	13.17±0.29	0.00	0.00	0.00
Staphylococcus aureus ATCC 25923	18.00 18.00 19.00	15.00 15.50 15.00	0.00	0.00
AVERAGE READINGS	18.33±0.58	15.17±0.29	0.00	0.00
B- Haemolytic staphylococcus aureus	18.00 18.50 18.00	13.00 13.00 13.50	0.00	0.00
AVERAGE READINGS	18.17±0.29	13.17±0.29	0.00	0.00
Listeria monocytogenes	15.00 15.00 16.00	13.00 13.00 13.50	0.00	0.00
Average Readings	15.33±0.58	13.17±0.29	0.00	0.00
Salmonella typhi ATCC 13311	14.00 14.50 14.00	0.00	0.00	0.00
Average Readings	14.17±0.29	0.00	0.00	0.00
Shigella flexneri ATCC 12022	20.00 20.00 20.50	17.00 17.00 18.00	15.00 15.00 16.00	0.00
Average Readings	20.17±0.29	17.33±0.58	15.33±0.58	0.00
Klebsiella pneumonia	18.00 18.00 18.50	15.00 15.00 15.50	0.00	0.00
Average Readings	18.17±0.29	15.17±0.29	0.00	0.00
Escherichia coli	0.00	0.00	0.00	0.00
Average Readings	0.00	0.00	0.00	0.00
Pseudomonas aeruginosa	17.00 17.00 18.00	0.00	0.00	0.00
Average Readings	17.33±0.58	0.00	0.00	0.00

3.5. *Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)*

Tables 8 and 9 provide MIC and MBC values for *P.* guajava against the tested bacteria, with values indicating the lowest concentrations necessary to inhibit and kill the bacterial strains, respectively. These tables are crucial for understanding the potency of the extracts and guiding dosages for potential therapeutic applications.

Table 8: Minimum Inhibitory Concentration Values (mg/ml) of Methanolic Guava Leaf Extract on Responsive Organisms

ASSAY ORGANISM	0.0025MG/	0.005MG/ML	0.01MG/ML	0.02MG/ML	0.04MG/ML	0.08MG/ML	0.16MG/ML	0.32MG/ML	0.64MG/ML	1.28MG/ML	2.56MG/ML	5.12MG/ML	10.24MG/ML	MINIMUM INHIBITORY CONCENTRA-TION VALUE (MG/ML)
CONCENTRATION CODE ON SAMPLE BOTTLE	A	B	C	D	E	F	G	H	I	J	K	L	M	
Staphylococcus saprophyticus DSM18669	+	+	+	+	+	+	+	+	+	-	-	-	-	1.28
Bacillus subtilis ATCC 6633	+	+	+	+	+	+	+	+	-	-	-	-	-	0.64
Staphylococcus aureus ATCC 25923	+	+	+	+	+	+	+	+	-	-	-	-	-	0.64
B- Haemolytic staphylococcus aureus	+	+	+	+	+	+	+	+	+	-	-	-	-	1.28
Listeria monocytogenes	+	+	+	+	+	+	+	+	+	-	-	-	-	1.28
Salmonella typhi ATCC 13311	+	+	+	+	+	+	+	-	-	-	-	-	-	0.32
Shigellaflexneri ATCC 12022	+	+	+	+	+	+	+	-	-	-	-	-	-	0.32
Klebsiella pneumonia	+	+	+	+	+	+	+	+	+	-	-	-	-	1.28
Escherichia coli	+	+	+	+	+	+	+	+	+	-	-	-	-	1.28
Pseudomonas aeruginosa	+	+	+	+	+	+	+	+	+	-	-	-	-	1.28

Table 9: Minimum Bactericidal Concentration Values (Mg/Ml) of Methanolic Guava Leaf Extract on Responsive Organisms

ASSAY ORGANISM	0.0025MG/ ML	0.005MG/ML	0.01MG/ML	0.02MG/ML	0.04MG/ML	0.08MG/ML	0.16MG/ML	0.32MG/ML	0.64MG/ML	1.28MG/ML	2.56MG/ML	5.12MG/ML	10.24MG/ML	MINIMUM BACTERICIDAL CONCENTRA-TION VALUE (MG/ML)
CONCENTRATION CODE ON SAMPLE BOTTLE	A	B	C	D	E	F	G	H	I	J	K	L	M	
Staphylococcus saprophyticus DSM18669	+	+	+	+	+	+	+	+	+	+	+	-	-	5.12
Bacillus subtilis ATCC 6633	+	+	+	+	+	+	+	+	+	+	-	-	-	2.56
Staphylococcus aureus ATCC 25923	+	+	+	+	+	+	+	+	+	+	-	-	-	2.56
B- Haemolytic staphylococcus aureus	+	+	+	+	+	+	+	+	+	+	+	-	-	5.12
Listeria monocytogenes	+	+	+	+	+	+	+	+	+	+	+	-	-	5.12
Salmonella typhi ATCC 13311	+	+	+	+	+	+	+	+	+	-	-	-	-	1.28
Shigella flexneri ATCC 12022	+	+	+	+	+	+	+	+	+	-	-	-	-	1.28
Klebsiella pneumonia	+	+	+	+	+	+	+	+	+	+	+	-	-	5.12
Escherichia coli	+	+	+	+	+	+	+	+	+	+	+	-	-	5.12
Pseudomonas aeruginosa	+	+	+	+	+	+	+	+	+	+	+	-	-	5.12

3.7. *Acute Toxicity Study*

The acute oral toxicity test was determined using ranges of low dose (10,000mg/kg), medium dose (15,000mg/ml) to a high dose (20,000mg/kg) of the methanolic extract. Oral administration of the extracts to the mice was observed to neither show signs of mortality, behavioural changes nor physical changes. Hence, the observation from the oral acute toxicity study suggests that the methanolic extract were safe for oral administration.

4.0. Conclusions

Among the three plants (*Vitellaria paradoxa, Psidium guajava* and *Morinda lucida*) understudied in this current research, *Psidium guajava* at different concentration showed more pharmacological activities as observed in the biochemical and haematological indices of the test animal (mice). The methanolic extracts of *Vitellaria paradoxa, Morinda lucida,* and *Psidium guajava* leaf revealed the presence of tannin, flavonoids, steroids, cardiac glycoside, alkaloids, phenol and anthraquinone, alkaloids, saponin, phlobatanin and terpenoids, which make the plants useful pharmacologically. This study proves that the methanolic extracts of the three plants are beneficial to the body system of the mice. Also, for the antimicrobial activities, only methanolic and aqueous extracts of *P. guajava,* among the three plants, and the antibiotics (levofloxacin) were active against the bacterial isolates used in this study. No serious or life-threatening side effects were observed on the hematological and biochemical parameters of mice exposed to different concentrations of *P. guajava, V.paradoxa* and *M. lucida* extracts used in this work. There was also no death or side effects associated with the acute toxicity study, but there were significant increases in the activities of some serum enzymes investigated.

References

Abdelrahim, S. I., Almagboul, A. Z., Omer, M. E. A. and Elegami, A. (2012). Antimicrobial activity of *Psidium guajava* L. *Fitoterapia*, **73**(7): 713-715.

Agunu, A. Ahmadu, A.A., Afolabi, S.O., Yaro, A.U., Ehinmidu, J.O. and Mohammed, Z. (2011). "Evaluation of the antibacterial and antidiarrhoeal activities of *Heeria insignis*." *Indian Journal of Pharmaceutical Sciences*, **73**(3): 328–332.

Akerele, J. O., Ayinde, B. A. and Ngiagah, J. (2011). Phytochemical and antibacterial and eyes. *Tropical Journal of Pharmaceutical Research,* **10**(2): 211-218.

Aneela, S., De, S., Kanthal, L.K., Choudhury, N.S., Das, B.L. and Sagar, K.V. 2011. Acute oral toxicity studies of *Pongamia pinnata* and *Annonas quamosa* on albino wistar rats. *International Journal of Research in Pharmacy and Chemistry*, **1**(4):820-824.

Arima, H. and Danno, G. I. (2012). Isolation of antimicrobial compounds from guava (*Psidium guajava* L.) and their structural elucidation. *Bioscience, Biotechnology, and Biochemistry*, **66**(8): 1727-1730.

Arunkumar, S. and Muthuselvam. (2009). Analysis of phytochemical constituents and antimicrobial activities of aloe vera L. against clinical pathogens. *World J. Agril. Sci*, **5**(5): 572-576.

Awoyinka, O.A., Balogun I.O. and Ogunnowa, A.A. (2007). Phytochemical screening and invitro bio activity of cnidoscolus asconiti folus (Europhiaceae). *Journal of Medicinal Plant Res,* **3**: 63-65.

Ayienda, C. K. (2016). Antibacterial activities of dichloromethane: methanolic leaf and stem bark extracts of *psidium guajava* linn against selected bacteria. A Thesis Submitted in Partial Fulfillment of the Requirements for the Award of the Degree of Master of Science (Medical Biochemistry) in the School of Pure and Applied Sciences of Kenyatta University.

Biswas, B., Rogers, K., McLaughlin, F., Daniels, D. and Yadav, A. (2013). Antimicrobial activities of leaf extracts of guava (*Psidium guajava* L.) on two Gram-negative and Gram-positive bacteria. *International Journal of Microbiology*, **43**(6): 102-112.

Bosha, J. A., Anaga, A. O. and Asuzu, I. U. (2018). Chemical Composition of *Newbouldia Laevis* (P. Bauv) Used in the Treatment of Diabetes Mellitus in Nigeria. Journal of Nutrition, **1**: 105

Chanda, S. and Kaneria, M. (2011). In vitro antioxidant property of some Indian medicinal plants. *Journal of Ethnopharmacology*, **5**(2): 169-179.

Chattopady, P., Sampa, D. M., Ghosh, I. J. (2013). Effect of dietary co-administration of sodium-selenite on sodium arsenite -induced ovarian and uterine disorders in mature albino rats, *Toxicology*, **75**: 412–422.

Chineke, C. A., Ologun, A. G. and Ikeobi, C. O. (2016). Haematological parameters in rabbit breeds and crosses in humid tropics. *Pakistan Journal of Biological Sciences*, **9**(11): 2102-2106.

Coles, E. H. (2016). Veterinary clinical pathology. W.B. Saunders Company, Philadelphia, 65pp.

Dilruba, M. M., Hasibuzzaman, M., Rahman, N. C., Mohanto, S., Aktar, A., Rahman, M. I. and Hossain, A. M. (2017). Ameliorating effects of Raphanus sativus leaves on sodium arsenite-induced perturbation of blood induced in Swiss albino mice, Asian Pacific Journal of Tropical Biomedicine, **7**(10): 915–920.

Ecobichon Ansari, S.H. *Essential of pharmacognosy*. 1st edition, New Delhi: Birla Publications Pvt. Ltd., 2007.

Ekom, S. E. and Tamokou, J. D. (2018). Methanol Leaves Extract of *Psidium guajava* Linn. Exhibited Antibacterial and Wound Healing Activities. *International Journal of Current Microbiology and Applied Science*, **7**(7): 4008-4023.

Esposito, F., Carli, I., and Del Vecchio, C. (2016). "Sennoside A, derived from the traditional Chinese medicine plant rheum L., is a new dual HIV-1 inhibitor effective on HIV-1 replication." *Phytomedicine*, **23**(12): 1383–1391.

Evans, W.C. and Trease, D. (2009). Pharmacognosy (15th Edition), London: WB Sacunders company Ltd.

Falana, M. B., Bankole, M. O., Ojo, D. A. and Omemu, A. M. (2016). Haematological parameters of Diarrhoea Induced Albino rats Treated with Leaf, bark and root of *V. paradoxa* extracted using four solvents. *International Journal of Multidisciplinary Research and Development*, **3**(2): 220-224.

Frederick, N. and Mani, J.V. (2016). Phytochemical screening and In-Vitro Antioxidant activity of different solvent extracts of *Musa rasthali* fruit. *International Journal of Chemical and Physical Sciences*, **5**(3):6-9.

Gbadegesin, A. M., Adegoke, E. G., Ewere, O. A. and Odunola, A. (2014). Hepatatoprotective and anticlastogenic effects of ethanol extract of *Irvingia gabonensis* (IG) leaves in sodium arsenite induced toxicity in male wistar rats, *Nigerian Journal of Physiological Science*, **29**: 029–036.

Gholamine, G., Houshmand, A., Hosseinzadeh, M., Kalantar, S., Mehrzadi, M. and Goudarzi, L. (2019). Gallic acid ameliorated sodium arsenite-induced renal and hepatic toxicity in rats. *Drug Chemistry and Toxicology*, **25**: 1–12.

Hossain, M. A. and Nagooru, M. R. (2011). Biochemical profiling and total flavonoids and contents of leaves crude extract of endemic medicinal plants *Corydyline terminalis* L Kunth. *Pharmacognosy Journal*, **3**(24): 25-29.

Ihedioha, J. I. and Chineme, C. N. (2014). Fundamentals of systemic veterinary pathology, Great AP Express Publishers Ltd., Nsukka, 128pp.

Irulandi, K., Geetha, S. and Mehalingam, P. (2016). Antioxidant, antimicrobial activities and phytochemical analysis of leaves extracts of *Dioscorea wallichii* Hook.f. *Journal of Applied Pharmaceutical Science*, **6**(11): 070-074.

Itelima, J. U., Agina, S. E. and Pandukur, S. G. (2017). Antimicrobial activity of selected plant species and antibiotic drugs against *Escherichia coli* O157: H7. *African Journal of Microbiology Research*, **11**(20): 792-803.

Joshi, B. C., Prakash, A. N. and Kalia, A. (2015). Hepatoprotective potential of antioxidant potent fraction from *Urtica dioica* Linn. (whole plant) in CCl4 challenged rats. *Toxicology Representative*, **2**: 1101–1110.

Kohanski, M. A., Dwyer, D. J. and Collins, J. J. (2010). How antibiotics kill bacteria: from targets to networks. *Nature Reviews Microbiology*, **8(**6): 423.

Krishnaiah, D., Sarbatly, R. and Bono, A. (2007). Phytochemical antioxidants for health and medicine: A move towards nature. *Biotechnology Molecular Biology Review*, **1:** 97-104.

Kumar, R. and Thakur, M. (2018). Syzygium cumini seed extract ameliorates arsenic-induced blood cell genotoxicity and hepatotoxicity in wistar albino rats. *Biochemical and Molecular Biology*, **7**(1): 110–118.

Meyer, D. J., Coles, E. H. and Rich, L. J. (2012). Veterinary laboratory medical interpretation and diagnosis. W.B. Saunders Company, Philadelphia, 13–40pp.

Nascimento, G. G., Locatelli, J., Freitas, P. C. and Silva, G. L. (2010). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian Journal of Microbiology*, **31**(4): 247-256.
Nasrabadi, M., Halimi, M. and Nadaf, M. (2013). Phytochemical screening and chemical composition of extract of Muscari neglectum. *Middle –East Journal of Science Research*, **14**(4): 566-599.

Nayak, B., Liu, R.H. and Tang, J. (2015). "Effect of processing on phenolic antioxidants of fruits, vegetables, and grains-a review." *Critical Reviews in Food Science and Nutrition*, **55**(7): 887–918.

Organization for Economic Co-operation and Development (OECD) 2014. Guidelines for the testing of new chemicals revised draft guideline; acute and subacute oral toxicity. http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/OECD/ OECDtg425.pdf.

Ojo, M. S., Nadro, I. O. and Tella, K. (2006). Protection of rats by extracts of some cpmmon Nigeria trees against acetaminophen – induced hepatotoxicity. *African Journal of Biotechnology*, **5:** 755–760.

Oyibo, A., Michael, A., Gbadegesin, H. and Oyeronke, A. O. (2021). Ethanol extract of *Vitellaria paradoxa* (Gaertn, F) leaves protects against sodium arsenite - induced toxicity in male wistar rats. *Toxicology Reports*, **8**: 774–784.

Parekh, J., Nair, R. and Chandas, O. (2005). Preliminary screening of some folklore plants from western India for potential antimicrobial activity. *Ind.J.Pharma Col* **37**,: 408-409.

Prashant, T. (2011). Phytochemical screening and extraction: A review, *Internationale pharmaceutica sciencia*, **1**: 98-106.

Sumathy, V., Jothy Lachumyl, S., Zakaria, Z. and Sasidharan, S. (2011). "In-vitro bioactivity and phytochemical screening of *Musa acuminate* flower". *Pharmacologyonline*, **2**:118-127.

Udem, C. S., Madubuike, U. and Anyanwu, R. (2011). The effects of Psidium guajava Linn. (Myrtaceae) leaf chloroform extract on some hematological and biochemical parameters in mice. Comparative Clinical Pathology, **20**: 47–51.

Ugwah-Oguejiofor, C. J., Okoli, C. O., Ugwah, M. O., Ogbulie, C. S., Mshellia, H. E., Umar, M. and Njah, A. A. (2019). *Heliyo*, **5(1)**: e01179.

Vieira, R., Rodrigues, D., Gonçalves, F. A., Menezes, J. S. and Sousa, O. V. (2011). Microbiocidal effect of medicinal plant extracts (*Psidium guajava* Linn. and *Carica papaya* Linn.) upon bacteria isolated from fish muscle and known to induce diarrhea in children. *Journal of Tropical Medicine*, **43**(3): 145-148.

Zaitsev, S. Y., Bogolyubova, N. V., Zhang, X. and Bienig, B. (2020). Biochemical parameters, dynamic tensiometry and circulating nucleic acids for cattle blood analysis: A review. *Peer J Life and Environment*, **8**: e8997

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