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Research Article

Antibacterial Activity of *Phyllanthus Amarus* (Schum and Thonn) Extract Against *Salmonella Typhi* Causative Agent of Typhoid Fever

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Abstract: The study was conducted to assess the antibacterial activity of *Phyllanthus amarus (*Schum and Thonn) extract against *Salmonella typhi* causative agent of typhoid fever at the laboratories of the Departments of Chemistry and Theoretical and Applied Biology of the College of Science, Kwame Nkrumah University of Science and Technology, Kumasi. The objectives were to determine the highest yield of crude extract of *P. amarus* using different proportions of water to ethanol and to determine the sensitivity of *Salmonella typhi* to these. Three different extraction procedures were carried out. In the first procedure, seven extraction setups each containing different proportions of the two extract (water and ethanol) were used with 10g of the plant sample. In the second procedure, eight setups were used for the two solvents. Ten grams of both fresh and dry plant sample were extracted in two different 200ml of water and in another two different 200ml of water; 20g of both fresh and dry plant sample were again extracted. The same procedure was repeated using ethanol as the solvent. In the third procedure, 10g each of fresh plant sample were boiled in 100ml and 200ml of water for 30 minutes. A sensitivity test to determine the zones of inhibition for the various plant extracts was done on *Salmonella typhi* isolated from human. Results from the crude yield of *P. amarus* using water only had the highest crude yield of 2.57g, followed by ethanol only which was 2.52g. The sensitivity studies conducted on the fresh *P. amarus* indicated that aqueous extract of *P. amarus* inhibited *S. typhi* to a zone of 5.00mm in 10g/200ml and 7.17mm in 20g/200ml. Ethanol extract also recorded an inhibition zone of 2.67mm and 5.33mm in 10g/200ml and 20g/200ml respectively. Again, sensitivity studies using dry *P. amarus* samples showed that the aqueous extracts recorded a zone of inhibition of 7.33mm in 10g/200ml and 13.50mm in 20g/200ml. Also ethanol extracts also recorded an inhibition zone of 6.83mm in 10g/200ml and 10.50mm in 20g/200ml. Significant differences were observed among the extracts and the control in both 10g/200ml and 20g/200ml concentrations ($P<0.05$). Aqueous and ethanol extracts of *P. amarus* proved inhibitory to *S. typhi*.

Keywords: *Phyllanthus Amarus*, *Salmonella Typhi*, Extract, Zone Of Inhibition

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1. Introduction

Medicinal plants have been used for the treatment of several human diseases over the century and have been very important in the health care delivery of every nation at one stage or the other [1]. Recent research has focused on natural plant product as alternatives to the existing drugs for disease remedy in developing countries [2]. Plant derived medicines have been part of traditional health care in most parts of the world for ages and there is increasing interest in them as sources of agents to fight microbial diseases [3-5].

The development of multiple antibiotic resistant organisms has constituted a global problem as far as treatment of some infectious diseases is concerned. Typhoid fever caused by *Salmonella typhi* has since 1989 developed simultaneously, resistance to conventional antibiotics of choice in several endemic areas [6].

The vehicles of transmission of this etiologic agent are mainly food and water. Several other disease-causing organisms of medical importance have also developed resistance to these conventional antibiotics.

Infectious diseases still remain an important cause of morbidity and mortality in man, especially in developing countries. Today, in Africa, many resort to the use of locally made herbal medicines prepared as infusions in hot water, decoction in cold water, concoction with food and as tinctures with alcohol as an alternative therapy for bacterial infections [7]. Plant parts such as the roots, leaves, shoots, barks, fruit peels, immature and unripe fruits have been used in most herbal preparations.

According to George and Pamplona-Roger, the therapeutic value of some common plants such as *Anthocleista vogelii* Planch, *Morinda lucida*, *Triplochiton scleroxylon*, *Alchornea cordifolia*, *Cassia sieberiana*, *Mangifera indica*, *Anacardium occidentale*, *Nauclea latifolia*, *Daniela oliveri,Citrus paradise*, *Ananas sativus* and *Carica papaya* have been used in the treatment of various ailments including enteric fever, diarrhoea, dysentery, malaria, common cold, convulsion, yellow fever, jaundice, dental caries, intestinal parasites, gastroenteritis, bacterial, viral and protozoan diseases [8]. Antiseptic, diuretic, antibacterial and anti-inflammatory properties have equally been reported [9].

Herbal medicine is readily available in our diverse vegetation, cheap and above all carries the potential for introducing new templates into modern medicine [10]. In many parts of the world, including Ghana, herbal medicine practitioners are still consulted as a first choice in the treatment of ailments, due to the fact that traditional medicine blends readily with the socio-cultural life of the people, and the fact that orthodox medicine are more expensive to procure and some orthodox pharmaceutical preparations are many times faked [11]. The use of water as the sole solvent yielded the highest crude extract of 2.57g. Ethanol only also yielded the second highest crude extract of 2.52g. A fraction of water/ethanol (3:2) yielded 2.37g of crude extract. Also a fraction of water/ethanol (7:3) yielded 2.21g of crude extract. 2.14g of crude extract was recorded by a fraction of water/ethanol (9:1) while a fraction of water/ethanol (4:1) yielded 2.01g of crude extract.

There is a vast array of medicinal plants used singly or in combination with other medicinal plants that confer synergistic effect in the treatments of various ailments.

The study was conducted to assess an extract against *Salmonella typhi* causative agent of typhoid fever at the laboratories of the Departments of Chemistry and Theoretical and Applied Biology of the College of Science, Kwame Nkrumah University of Science and Technology, Kumasi.

A Completely Randomised Design with three replications was used.

The study was guided by these research questions:

1. Determine the yield of the crude extract of *Phyllanthus amarus* using different proportions of water: ethanol
2. Determine the sensitivity of *Salmonella typhi* to the various concentrations of the crude extract of *P. amarus*

1.1. Description of Phyllantus Amarus

Phyllantus amarus is a small erect tropical herb that grows to a height of 10-60cm. It is an annual and widespread throughout the tropics and subtropics. It is found in sandy regions as a weed in cultivated and wastelands. The plant is a common tropical weed that grows well in moist, shady and sunny places [12]. Some common names of *Phyllanthus* *amarus* in North, Central and South America are black catnip, carry-me seed, egg woman, hurricane weed, quinine creole, quinine weed, seed-under-leaf, stone breaker among others [13]. The name 'Phyllanthus' means "leaf and flower" since both the flower as well as the fruit seems to come one with the leaf [14].

P. amarus is a member of the Euphorbiaceae family (Spurge family), with over 6500 species in 300 genera. Euphorbiaceae family consist of upright or prostrate herbs or shrubs, often with milky acrid juice [15]. And is mainly a pan-tropical family with some species either more or less temperate. Numerous species of this family are native to North, Central and South America [16]. The plants are monoecious or monogamous. It has smooth cylindrical stem (1.5-2mm) thick and deciduous horizontal branch lets (4-12cm) long and 0.5cm thick, with 15 to 30 leaves. The leaves are simple, alternate or opposite and leathery, and borne on petioles 0.3 to 0.5 mm long. The flowers are very small and diclinous, often in clusters borne in greenish cup-shaped structures with glands. The fruit is a three-lobed capsule containing six seeds and extends from the cup with a long stalk pendant about 1-2mm [15]. The seeds are triangular (like an orange segment), light brown, 1mm long, with 5-6 ribs on the back and the seeds are hurled away when the fruits burst open [13].

Figure 1. Phyllantus amarus

1.2. Pathology of Salmonella typhi

Salmonella typhi has killed over 600,000 people annually all over the world [17]. It is a deadly bacterial disease that causes typhoid fever and is transmitted through food and water. It has become an epidemic in South Asian countries where sanitation is lacking. *S. typhi* usually invades the surface of the intestine in humans, but have developed and adapted to grow into the deeper tissues of the spleen, liver, and the bone marrow. Symptoms most characterized by this disease often include a sudden onset of a high fever, a headache, and nausea. Other common symptoms include loss of appetite, diarrhoea, and enlargement of the spleen (depending on where it is located). *S. typhi* involves colonization of the reticuloendothelial system. Some individuals who are infected with *S. typhi* become life-long carriers that serve as the reservoir for these pathogens. *S typhi* has an endotoxin (which is typical of Gram negative organisms), as well as the VI antigen, which increases virulence. It also produces a protein called invasion that allows non-phagocytic cells to take up the bacterium and allows it to live intra cellular. *S. typhi* is a strong pathogen for humans due to its resistance to the innate immune response system [17].

Recently, strains of MDR (multi-drug resistant) Salmonella have been identified and grouped together in a single haplotype named H58. It has been found that these strains are now resistant to nalidixic acid and have reduced susceptibility to fluoroquinolones.

This strain has been recently found in Morocco, which shows that the MDR strain has reached as far as Africa [18].

1.3. Current Research on Salmonella typhi

Much research is going on since the global emergence of multi drug resistant strains. In India, samples of 21 *Salmonella typhi* strains were tested for their vulnerability to antimicrobial agents. Three different antibiotics were tested; chloramphenicol (256mg/l), trimethoprim (64mg/l), and amoxicillin (>128mg/l). Eleven of the *S. typhi* strains were resistance to chloramphenicol, trimethoprim and amoxicillin. Four of the isolates were resistant to all of them except for amoxicillin. Six of the isolates were completely sensitive to all of the antimicrobial agents tested. All the *S. typhi* isolates were susceptible to cephalosporin agents, gentamicin, amoxicillin plus clavulanic acid, and imipenem. The genes responsible for the resistance of the antibiotics included chloramphenicol acetyltransferase type I, dihydrofolate reductase type VII, and TEM-1 β-lactamase. Pulsed-field gel electrophoresis analysis of XbaI-generated genomic restriction fragments identified a single distinct profile (18 DNA fragments) for all of the resistant isolates. After comparing this, six different profiles were recognized among the sensitive isolates. It was found that a single strain containing a plasmid having multi drug-resistance has emerged in the *S. typhi* population and has been able to adapt and survive the antibiotics as they are introduced into clinical medicine [19].

With the current spread of Salmonella, researchers are looking for easier ways to detect typhoid fever in order to better treat patients. Another project has to do with dipstick assay which detects antibodies and analyses the effect of typhoid fever in patients. It found specific IgM antibodies on patients in 43.5%, 92.9%, and 100% for samples collected 4-6 days, 6-9 days, and greater than 9 days after the onset of fever, respectively, the number of antibodies increasing during the length of the duration. Testing of serum samples from culture negative patients with a clinical diagnosis of typhoid fever resulted in staining of the dipstick in 4.3% of the samples collected on the day of admission and in 76.6% one week later. This shows the late development of antibodies in the blood for a large number of patients. The advantages of the dipstick assay are that the result can be obtained on the same day allowing a prompt treatment. No special laboratory equipment is really needed to perform the assay and one would only need a small amount of serum. What makes it even better is that the simplicity of the assay would allow it to be used in places that lack laboratory facilities, such as third world countries that lack modern facilities and where disease is running high [20].

More people have taken a greater interest in *Salmonella typhi* since the decreasing effects of antibiotics. In 2006, more research was done in order to find the global gene expression by *Salmonella typhi* during infection. Global expression profiles of *S. typhi* grown *in vitro* and within macrophages at different time points were obtained and studied. Virulence factors, such as the SPI-1- and SPI-2-encoded type III secretion systems, were found as expected during infection by Salmonella. The results concluded that *S. typhi* had an increased expression of genes encoding resistance to antimicrobial peptides, which used the glyoxylate bypass for fatty acid utilization, and did not induce the SOS response or the oxidative stress response. It was also found that genes coding for the flagella apparatus, chemo taxis, and iron transport systems were down-regulated in vivo. This experiment allowed a better understanding of Salmonella and found a safer and more effective way to determine the bacterial transcriptome *in vivo*. This could possibly lead to the investigation of transcriptional profiles of other bacterial pathogens without the need to recover much bacterial mRNA from the host [21].

Typhoid fever is an acute illness associated with fever that is most often caused by the *Salmonella typhi* bacteria. It can also be caused by *Salmonella paratyphi*, a related bacterium that usually leads to a less severe illness. The bacteria are deposited in water or food by a human carrier and are then spread to other people in the area. Typhoid fever is rare in industrial countries but continues to be a significant public-health issue in developing countries [22, 23].

1. Materials and Methods

The study was conducted at the laboratories of the Departments of Chemistry and Theoretical and Applied Biology, College of Science, Kwame Nkrumah University of Science and Technology, Kumasi.

2.1. Source of Plant Materials

The plant used for the study was *Phyllanthus amarus*. Plant samples were collected from Kwame Nkrumah University of Science and Technology, Kumasi campus and Saint Louis College of Education campus, all in the Kumasi Metropolis. It was observed that the plant was shade loving and grew best in moist areas and sandy soils, especially at the edges of concrete floors. Identification of plant sample collected was done at the Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology, Kumasi.

2.2. Preparation of Plant Samples

Plant materials collected were washed under running tap water and were allowed to drain before air drying under shade for two weeks. The roots were separated from the leaves and the stem, because that is what is traditionally done. The leaves together with the stem and the small branches were then grinded mechanically with mortar and pestle.

2.3. Extraction Procedure

Three different extraction procedures were carried out. The first extraction process consisted of using different proportions of the two main extraction solvents namely water and ethanol. The ratios of water to ethanol used for the proportions are presented in Table 1.

Table 1. Proportions of water to ethanol used in extraction

Label	Ratio	Description
W9E1	9:1	90% water : 10% ethanol (180ml of water to 20ml of ethanol)
W4E1	4:1	80% water : 20% ethanol (160ml of water to 40ml of ethanol)
W7E3	7:3	70% water : 30% ethanol (140ml of water to 60ml of ethanol)
W3E2	3:2	60% water : 40% ethanol (120ml of water to 80ml of ethanol)
W1E1	1:1	50% water : 50% ethanol (100ml of water to 100ml of ethanol)
W1	1	100% water (200ml of water)
E1	1	100% ethanol (200ml of ethanol)

Ten grams (10g) of the *P. amarus* plant samples was placed into each of the seven flasks containing the different proportions of the two extraction solvents. The flasks were then connected to a setup consisting of Soxhlet Extractor Apparatus and a condenser. Power was applied for the extraction process to begin.

In the second extraction process, eight extraction setups were used for the two solvent (water and ethanol), four for each solvent. For water extraction, 10g of both fresh and dry *P. amarus* plant samples were extracted in 200ml of water and another 20g of both fresh and dry P. amarus plant sample was also extracted in 200ml of water. The same procedure was repeated for ethanol extraction.

In the third extraction process, 10g of fresh *P. amarus* plant sample was boiled in 100ml and 200ml of water for 30minutes.

2.4. Isolation of Salmonella From Human Faeces

2.4.1. Preparation of Buffered Peptone Water

Twenty grams (20g) of powdered peptone water was weighed and dissolved in 1 litre of distilled water in an Erlenmeyer flask. The solution was thoroughly mixed before distributing into universal bottles. The universal bottles with their contents were sterilized by autoclaving at 121 ℃ for 15 minutes.

2.4.2. Preparation of Non-Selective Pre-Enrichment Medium:

Twenty-five grams (25g) of faeces was weighed and placed into an Erlenmeyer flask. 225ml of buffered peptone water was added to the Erlenmeyer flask to obtain 1 part sample and 9 parts buffered peptone water. This was thoroughly mixed and incubated at 370 C overnight (16-20 hours).

2.4.3. Preparation of Selective Enrichment Medium

2.4.3.1. Selenite broth preparation

Twenty-three grams (23g) of selenite broth powder was suspended in 1 litre of distilled water. The suspension was thoroughly mixed before heating to boiling point. The resulting solution was then distributed into universal bottles using syringe.1ml of the non-selective pre-enrichment medium was transferred with a pipette into 10ml of the selenite broth and incubated at 44°C for 48 hours.

2.4.3.2. SS agar preparation

Fifty-two grams (52g) of SS agar powder were suspended into one litre of distilled water. The solution was boiled until completely dissolve. 10ml of the resulting solution was poured unto sterile petri dishes (plates) and allowed to solidify.

2.4.3.3. Inoculation of Salmonella on Selective Agar Plates

Using an inoculating loop, streaks from the selenite broth were made on the solidified SS agar on the plate. The inoculated plates were then incubated at 37 C overnight (18-24 hours). Black colonies on the SS agar were seen after 48 hours confirming the presences of *Salmonella typhi*.

2.4.3.4. Storing of *Salmonella typhi*

Black colony of *Salmonella typhi* identified was washed with 10ml distilled water before adding to a selective SS agar medium in a universal bottle. This was incubated at 37°C for 48hours before storing in a refrigerator for future use.

2.5. Experimental Design

A Completely Randomised Design with three replications was used. Three sets of experiment were carried out. In the first experimental setup, different ratios of the extraction solvent were used to determine which combination gives the highest plant extract. In the second experimental setup, sensitivity of *S. typhi* to fresh plant extract was investigated. The treatments used consisted of (i) boiled plant sample in water, (ii) crude extract of plant with water, (iii) crude extract of plant with ethanol and (iv) a control treatment consisting of a standard antibiotic (Chloramphenicol tablet).

In the third experiment, sensitivity of *S. typhi* to dry plant sample was investigated. The treatments used consisted of (i) crude extract of plant with water, (ii) crude extract of plant with ethanol and (iii) a control treatment consisting of a standard antibiotic (Chloramphenicol tablet).

2.6. Sensitivity Test

To determine the effect of the plant extracts and chloramphenicol on the test organisms, a disc diffusion technique using the Kirby- Bauer method was applied in testing pure cultures of the test organism for their antimicrobial sensitivities based on zones of inhibition on agar plates [46]. In this method, circular discs from filter paper were sterilized in a hot air oven for 1hour. The discs were then impregnated by soaking with each plant extract and air-dried for a few minutes. 1ml of *Salmonella typhi* culture suspension was placed onto solidified SS agar plates using a sterile micropipette. A glass spreader was used to evenly distribute the test suspension on the SS agar. A sensitivity disc of each plant extract and the standard chloramphenicol disc of 10μg potency were aseptically transferred onto the SS agar plates using a sterilized forceps. The plates were labelled accordingly. The plates were then incubated at 37°C for 24-48 hours. The plates were later observed for zones of inhibition.

2.7. Statistical Analysis

The data collected were subjected to analysis of variance (ANOVA) using Statistic 9 statistical package. Differences in means were done using the least significant difference (LSD) at P=0.05. The results were expressed as mean ± standard error of mean (S.E.M.).

3. Results

3.1. Yield of Crude Extract of Phyllanthus Amarus

Table 2 indicates the yield of crude plant extract from different fractions of extraction solvent. Using water as the sole solvent yielded the highest crude extract of 2.57g. Ethanol only also yielded the second highest crude extract of 2.52g. A fraction of water/ethanol (3:2) yielded 2.37g of crude extract. Also a fraction of water/ethanol (7:3) yielded 2.21g of crude extract. 2.14g of crude extract was recorded by a fraction of water/ethanol (9:1) while a fraction of water/ethanol (4:1) yielded 2.01g of crude extract. However, a fraction of water/ethanol (1:1) yielded the lowest crude extract of 1.05g.

Table 2. Yield of crude plant extract from different fractions of extraction solvent.

Proportion of extraction solvent	Ratio	Yield of plant extract (g)
Water/Ethanol (90:10)	9:1	2.14
Water/Ethanol (80:20)	4:1	2.01
Water/Ethanol (70:30)	7:3	2.21
Water/Ethanol (60:40)	3:2	2.37
Water/Ethanol (50:50)	1:1	1.05
Water only	1	2.57
Ethanol only	1	2.52

Sensitivity of Fresh *Phyllanthus Amarus* to Salmonella Typhi: Sensitivity of *S. typhi* to fresh *P. amarus* sample in 10g/200ml is presented in Table 3. The results showed that water extract of fresh *P. amarus* was able to suppress activities of *S. typhi* and recorded the largest zone of inhibition of 5.00mm. This was followed by ethanol extract of fresh *P. amarus* which recorded an inhibition zone of 2.67mm. However, boiling of plant sample in water and the standard antibiotic, chloramphenicol recorded the smallest zone of inhibition of 1.83mm each. Statistically, there were significant differences observed among the extraction methods and the standard antibiotic (P<0.05).

Table 3. Sensitivity of *S. typhi* to fresh *P. amarus* sample in 10g/200ml

Extraction method	Zone of inhibition (mm)
Boiling with water	1.83 ± 0.29
Water extract	5.00 ± 1.00
Ethanol extract	2.67 ± 0.58
Chloramphenicol (control)	1.83 ± 0.29

Lsd (P=0.05): 1.15; P-value: 0.001

Table 4 shows the sensitivity of *S. typhi* to fresh *P. amarus* sample in 20g/200ml. The results of the experiment showed that *S. typhi* was very sensitive to the water extract of P. amarus with the largest inhibition zone of 7.17mm. The ethanol extracts recorded the second largest zone of inhibition of 5.33mm. The standard antibiotic, chloramphenicol had an inhibition zone of 5.00mm. However, boiling of fresh *P. amarus* in water recorded the smallest zone of inhibition of 3.33mm. There were significant differences observed among the different extraction methods and the standard antibiotic ($P<0.05$).

Table 4. Sensitivity of *S. typhi* to fresh *P. amarus* extract in 20g/200ml

Extraction method	Zone of inhibition (mm)
Boiling with water	3.33 ± 0.76
Water extract	7.17 ± 1.00
Ethanol extract	5.33 ± 1.00
Chloramphenicol (control)	5.00 ± 1.53

Lsd (P=0.05): 1.88; P-value: 0.008

3.2. Sensitivity Of Dry Phyllanthus Amarus to Salmonella Typhi

Sensitivity of *S.typhi* to dry *P. amarus* in 10g/200ml is presented in Table 5. The results showed that activities of *S. typhi* were inhibited by water extract of dry *P. amarus* with the largest zone of inhibition of 7.33mm. Ethanol extract of dry *P. amarus* was able to suppress activities of *S. typhi* to a zone of 6.83mm. The control treatment, chloramphenicol however, recorded the smallest zone of inhibition of 1.00mm. There were significant differences observed among the extraction methods and the control ($P<0.05$).

Table 5. Sensitivity of *S. typhi* to dry *P. amarus* extract in 10g/200ml

Extraction method	Zone of inhibition (mm)
Water extract	7.33 ± 0.17
Ethanol extract	6.83 ± 0.17
Chloramphenicol (control)	1.00 ± 0.00

Lsd (P=0.05): 0.47; P-value: 0.000

Table 6 depicts the sensitivity of *S .typhi* to dry *P. amarus* in 20g/200ml. The results showed that water extract of dry *P. amarus* suppressed activities of *S. typhi* and recorded the largest zone of inhibition of 13.50mm. Again, activities of *S. typhi* were inhibited by ethanol extract of dry *P. amarus* and recorded an inhibition zone of 10.50mm. However, chloramphenicol recorded the small zone of inhibition of 6.33mm. There were significant differences observed among the extraction methods and the control ($P<0.05$).

Table 6. Sensitivity of *S. typhi* to dry *P. amarus* extract in 20g/200ml

Extraction method	Zone of inhibition (mm)
Water extract	13.50 ± 0.87
Ethanol extract	10.50 ± 0.87
Chloramphenicol (control)	6.33 ± 1.20

Lsd (P=0.05): 3.43; P-value: 0.006

4. Discussion

4.1. Sensitivity of Phyllanthus Amarus to Salmonella Typhi

The sensitivity tests of *Phyllanthus amarus* to *Salmonella typhi* showed that both aqueous and ethanolic extracts of the plant material showed antimicrobial activity on the test organism. The test pathogen, *S. typhi* was more susceptible to the aqueous extract of *P. amarus* than the ethanol extract at all concentrations. The highest susceptibility was recorded with the dry plant aqueous extract at 20g/200ml, followed by the ethanol extract at 20g/200ml. The susceptibility of the test inoculum to the extract of the fresh and dry plant material increased with increasing concentration of the extract. The antibacterial efficacy of the plant extracts were very high compared to the low activities recorded with chloramphenicol. Chlorampenicol showed different sensitivity to the test organism, this indicates that it still has the potential of curing typhoid fever. But it was realized that, the sensitivity increased with increasing concentration. According to Oluduro and Omoboye in 2010, the antibacterial activities of most plant extracts are concentration dependent as zone of growth inhibition increased with increasing concentration of the extracts [7]. Ekwenye and Elegalam and Azu and Onyeagha reported that the efficacy of most plant extracts is concentration dependent [24, 25].

Assessing the quality of *Phyllanthus amarus* leaves extract for its hypolipidemic activity found the presence of four phyto-constituent namely alkaloids, flavonoids, saponins and tannins in the plant sample [26]. Flavonoids, tannins, alkaloids, steroids, terpenoids, saponins and glycosides were also obtained by Obianime and Uche, 2009 in their comparative study of the methanol extract of P. amarus leaves [27]. The presence of phytochemicals in plant extracts is a function of their antimicrobial activities against the test pathogen as they play important roles in bioactivity of medicinal plants [7]. They further explained that phytochemicals exert antimicrobial activity through different mechanisms. Chonoko and Rufai in 2011 also indicated that there was a link between the antibacterial activity exhibited by the plant extracts to the presence of steroids flavonoids, tannins, alkaloids and saponins [28].

Tannins, for example, act by iron deprivation, hydrogen binding or specific interactions with vital proteins such as enzymes in microbial cells [29], [30]. Herbs that have tannins as their component are astringent in nature and are used for the treatment of gastrointestinal disorders such as diarrhoea and dysentery; [31, 32]. Saponinis believed to be responsible for numerous pharmacological properties [33] and has been shown to have immense significance as anti hypercholesterol, hypotensive and cardiac depressant properties [34]. This perhaps justifies the already locally established function of the plant in the treatment and management of hypertensions. Alkaloids on the other hand are detoxifying and have antihypertensive properties; [35, 36]. It's toxicity against cells of foreign organisms has been reported by Akinpelu and others [30]. Waterman 1992 reported that alkaloids and flavonoids were useful as antimicrobial, anti-inflammatory and anti-oxidant agents [37]. Okwu and Josiah in 2006 also indicated that flavonoids are antioxidants [38]; hence, Adeneye and others in 2006b reported that the flavonoids contributed to the antioxidant activity of the *P. amarus* plant [39].

The choice of antimicrobial drugs in the absence of susceptibility information is often influenced by the signs and symptoms of disease, site of infection, and history of illness including patient's age. In many medical set-ups in the developing nations where laboratory facilities are inadequate, broad spectrum antibiotics are often used for suspected bacterial infections. These practices are not without danger which could worsen the disease prognosis. Intensive use of antibiotics often results in the development of resistant strains creating a problem in the treatment of infectious diseases [40].

Harvey and others in 2006 reported chloramphenicol as the drug of choice in the management of typhoid fever although its toxicity is well known. According to them, higher doses of chloramphenicol may not be favourable to the host because its toxicity is known to precipitate some serious adverse reactions [41]. Cunha in 2001, reiterated that intake of antibiotics are associated with side effects [42]. The increasing emergence of antibiotic-resistant strains to traditional antimicrobials makes it necessary that sensitivity tests should be carried out prior to initiation of antibiotic therapy [43]. Disk diffusion and subsequent Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC) tests have remain the most commonly used methods to test organisms' susceptibility to antibiotics although they were outside the scope of the present study [44].

The present work has provided the basis for selecting plant extracts that are likely to be effective against *Salmonella typhi*. The problem of increasing emergence of resistance to antibacterial drugs makes these studies unavoidable, and equally highlights the need for periodic review of antimicrobial activities of common antibiotic drugs available on the market since chloramphenicol was found to be less effective at a lower concentration or dosage than at a higher one. Moreover, medicinal plants often have fewer side effects, better patient tolerance, relatively less expensive, acceptance due to long history of use and being renewable in nature [45].

Generally, the present study has shown that all the different concentrations of the plant extract tested possess a measure of antimicrobial properties and is concentration dependent. The plant material examined in the present study have been in used in Ghana in the preparation of decoction and concoction for the treatment of typhoid fever caused by the test pathogen particularly, when modern drugs of choice failed in achieving the therapy.

This study has confirmed the antibacterial potentials of *P. amarus*, thus supporting their folklore application as a medical remedy for typhoid fever. Hence, supports the usefulness of the plant in the treatment of other ailments caused by microorganisms.

4.2. Yield of Crude Extract of Phyllanthus Amarus

The use of water as the sole solvent yielded the highest crude extract of 2.57g. Ethanol only also yielded the second highest crude extract of 2.52g. A fraction of water/ethanol (3:2) yielded 2.37g of crude extract. Also a fraction of water/ethanol (7:3) yielded 2.21g of crude extract. 2.14g of crude extract was recorded by a fraction of water/ethanol (9:1) while a fraction of water/ethanol (4:1) yielded 2.01g of crude extract. However, a fraction of water/ethanol (1:1) yielded the lowest crude extract of 1.05g. Comparing the use of the different fractions of water to ethanol, it was identified that there was no relation between the ratio of water to ethanol used and the yield of the crude plant extract. Factors like age of the plant and the polarity of the solvent used may affect the yield. Water and ethanol seems to be a good solvent for this plant which supports the use of water and alcohol as traditional solvent.

5. Conclusions

Generally, water was the best solvent used for the crude extraction of *Phyllanthus amarus* since it gave the highest crude extract. Ethanol gave the second highest yield of crude extract of *P. amarus*. The different proportions of water to ethanol gave different yields of the crude plant which has no relation. The proportion of 1:1 of water to ethanol gave the least yield.

The water extract of fresh *P. amarus* showed the largest and significant zone of inhibition of 5.00mm and 7.17mm in 10g/200ml and 20g/200ml concentration respectively compared to the other extracts antibacterial activity on *S. typhi* investigated in the study.

The ability of the dry *P. amarus* extract showing sensitivity to *S. typhi* was observed in the water extract which also showed the largest and significant zone of inhibition of 7.33mm and 13.50mm in both 10g/200ml and 20g/200ml concentration respectively.

However, the ethanolic extracts of both the fresh and dry *P. amarus* in 10g/200ml and 20g/200ml concentration also showed significant sensitivity on the test organism. Chloramphenicol which was the control showed different sensitivity on the test organism.

The present study showed that fresh and dry extracts of *Phyllanthus amaru s*howed antimicrobial activity on *S. typhi*, hence this provides a scientific basis that reflects the idea of traditional healers for using this plant for curing of typhoid fever as well as other ailments.

In conclusion, the results of the present study support the folkloric usage of the plant and suggest that both the water and ethanolic plant extracts possess compounds with antimicrobial properties that can be further explored for antimicrobial activity. This antibacterial study of the plant extracts demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The use of the plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases.

6. Recommendation

Based on the findings from this study, it is recommended that:

1. Further studies should be conducted to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Phyllanthus amarus a*gainst *Salmonella typhi* because of the antibacterial activity exhibited by the fresh and dry plant extracts.
2. Evaluation of the phytochemical properties and toxicological studies of *Phyllanthus amarus* must be carried out to ascertain its relative safety as a possible antimicrobial agent.

Authors Contribution

Conceptualization, C,P.D. and R.O; methodology, C.P.D . and R. O.; validation, R. O, C.P.D. formal analysis, C.P.D.; investigation, C.P.D.; resources, R. O.; data curation, R.O; writing—original draft preparation, C.P.D.; writing—review and editing,C.P.D.; visualization, R.O; supervision, R. O.; project administration, C. P.D and R.O. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

Data is available on request from the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest. No funders had any role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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