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Research Article

Evaluation of the Phytochemical Constituents and Antimicrobial Activities of Methanolic, Ethyl Acetate and Petroleum Ether Extracts of the Stem Bark of *Heisteria parvifolia*

Sylvenus Aguree[1*] and Peter Jagri Onilimor[2]

[1]*Department of Pharmaceutical Sciences, School of Applied Science and Technology, Wa Polytechnic, Wa, Ghana*

[2]*Quality Control Department, Salom Pharmacy Limited, Kumasi, Ghana*

ABSTRACT

The search for novel drugs is gaining prominence from plant sources due to the abundance of bioactive constituents in plants. This research established the bioactive constituents and antimicrobial activities of the stem bark extracts (Methanol, ethyl acetate and petroleum ether) of *Heisteria parvifolia*. The Phytochemical screening revealed the presence of tannins, flavonoids, glycosides, reducing sugars, saponins and anthraquinones. The methanolic extract tested positive for tannins, flavonoids, glycosides, cardiac glycosides, reducing sugars, anthraquinones and saponins while the petroleum ether extracts tested positive for glycosides, reducing sugars, terpenoids and cardiac glycosides. The three different extracts were tested against the common micro-organisms that affect human. In all, three gram positive organisms, six gram negative organisms were used. The methanolic extract showed a very good zone of inhibition at 14.50 mm against Proteus vulgaris and the ethyl acetate extract showed inhibition against Escherichia coli at a zone of 11.30 mm with the petroleum ether extract showing no activity against all the nine micro-organisms used. These findings confirmed the rational in its traditional usage in the treatment of diarrhea and convulsions which are the causes of microbial infections.

Keywords: Medicinal plants; Herbal medicine; Phytochemical screening; *Heisteria parvifolia*; Antimicrobial activities; Crude extracts; Mampong-akuapim; *Heisteria parvifolia*; MIC; Bioactive constituents

INTRODUCTION

The use of traditional plants as a source of medicine is not limited to one region of the world. It is an age-long practice throughout the world, used to treat and cure illnesses and diseases. The reliance on medicinal plants for medication is still actively practiced by most rural populace due to its cheaper cost and accessibility [1]. History and with its evidence of clinical applications supported by the origin of these medicinal plants has proven that herbal preparations have negligible toxicity and are more effective in the treatment of most health conditions by the rural populace [1]. Recent interest in medicinal products has been focused on the inadequate evidence of quality control measures and experimental evidence on the potency and efficacy of these herbal products [2]. Among some of the reported effects of herbal preparations are nephrotoxicity and hepatotoxicity [3,4].

Notwithstanding this, medicinal plants are still the most dependable source of life saving drugs for majority of the world's population [5]. The World Health Organization has estimated that about 80% of the world's population depends on plants for the treatment of most health conditions and this can be attributed to the availability of these medicinal plants such as herbs or shrubs which are the main source for the development of modern drugs and medicines [6]. These medicinal plants typically contain one or more pharmacologically active principles (phenolic, flavonoids, alkaloids, saponins and tannins) that may act, in isolation, collectively or in synergy to provide an improvement in the health of the consumers [7,8].'Bitters' which is typically obtained from plants is known to accelerate digestion while medicinal plants that contain phenols or related derivatives could be responsible for the reduction in inflammation and the antioxidative activities of such plants. This may be enhanced by isolating and characterizing the active principles in these plants which are the source of potential medicines [9]. Medicinal Plants generate bioactive products that protect them against predators and in some occasions, can be poisonous in nature [10,11]. Undoubtedly, the plant kingdom still holds many species of plants containing substances of medicinal value which are yet to be discovered.

Correspondence to: Sylvenus Aguree, Department of Pharmaceutical Sciences, School of Applied Science and Technology, Wa Polytechnic, Wa, Ghana, Tel: +233-549738988; E-mail: sdaguree@gmail.com

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With the high demands for medicinal plants products, there is a need for thorough scientific investigations into these medicinal plants for their health benefits and the likely possible toxic effects [12]. Among the vast range of medicinal plants used for medications is *Heisteria parvifolia* which is used in the treatment of many health conditions in Ghana [13].

Heisteria parvifolia is a shrub or small tree that grows up to about 20 to 60 m tall and has a bole up to about 60 cm in average diameter with slight fluttering at the base and thin buttress. The genus *Heisteria* comprises about 65 species in tropical Africa. Some species of this plant include, *Heisteria trillesiana*, *Heisteria zimmereri* and many others in tropical Africa [14]. *Heisteria parvifolia* occurs in different types of habitats, evergreen moist rainforest, coastal and riverine forest and primary upland forest, seasonally flooded forest and can also occur in savanna and secondary forest [15], in Ghana it is strongly associated with acid and base-poor soils. It is a dicotyledonous plant belonging to the family Olacaceae. The specie can also be found in Senegal, South-West Mali, Democratic Republic of Congo (DRC), Angola, Uganda, South Sudan and many other African countries [16]. The wood of *Heisteria parvifolia* is used for building poles, piles, palisades and tool handle. The flexibility of the stem and wood make them suitable for making bows, the twigs are used as chew-sticks and arrows. It has a glossy, dark green leaves with enlarged scarlet calyx persisting on the developing fruits. In some areas, the fruits are eaten fresh, the oil-rich seeds are eaten fresh or roasted or cooked before eaten [17]. The plant is known for its medicinal uses in various countries for instance in Ghana, the ground roots are used as enema against stomach-ache, stem bark is also used for treating diarrhea, convulsion and as a cough medication [18]. In Democratic Republic of Congo, the root bark is used against migraine by dropping it into the nose and into the eyes to treat painful or infected eyes and against cough [19]. The stem bark is applied to circumcision wounds in Gabon. In Cote d'Ivoire the leaves are taken against convulsion [20].

The seed kernel contains about 50% fat and a nice flavor scent [21]. The fatty acid composition is reported as mainly long chain saturated fatty acid (18.5%) with carbon length of C_{16}-C_{28} and 31% of oleic acid with other mono and di-enoic fatty acids [21].

The plant is of great prospects and most likely to remain a producer of timber, fruits, kernels and as source of food for local use. The seed oil is on a high demand as a source of rare fatty acids [21]. There is however, limited scientific research into the medicinal importance of this plant despite its widespread traditional usage.

MATERIALS AND METHODS

Materials

The following chemicals/reagents were secured from the indicated sources, methanol, ethyl acetate, petroleum ether (Fisher Scientific, UK), chloroform (Fisher Scientific, UK), hexane (Fisher Scientific, UK), ethanol (Sigma Aldrich, UK), ferric chloride, Meyers' reagent, hydrochloric acid (Sigma Aldrich, UK), sulphuric acid (Sigma Aldrich, UK), diethyl ether (Fisher Scientific, UK).

Authentication of collected sample

The stem bark of *Heisteria parvifolia* was harvested from some farm lands in Mampong-Akwapim (Eastern region of Ghana) and authenticated by a Botanist of the Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Science and was assigned the voucher specimen number KNUST/HMI/002 and deposited at the Faculty's herbarium for record purposes.

Plant, organisms and media used

The stem bark of *Heisteria parvifolia* was used as the plant material. The organisms used were: *Staphylococcus aureus* (ATCC-25923), *Proteus vulgaris*, *Streptococcus pyogenes* (clinical strain), *Salmonella paratyphoid* (Clinical strain), *Enterococcus faecalis* (ATCC-29212), *Klebsiella pneumonia* (Clinical strain), *Escherichia coli* (ATCC-25922), *Pseudomonas aeruginosa* (ATCC-4853), *Neisseria gonorrhoea* (clinical strain), and nutrient agar and nutrient broth media.

Sample preparations

The stem bark of *Heisteria parvifolia* was harvested, washed and chopped into smaller pieces, air-dried under room temperature for about two weeks and pulverized into fine powder. The powdered sample (150 g) was extracted with methanol, ethyl acetate and petroleum ether by cold maceration for three days (72 h) separately. The macerated samples were filtered and concentrated to dryness which gave the following yields, 6.56 g for methanol extract, and 5.45 g for ethyl acetate and 3.11 g for petroleum ether. The extracts were stored in a deep fridge until used.

Phytochemical screening

In order to determine the secondary metabolites in the stem bark of the above-mentioned plant, phytochemical analyses were conducted on the various extracts (methanolic, ethyl acetate and petroleum ether) using standard protocols employed [22].

Test for tannins

25 mL of distilled water was added to 0.5 g of powdered sample and boiled in a water bath for 5 min. It was then cooled and filtered. 15 mL of distilled water was added to 1 mL of the filtrate and 5 drops of 1% lead acetate solution added. The presence of white precipitate showed the presence of tannins.

Test for saponins

10 mL of distilled water was added to 0.3 g of the sample and shaken strongly for about 3 min in a test tube and observed for the presence of persistent foam for at least 5 min. The persistence of the foam shows the presence of saponins.

Test for anthraquinones

5% and 2 mL of aqueous $FeCl_3$ and dilute H_2SO_4 successively were added to 0.2 g of the powdered sample and boiled, for 5 min. The mixture was filtered, cooled and shaken with equal volumes of chloroform. The chloroform layer was then shaken with half the volume of dilute ammonia solution and the presence of the pink rose coloration indicate the presence of Anthraquinones.

Test for terpenoids

5 mL of the extract solution was added to 2 mL of $CHCl_3$ in a test tube followed by 3 mL of concentrated sulphuric acid. The formation of a reddish-brown layer coloration shows the presence of terpenoids.

Triterpenoids

0.2 g of powdered sample was shaken in chloroform to obtain a chloroform layer and filtered. 3-4 drops of concentrated (2 M) H_2SO_4 was added to the filtrate, it was then shaken and allowed to stand for 10 min. The formations of a clear reddish-brown colour at the interface with effervescence indicate the presence of triterpenoids.

Test for sterols

Chloroformic extract of the sample was prepared and 5 mL of the Chloroformic extract was measured into a test tube and acetic anhydride followed by concentrated sulphuric acid were added carefully down the side of the test tube to form a layer. The absence of a bluish colour at the interface indicate the absence of steroids.

Test for flavonoids

Aqueous form of the extract was prepared and filtered with a Whatman. No 1 filter paper dipped into the filtrate and dried. The filter paper was then exposed to ammonia solution and was observed for the appearance of an intense yellow colour. The appearance of an intense yellow colour shows the presence of flavonoids.

Test for glycosides

Approximately 0.2 g of the extract was weighed into 5 mL of dilute hydrochloric acid (0.05 M) and boiled on a water bath for 2 min. The mixture was filtered and made distinctly alkaline by adding three drops of 20% sodium hydroxide. The alkalinity of the mixture was then tested using a litmus paper. 1 mL of Fehling's solution A and B were added to the filtrate and heated on a water bath for two minutes. The presence of brick-red precipitate shows the presence of glycosides.

Test for phenols

0.5 g of the sample was extracted with ethanol and filtered. 3 drops of ferric chloride solution was added and the appearance of a bluish-black color indicate the presence of phenols.

Test for alkaloids

The sample was extracted with ammoniacal alcohol, filtered and evaporated to dryness. The residue was then extracted with 1% sulphuric acid. The filtrate was then rendered distinctly alkaline with dilute ammonia solution. The mixture was then shaken with $CHCl_3$ to separate the Chloroformic phase and evaporate off the $CHCl_3$. The residue was then dissolved in 1% sulphuric acid. 1 drop of Dragendorff's reagent was added to the 1% sulphuric acid and the absence of an orange-red precipitate indicate the absence of alkaloids.

Agar well diffusion method

The antimicrobial activities of the various plant extracts were determined using agar well diffusion method which has been recommended as a standard protocol for conducting antimicrobial screening [23]. The microbes were cultured with nutrient broth and incubated at a temperature of 37°C for a period of 24 h. The cultured microbes were sub-cultured again for another 24 h to obtain a purer microbial colonies. Mueller-Hinton agar powder (14 g) was prepared in 500 mL distilled water and sterilized in an autoclave for 15 min at a temperature of 120°C in order to get rid of any possible contamination of the agar.

The petri dish together with the cork borer, wire loop and other materials needed for the work were sterilized to eliminate unwanted contaminations. About 1 mL colony forming units (cfu) each of the microbes were respectively transferred into the sterilized petri dish using syringes. A 100 mL of the prepared Mueller-Hinton agar were poured into the petri dish containing the various microbes and allowed to solidify, and this was repeated for all the three extracts and the controls (negative control, DMSO and positive control, ciprofloxacin). A size seven cork borer was used to bore wells on the media. Clear labels were made on each of the petri dishes based on the concentration and type of extract.

Crude extracts (0.15 µg to 0.25 µg) of methanolic, ethyl acetate and petroleum ether were dissolved in 10 mL each of 2% dimethyl sulfoxide (DMSO).

A 100 µL of the various concentrations of each extract were inoculated into each well and allowed for the extract to diffuse into the Muller-Hinton agar and subsequently placed in the incubator at 37°C for 24 h. Each analysis was repeated three times.

The antimicrobial activities of the extracts were monitored by observing the various zones of inhibition after the incubation by measuring the diameter of the zones of inhibition using calipers. Ciprofloxacin was used as a positive control at a concentration of 0.001 µg/mL and 2% DMSO was also used as a negative control.

Broth dilution

Broth dilution method was used to further investigate the antimicrobial activities of the extracts that shows good zones of inhibition from the agar well diffusion method using the protocol of [23]. The microbes were prepared from a 24 h cultured nutrient broth and by adjusting the microbes to a suspension of 10^8 cfu/mL.

The various extracts (2%) were dissolved in 2% dimethyl sulfoxide (DMSO) and serially diluted with (DMSO) to obtain concentrations ranging between 0.15 µg/mL to 20 µg/mL. A 96-well sterilized micro-plate was employed for this analysis. Double strength nutrient broth (100 µL), 80 µL of the various prepared extracts and 20 µL of the selected cultured microbes were placed in each well and repeated for the controls (negative control, DMSO, and positive control, ciprofloxacin). The plates were incubated at a temperature of 37°C for a period of 24 h. The growth of the organisms was determined by adding 20 µL of tetrazolium salt solution (MTT) and incubated for another 15 min. The appearance of the dark colour in the wells after adding the tetrazolium salt shows the presence of microbes, since microsomal enzymes in the micro-organisms react with the extracts to form a dark complex with the tetrazolium salt.

The Minimum inhibitory concentration of the extracts were read as the lowest concentration of the extracts without a visible growth (well without a dark colour). Ciprofloxacin was used as the positive control and dimethyl sulfoxide as the negative control. The experiment was repeated three times.

RESULTS

Phytochemical screening

The Phytochemical constituents of the stem bark extract of the

plant *Heisteria parvifolia* revealed the present and absent of the following results as shown in the table below.

The + indicated in the table above shows the presence of that secondary metabolite in that particular extract and indicates the absence of that particular secondary metabolite.

Table 1 is an illustration of the results of the phytochemical screening conducted on the stem bark of the plant *Heisteria parvifolia*.

Table 2 shows the Mean ± standard deviation of the various zones of inhibition of the tested organisms against the various extracts as well as the positive and negative controls of the agar well diffusion method of the anti-microbial screening.

Table 3 shows the various concentrations of the extracts and standard that inhibited the growth of the tested organisms in the broth dilution method of the anti-microbial screening. The lower the value, the more potent that extract is against the tested organism and the higher the value less potent the extract is against the organism tested.

DISCUSSION

This research investigated the important secondary metabolites and antimicrobial activities of the crude extracts (methanolic, ethyl acetate and petroleum ether) of the stem bark of *Heisteria parvifolia*. The report therefore discusses the findings obtained from the phytochemical screening and antimicrobial screening of the crude extracts of the stem bark of *Heisteria parvifolia*.

Phytochemical constituents

The pulverized powder and the crude extracts (methanolic, ethyl acetate and the petroleum ether) of the stem bark of *Heisteria parvifolia* were screened for the presence and absence of phytochemical constituents in the stem bark of *Heisteria parvifolia*.

The phytochemical screening revealed the presence of tannins, flavonoids, glycosides, terpenoids, anthraquinones, saponins and reducing sugars in the pulverized powder and the absence of alkaloids and steroids as shown in Table 1.

The phytochemical screening of the methanolic extracts showed positive for tannins, glycosides, reducing sugars, flavonoids, cardiac glycosides, saponins and anthraquinones while cynogenic glycosides, terpenoids, steroids, and alkaloids were absent. The ethyl acetate extracts tested positive for tannins, glycosides, reducing sugars, flavonoids, saponins and cardiac glycosides and negative for anthraquinones, cynogenic glycosides, terpenoids, steroids and alkaloids. Petroleum ether extract of the stem bark of *Heisteria parvifolia* showed positive tests for glycosides, reducing sugars, terpenoids and cardiac glycosides but tested negative for anthraquinones, cynogenic glycosides, flavonoids, steroids, tannins, saponins and alkaloids.

Phytochemicals that are polar are mostly extracted with polar solvents such as methanol, those constituents with intermediate polarities are extracted with solvents of intermediate polarity such as ethyl acetate and purely non-polar compounds were extracted into the petroleum ether [24].

The presence and content of these phytochemical constituents may be responsible for the medicinal properties of the plant in traditional applications for the treatment of diseases as described in the use of *Heisteria trillesiana* and other plants in the same genus for the preparation of concoctions as a remedy for stomach upsets, convulsion in children and in wound healings [21].

Antimicrobial activities

Two methods were employed to assess the antimicrobial activities of the extracts, thus, the agar well diffusion method and the broth dilution method (minimum inhibitory concentration).

The agar well diffusion method was used as a preliminary assessment of the antimicrobial potential of the extracts [23]. The results (Table 2) showed various zones of inhibition among the micro-organisms used with *Proteus vulgaris* showing the largest zone of inhibition (14.50 mm) and the least zone of inhibition exhibited by *Neisseria gonorrhea* at 9.45 mm. This is as a result of the type and content of the phytochemical constituents present in the methanolic extract such as tannins and flavonoids which have proven to show some activities against micro-organisms especially gallotannins [25]. The ethyl acetate extract showed a zone of inhibition of 11.30 mm for *Escherichia coli*, while the smallest zone of inhibition at 3.42 mm was obtained for *Klebsiella pneumonia*.

The petroleum ether extract was equally tested for its antimicrobial potentials using the same test organisms as shown in Table 2. The petroleum ether at the various test concentrations did not show any antimicrobial activity and this may be a reflection of the absence of alkaloids, tannins and flavonoids which are known to have some

Table 1: Phytochemical constituents present in the extract.

Test powdered sample		Crude extract		
		Methanol	Ethyl acetate	Petroleum ether
Alkaloids	-	-	-	-
Saponins	+	+	+	-
Tannins	+	+	+	-
Glycosides	+	+	+	+
Steroids	-	-	-	-
Reducing sugars	+	+	+	+
Terpenoids	+	-	-	+
Flavonoids	+	+	+	-
Cardiac glycosides	+	+	+	+
Cynogenic glycosides	-	-	-	-
Anthraquinones	+	+	-	-

Table 2: Zones of inhibition (mean ± standard deviation).

Test organisms	Crude extract			Standard drug	Negative control
	Methanol (mm)	Ethyl acetate (mm)	Petroleum ether (mm)	Ciprofloxacin (mm)	2% DMSO (mm)
Streptococcus pyogenes	10.23 ± 0.023	6.54 ± 0.895	0	15.30 ± 0.004	0
Proteus vulgaris	14.30 ± 0.002	6.94 ± 1.673	0	16.42 ± 0.011	0
Salmonella typhi	12.40 ± 0.072	10.30 ± 0.721	0	15.30 ± 0.007	0
Pseudomonas aeruginosa	13.56 ± 0.059	4.50 ± 0.956	0	16.25 ± 0.002	0
Klebsiella pneumonia	10.30 ±1.221	3.42 ± 0.993	0	20.45 ± 0.043	0
Staphylococcus aureus	11.87 ± 0.067	6.15 ± 1.116	0	15.50 ± 0.006	0
Escherichia coli	12.00 ± 0.091	11.30 ± 0.274	0	15.20 ± 0.046	0
Enterococcus feacalis	10.34 ± 1.07	8.45 ± 0.193	0	16.50 ± 0.051	0
Neisseria gonorrhoea	9.45 ± 0.073	5.86 ± 0.008	0	15.45 ± 0.021	0

Table 3: Broth dilution method of antimicrobial screening (MIC Determination).

Test organisms	Plant extracts		Standard drug
	Methanol (µg/mL)	Ethyl acetate (µg/mL)	Ciprofloxacin (µg/mL)
Streptococcus pyogenes	5	20	0.03
Proteus vulgaris	2.5	10	0.01
Salmonella typhi	2.5	5	0.05
Pseudomonas aeruginosa	2.5	20	0.01
Klebsiella pneumonia	10	20	0.01
Staphylococcus aureus	2.5	20	0.01
Escherichia coli	2.5	5	0.05
Enterococcus feacalis	10	10	0.05
Neisseria gonorrhoea	10	20	0.01

antimicrobial activities against the common micro-organisms that have threatened survival of man [26].

The plants with phenolic compounds such as tannins, flavonoids can be used as therapeutic agents against micro-organisms because of the numerous antimicrobial activities exhibited [27]. They can affect the growth and metabolism of bacteria, inactivating or inhibiting the microbial growth according to their secondary metabolites and concentration.

Although the methanol and the ethyl acetate extracts share similar phytochemical profile, the difference in zones of inhibitions observed between the methanolic and the ethyl acetate extracts on the same set of organisms may be due to the quantity of the phytochemical constituents present in each extract. Analysis of phytochemical constituents [28,29] have reported that different crude extracts having the same phytochemical constituents produce different biological activities as a result of the different concentrations of bioactive substances in the different extracts.

Further research on phytochemical constituents have discussed that, phenolic compounds such as flavonoids, tannins,

and phenolic acids (which is present in the methanol and ethyl acetate extracts) play important roles in microbial growth inhibitions by inactivating the cellular enzymes of micro-organisms because of their ability to penetrate microbial cell membrane and cause membrane permeability defects [30].

Also, the assessment of possible toxicity of secondary metabolites of plants [31] have explained that the toxicity of phenolic compounds to micro-organisms can be due to adsorptions and disruption of microbial cell membrane, interference with microbial cell enzymes, and the deprivations of metal ions.

Ciprofloxacin which was the positive control showed the largest zones of inhibition against all the nine micro-organisms. Ciprofloxacin is known to act by inhibiting a subunit of the DNA grase, causing a relaxation of the supercoiled DNA leading to the termination of the chromosomal replication, recombination and transcription of DNA and subsequently killing the micro-organisms [32]. The extracts that showed activity against the micro-organisms were further analyzed to determine the minimum inhibitory concentrations.

The minimum inhibitory concentrations of the extracts were carried out using the methanolic and ethyl acetate extracts since the petroleum ether extract did not show any antimicrobial activities from the agar well diffusion method as shown in Table 2. The extract concentrations prepared ranged from 1.5 µg/mL to 20 µg/mL with the methanolic extract showing antimicrobial activities at concentrations of 2.5 µg/mL against *Proteus vulgaris, Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* at 5.0 µg/mL against *Streptococcus pyogenes* while there were no antimicrobial activities at a concentration of 10.0 µg/mL against *Neisseria gonorrhea, Enterococcus feacalis* and *klebsiella pneumonia*. The ethyl acetate extract on the other hand did not show much antimicrobial activities after the extract concentration was further reduced, the lowest concentration at which antimicrobial activities was observed at 5.0 µg/mL against *Salmonella typhi* and *Escherichia coli* and at 10.0

µg/mL against *Enterococcus faecalis* and *Proteus vulgaris*. From the minimum inhibitory concentration (MIC) results obtained from the screening, it has shown that the methanolic extract possesses antimicrobial activities at very low concentrations against the selective micro-organisms as shown in Table 3 as compared to that of the ethyl acetate which gave antimicrobial activities at higher concentrations. These findings may provide the justification for the use of *Heisteria parvifolia* in traditional medical practice.

CONCLUSION

The study conducted on the stem bark of this plant is the first scientific work conducted based on the literature available despite its wide spread traditional usage. This research has confirmed the rationale in its usage traditionally. The study has revealed the presence of important phytochemical constituents in the methanolic and ethyl acetate extracts. Further works conducted on the extracts shows that, the methanolic and the ethyl acetate possess good antimicrobial activities with the methanolic showing better activities at lower concentrations against the most common infectious micro-organisms.

AUTHOR'S CONTRIBUTION

The concept of this research was initiated by Sylvenus Aguree and made possible through his hard work. All laboratory analysis was conducted by same including the drafting of this manuscript. Samuel Asare-Nkansah and Peter Jagri provided critical review of the manuscript. Both read and approved the manuscript for submission.

CONFLICTS OF INTEREST

No conflict of interest exists.

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