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CASE REPORT

Prune Belly Syndrome - A Rare Presentation in Ghanaian Neonate

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Prune Belly Syndrome (PBS) is a rare congenital anomaly characterized by deficiency or absence of abdominal muscles, cryptorchidism and severe urinary tract abnormalities. Although it is thought to be more common in people of African descent in the USA, there are few reports of the syndrome from countries in Africa including Nigeria and Rwanda. Prenatal diagnosis through ultrasonography where the cardinal signs of hydronephrosis, bilateral hydroureters, megacystis and oligohydramnios are detected is increasingly becoming the norm. However, in resource limited settings where prenatal ultrasound services are not readily accessible or available, late postnatal presentations with pulmonary hypoplasia are encountered. This study reports of a neonate who presented with difficulty in breathing and wrinkled abdomen to a tertiary center in the Northern region of Ghana.

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Keywords*:* Prune Belly Syndrome, Ghanaian neonate, abdominal wall muscle deficiency

INTRODUCTION

Prune belly syndrome (PBS), also known as Eagle-Barrett syndrome or the Triad syndrome, is a rare congenital anomaly characterized by congenital abdominal wall muscle deficiency, cryptorchidism and severe urinary tract abnormalities (Hassett *et al.*, 2012). The syndrome is sometimes termed pseudo-prune belly syndrome when it occurs in females, in view of the fact that by definition, affected females cannot have the complete triad and the urologic manifestation may be less severe (Fotter *et al.*, 2001; Kristoff *et al.*, 2012). The syndrome was first reported in the 19th century but only got its current name in the early part of the 20th century (Osler, 1901). The incidence has been reported to range between 1/29000 and 1/40000 in different studies (Woods and Brandon, 2007). About 95% of patients with PBS are male, making the syndrome an almost exclusively male disorder (Woods and Brandon, 2007; Ademola *et al.*, 2012) with the incidence being quadrupled in twins compared to single deliveries (Balaji *et al.*, 2000). PBS is thought to be more common in African-American/Afro-Caribbean population than Caucasians in the USA although there are very rare reports of the syndrome from Nigeria (Okeniyi, 2005; Ademola *et al.*, 2012). The exact etiology of the syndrome is not well defined but there are a number of theories to explain its etiopathogenesis. The mesodermal developmental defect during early pregnancy and proximal urethral obstruction are the two main theories advanced (Straub and Spranger, 1981; Moerman *et al.*, 1984) but none of these theories fully explains the syndrome.

The most common mode of diagnosis is an obstetric ultrasound scan (USG) in the second trimester of pregnancy. Features that may point to the syndrome include hydronephrosis, bilateral hydroureters, megacystis and oligohydramnios (Hoshino *et al.*, 1998; Hassett *et al.*, 2012). The initial postnatal management depends on the presentation and whether or not there is pulmonary involvement, but all surviving patients will eventually require a multi-disciplinary approach in long term management. Prognosis depends on severity of renal impairment and degree of pulmonary involvement but these have significantly improved over the years

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(Woods and Brandon, 2007). About 1/3 of all cases surviving beyond the neonatal period will need dialysis and subsequent renal transplant due to chronic renal failure (Crompton *et al.*, 1994) making renal and urinary tract care a central part of the management of these patients. Furthermore, patients may benefit from cosmetic surgery to reduce the redundant, floppy skin over the abdomen (Kristoff *et al.*, 2012).

In order to create awareness of this rare syndrome and to sensitize medical practitioners and sonographers of the importance of early referral for expert review when abnormal findings are suspected on obstetric USG, a case report of a day old neonate who was presented to the Neonatal Intensive Care Unit (NICU) of the Tamale Teaching Hospital after delivery at home due to difficulty in breathing and abnormally-appearing abdomen was reviewed.

CASE REPORT

A day old male baby who was delivered at home by spontaneous vaginal delivery (SVD) at term was brought to the NICU of the Tamale Teaching Hospital by his parents on the same day because of difficulty in breathing and abnormally-appearing abdomen. The weight at presentation was 3.2 kg and according to the mother the baby cried immediately after birth although the baby had not been fed prior to presentation at the hospital.

The mother was 30 years old, (gravida 4 para 3), with regular antenatal clinic attendance but none of the routine laboratory investigations (e.g blood group, HBsAg, G6PD, retroviral screen and VDRL) was carried out during the pregnancy. A single obstetric ultrasound scan performed in the second trimester of pregnancy at a radio-diagnostic center reported an enlarged yolk sac with sufficient liquor.

At the initial physical examination on presentation to the NICU, the weight was 3.2 kg, axillar temperature was 37.3^{o}C and SpO_2 was 37% in room air with central cyanosis. There was flaring of the alae nasi and lower chest in-drawing. Air entry was bilaterally reduced on auscultation of the chest. No cardiac murmurs were heard. The abdomen was soft, bulging to the flanks and wrinkled (Figure 1) with visible peristalsis. The baby had normal external male genitalia with bilateral undescended testes.

The patient was diagnosed as having PBS with severe respiratory distress, probably secondary to pulmonary hypoplasia. His airways were immediately suctioned to clear it of secretions and he was placed on a bubble C-PAP (Pressure of 6 cm H_2O) with which the SpO_2 rose to a maximum of 88%. The patient was also started on parenteral ampicillin (50 mg/kg, twice daily), gentamycin (3.5 mg/kg daily), intravenous fluids (1/5 normal saline) and a statum dose of 1 mg vitamin K administered intramuscularly. Eight hours into admission in the hospital, the baby's condition began to deteriorate and the SpO_2 dropped to less than 50% with bradycardia on the bubble C-PAP. Cardiopulmonary resuscitation was initiated immediately but was not successful and the baby passed away after 9 hours on admission.

DISCUSSION

PBS is a rare congenital disorder characterized by abdominal wall muscle defects, cryptorchidism and urinary tract anomalies. The condition is a predominantly male disorder as seen in the present case and others (Ademola *et al.*, 2012). It is thought to be more common in the African-American population in the USA but there has been very few reports in literature from Africa (Ademola *et al.*, 2012). The condition is commonly diagnosed through obstetric ultrasound during the second trimester of pregnancy and in most resource-limited settings this procedure is not generally available or readily accessible. Indeed, very little is known about the condition and this coupled with the fact of non-availability or ready accessibility of diagnostic tools may lead to misdiagnosis hence the resultantly low number of cases recorded in Africa. It could also be due to the negative socio-cultural beliefs that perceive babies with congenital anomalies to be bad omen to the family, precluding families from bringing them to health facilities (kotei, 1990; Okeniyi, 2005). An extensive search through available literature has yielded no published reports of PBS in Ghana and as such makes this case a novelty. Prenatal USG is by far the most common diagnostic method for this

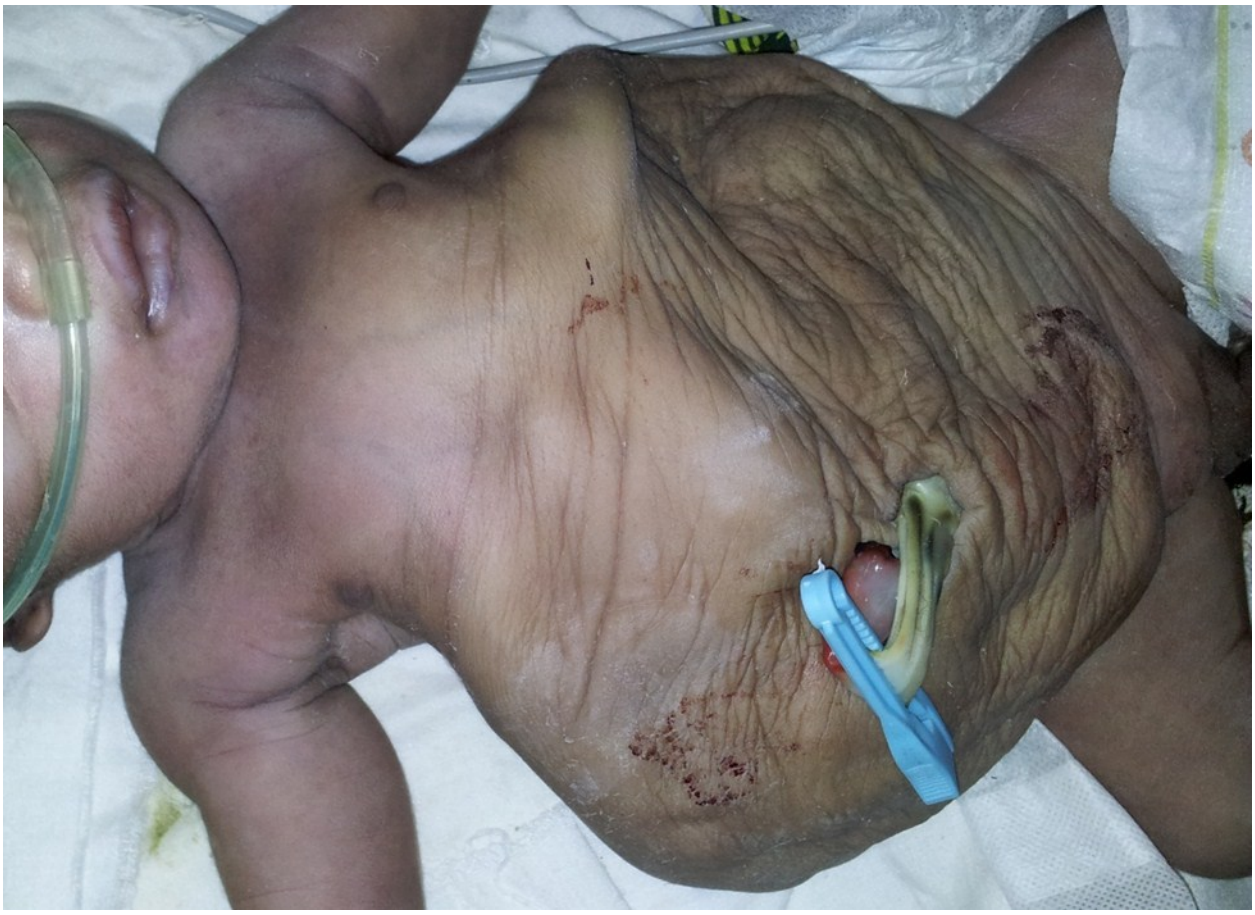

Figure 1: Wrinkled abdominal wall resembling a dry prune in a patient with PBS

complex syndrome and can detect the presence of the syndrome as early as 12-14 weeks of gestation (Hoshino *et al.*, 1998; Papantoniou *et al.*, 2010). In formulating a diagnosis though, other causes of lower urinary tract obstruction which may lead to distended bladder, megaureters and hydronephrosis should be ruled out. With respect to the case under review, the scan was carried out in a peripheral diagnostic center by a technician sonographer who made a record of and reported an enlarged yolk sac but the pregnant woman was not referred for a more detailed scan by an obstetrician until delivery. Termination of the pregnancy may be offered if diagnosis is made before viability (Hoshino *et al.*, 1998; Agarwal, 2005; Papantoniou *et al.*, 2010) and rightly so, in this case,the prenatal diagnosis could have helped in taking a decision regarding termination of the pregnancy.

Pulmonary hypoplasia, associated with approximately 60% of all cases, is the most common respiratory condition encountered in PBS (Hassett *et al.*, 2012; Tonni *et al.*, 2013). It is thought to be secondary to oligohydramnios which was one of the features of this syndrome (Ome *et al.*, 2013). Although the only obstetric USG performed during the second trimester in this case did not report oligohydramnios, the patient presented hours after delivery with severe respiratory distress, central cyanosis and very low oxygen saturation in room air. Majority of newborns with PBS who have pulmonary hypoplasia would die within the first week of life and in conformity to this fact, the patient in the present case died 9 hours into admission due to severe respiratory distress.

In Prune Belly syndrome, the presence of pulmonary hypoplasia in addition to severe renal dysfunction is a predictor of very high mortality in early days after birth (Woods and Brandon, 2007; Hassett *et al.*, 2012; Tonni *et al.*, 2013). Home delivery, inadequate management and delayed presentation as was done in this case might have contributed significantly to the high mortality.

CONCLUSION

Prenatal diagnosis through USG is the contemporary practice and needs to be enhanced particularly in resource-limited settings in order to increase availability and ready accessibility. Sonographers should be encouraged to refer suspicious cases for more detailed scans and expert opinions. In cases where early diagnosis is made in gestation, termination of pregnancy could be offered. Furthermore, early detection and management of pulmonary hypoplasia which is one of the predictors of early and high mortality in Prune Belly Syndrome is very vital to survival.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Preliminary Phytochemical Screening and *In vitro* Antioxidant Properties of *Trichilia monadelpha* (Thonn.) J. J. de Wilde (*Meliaceae*)

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The study evaluated the antioxidant potential and phytochemical constituents in the stem bark extracts of *Trichilia monadelpha* (Thonn) JJ De Wilde (Family: Meliaceae). Petroleum ether (PEE), ethyl acetate (EthE) and ethanol extracts (EAE) of the stem bark of *T. monadelpha* were screened for the presence of phytochemicals. The *in vitro* antioxidant potential of the extracts were also determined using the reducing power and 2, 2-diphenyl-1-picryl-hydrazyl radical (DPPH) scavenging tests respectively. Total phenol content of the extracts was also estimated. Phytochemical analysis revealed the presence of important secondary metabolites. Alkaloids, terpenoids, phytosterols, reducing sugars and coumarins were present in PEE. EAE had tannins, alkaloids, terpenoids, phytosterols, reducing sugars, flavonoids, cardiac glycosides, anthraquinones and saponins whiles EthE contained tannins, alkaloids, reducing sugars, cardiac glycosides, anthraquinones, terpenoids and phytosterols. Total phenol contents were estimated to be 7.51±0.87 mg tannic acid equivalent/g of petroleum ether extract, 34.14±0.78 mg tannic acid equivalent/g of ethyl acetate extract and 119.30±3.20 mg tannic acid equivalent/g of hydroethanol extract. The extracts showed a concentration-dependent reduction of Fe^{3+} to Fe^{2+} in the reducing power test as well as concentration-dependent DPPH radical scavenging. Of the three extracts, EAE had the most antioxidant activity. Findings of this study suggests that the stem bark of *Trichilia monadelpha* may be a good source of natural antioxidants and might be useful in treating the diseases associated with oxidative stress.

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Keywords*:* Phytochemicals, total phenol content, reducing power, DPPH, antioxidant, free radicals.

INTRODUCTION

Free radicals and reactive oxygen species have received a lot of attention especially in experimental or clinical medicine and biology because of their role in the aetiology of various chronic and degenerative diseases, including aging, coronary heart disease, inflammation, stroke, diabetes mellitus and cancer (Halliwell, 2011; Halliwell, 2012; Halliwell *et al.*, 1992). The damaging effects of reactive oxygen species (e.g. singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite) on cells has been shown to be abrogated by plants with antioxidant compounds (Dasgupta *et al.*, 2007). Plants are endowed with antioxidant and free radical scavenging molecules including vitamins, terpenoids, phenolic acids, tannins, flavonoids, coumarins, and other secondary metabolites. The search for compounds, that can protect the human body from oxidative damage and retard the progress of many chronic diseases, has therefore greatly focused on plant sources as they produce significant amount of antioxidants and represent a potential source of new compounds with antioxidant activity.

Trichilia monadelpha (Thonn) JJ De Wilde (Family: Meliaceae), known locally as Otanduro (Twi) or Tenuba (Nzema), is a tree that grows 12-20 m high and establishes itself well in the lowland high forest and evergreen semi-deciduous secondary jungles, often near river banks (Abbiw, 1990). Preparations

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(decoctions, infusions and tinctures) of the stem bark of the plant have been used in Ghanaian traditional medicine to treat pain, psychoses, epilepsy and inflammation for many years and their efficacies are widely acclaimed in different communities in Ghana (Abbiw, 1990; Dokosi, 1998). Pharmacological studies have revealed that the ethanolic stem bark extract of *T. monadelpha* has anti-trypanosomal and antiplasmodial activities (Kamanzi Atindehou *et al.*, 2004). Also, various stem bark extracts of the plant have been shown to have anti-inflammatory (Ainooson *et al.*, 2012) and analgesic (Woode *et al.*, 2012) properties. In the present study, the phytochemical constituents and the *in vitro* antioxidant and free radical scavenging potential of stem bark extracts of *Trichilia monadelpha* are determined.

MATERIALS AND METHODS

Chemicals

Acetic anhydride, ammonia, chloroform, Dragendorff's reagent, ethanol, ferric chloride, gelatin, hydrochloric acid, lead acetate, magnesium metal strips, methanol, *n*-propyl gallate, potassium ferricyanide, sodium chloride, sodium carbonate, sulphuric acid, sodium hydroxide and tannic acid were obtained from British Drug House (BDH) Ltd (Poole, England) while2, 2-diphenyl-1-picrylhydrazyl (DPPH), trichloroacetic acid (TCA), Folin-Ciocalteau reagent and Wagner's reagent were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). All chemicals were of highest purity (≥ 99.0%).

Plant collection

The stem bark of *Trichilia monadelpha* was obtained from Bomaa (7°05'06.82" N; 2°10'01.63" W) in the Tano North District of the Brong Ahafo Region of Ghana between August and October, 2010. The leaves of the plant were authenticated by Dr. Kofi Annan of the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. A voucher specimen was kept in the Faculty of Pharmacy Herbarium (No. FP/079/10).

Preparation of stem bark extracts

The plant bark was chopped into pieces, sun dried for fourteen days and pulverized into fine powder. The powdered plant bark was serially extracted with 40-60ºC petroleum ether, ethyl acetate and 70% ethanol over a 24-hour period using the Soxhlet apparatus. The extracts obtained were labelled as follows: EAE (ethanol extract), PEE (petroleum ether extract) and EthE (ethyl acetate extract). The resulting extracts were concentrated under reduced pressure at 40-60˚C to a dark brown syrupy mass in a rotary evaporator. The syrupy mass was further dried using water bath and kept in a desiccator. The final yields were 9.6 % (EAE), 0.9 % (PEE), and 0.7 % (EthE).

Phytochemical analysis

Phytochemical analysis of the extracts was performed according to standard methods (Kokate, 2005; Tiwari *et al.*, 2011; Trease *et al.*, 1989).

Test for Tannins and Phenolic compounds

About 0.5 g of each of the plant extract was boiled with 25 ml of water for 5 minutes. It was then cooled, filtered and the volume adjusted to 25 ml.

Lead acetate test: To 1 ml aliquot of each of the extracts, 10 ml of water and 5 drops of 1% lead acetate solution was added. The formation of white precipitate indicated the presence of tannins (Kokate, 2005).

Ferric chloride test: To 1 ml aliquot of each of the extracts 3-4 drops of neutral 5% ferric chloride solution was added. Formation of dark green colour indicated the presence of phenols (Kokate, 2005).

Gelatin test: To about 1 g of each of the extracts, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicated the presence of tannins (Tiwari *et al.*, 2011).

Test for Alkaloids

Five (5) grams of each of the extracts was stirred with 5 ml of 1% aqueous hydrochloric acid (HCl) on water bath and then filtered. Of the filtrates, 1 ml of each extract filtrates were taken into test tubes to be tested for the presence of alkaloids.

Dragendroff's test: To 1 ml of each of the extracts, 1 ml of Dragendroff's reagent (potassium bismuth

iodide solution) was added. An orange-red precipitate indicated the presence of alkaloids.
Wagner's test: To 1 ml of each of the extracts, 2 ml of Wagner's reagent (iodine in potassium iodide) was added. A reddish brown coloured precipitate indicated the presence of alkaloids.

Test for carbohydrates
One gram of each of the extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates (Tiwari *et al.*, 2011).
Benedict's test: 1 ml of each of the filtrates were added to 5 ml Benedict's reagent and heated gently for 2 minutes and cooled. Orange red precipitate indicated the presence of reducing sugars.
Fehling's Test: 1 ml of each of the filtrates was hydrolyzed with dilute HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicated the presence of reducing sugars.

Test for Phytosterols
Salkowski's test: One gram of each of the extracts were dissolved in 10 ml of chloroform and filtered. The filtrates were treated with few (3-4) drops of concentrated sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicated the presence of triterpenes (Tiwari *et al.*, 2011).
Libermann-Burchard's test: One gram of each of the extracts was dissolved in few drops of chloroform, 3 ml of glacial acetic acid and 3 ml of acetic anhydride were added. This solution was warmed and cooled under running tap water. Few drops of concentrated sulphuric acid were added along the side of the test tubes. The appearance of a reddish violet colour at the junction of the two layers and a bluish green colour in the acetic acid layer indicates the presence of unsaturated sterols and/or triterpenes (Wall *et al.*, 1954).

Test for Flavonoids
Shinoda's test: About 1 g of each of the extracts was further dissolved with 5 ml of ethanol (98 %). To this was added a small piece of magnesium foil metal, this was followed by drop wise addition of concentrated hydrochloric acid. Intense cherry red colour indicated the presence of flavonones. Orange red colour indicated the presence of flavonols (Brain *et al.*, 1975).
Lead acetate test: Few drops of lead acetate solution were added to each of the extracts in test tubes. Formation of yellow coloured precipitate indicated the presence of flavonoids (Tiwari *et al.*, 2011).

Test for Coumarins
In a test tube, 1 g of each of the extracts were placed and covered with filter paper moistened with dilute sodium hydroxide (NaOH), then heated on water bath for a few minutes. The filter paper was examined under UV light, yellow fluorescence indicated the presence of coumarins (El-Tawil, 1983).

Test for Glycosides
Extracts were hydrolyzed with dilute HCl, and then subjected to test for glycosides.
Keller-Killiani's test: One ml of each of the extracts was mixed with 5 ml of 70% alcohol for 2 minutes. This was filtered and to the filtrates was added 10 ml of water and 0.5 ml of lead acetate. This was filtered and the filtrate was shaken with 5 ml of chloroform. The chloroform layers were separated in a porcelain dish and the solvent removed by evaporation. This was cooled and dissolved in 3 ml glacial acid containing 2 drops of 5 % ferric chloride solution. The solution was carefully transferred to the surface of 2 ml concentrated sulphuric acid. A reddish brown layer formed at the junction of the two liquids and the upper layer which slowly became bluish green and darkening with standing indicated the presence of cardiac glycosides (Harborne, 1998).
Borntrager's test: Few drops of dilute sulphuric acid were added to 1 ml of each of the extracts. This was boiled and filtered. The filtrate was extracted with chloroform. The chloroform layer was treated with 1 ml of ammonia. The formation of red colour on the ammoniacal layer showed the presence of anthraquinone glycosides (Harbourne, 1984; Sofowora, 1993).

Test for Saponins
Froth Test: Extracts (1 g) were diluted with distilled water to 20 ml and this was shaken in a gradu-

ated cylinder for 15 minutes. Formation of 1 cm layer of foam indicated the presence of saponins (Tiwari *et al*., 2011).

Foam Test: The extract (0.5 g portions) was shaken with 2 ml of water. Foam produced which persisted for ten minutes indicated the presence of saponins (Trease *et al*., 1983).

In vitro anti-oxidant assay

Total Phenolic Content

The total soluble phenolic content of the three extracts (0.3-1 mg ml^{-1}) were quantified using the Folin -Ciocalteu's phenol reagent (Singleton and Rossi, 1965) with tannic acid (0.01-0.1 mg ml^{-1}) as standard. The extracts (1 ml) were added to 1 ml Folin-Ciocalteu's reagent (diluted tenfold in distilled water) in separate test tubes. The content of each test tube was mixed and allowed to stand for five minutes at 25°C in an incubator. One millilitre (1 ml) of 2 % sodium carbonate solution (Na_2CO_3) was added to the mixture. This was allowed to stand for 2 hours at 25°C in an incubator and centrifuged at 1000 ×*g* for 10 minutes to get a clear solution. The absorbance of the supernatant was then determined at 760 nm using UV mini-1240 single beam spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan). Distilled water (1 ml) was added to 1 ml Folin-Ciocalteu's reagent (diluted ten-fold in distilled water) processed in the same way as done for the test samples and used as blank. All measurements were done in triplicates. The total phenolics were expressed as milligrams per milliliter of tannic acid equivalents (TAEs) through the calibration curve with tannic acid.

Reducing power

The reducing power of the three extracts (0.1-3 mg ml^{-1}) was determined according to the method of Oyaizu (1986), with tannic acid (0.1-3 mg ml^{-1}) as a reference antioxidant. The reference antioxidant/ extract (1 ml) was mixed with 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1 % potassium ferricyanide solution ($K_3Fe[CN]_6$) in a test tube. The mixture was incubated at 50°C for 20 minutes. Following this, 1.5 ml of 10% trichloroacetic acid solution (TCA) was added to the incubated mixture, and centrifuged at 865 ×*g* for 10 min. The supernatant (2.5 ml) was then mixed with 2.5 ml distilled water and 0.5 ml of 0.1 % ferric chloride solution in a test tube. The absorbance was measured at 700 nm using UV mini-1240 single beam spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan). The blank was prepared by adding distilled water (1 ml) to 2.5 ml sodium phosphate buffer and 2.5 ml 1% potassium ferricyanide ($K_3Fe[CN]_6$) in a test tube. Three replicates were used. Results were then expressed as percentages of blank and presented as concentration-absorbance curves.

DPPH Scavenging Activity

The scavenging of the stable 2, 2-diphenyl-1-picrylhydrazil (DPPH) radical is a widely used method to evaluate the free radical scavenging ability of various samples, including plant extracts (Chang *et al*., 2002). The experiment was carried out as described in literature (Blois, 1958) with a few modifications. The extracts (0.1-3 mg ml^{-1} in methanol) were compared to *n*-propyl gallate (0.01-0.3 mg ml^{-1} in methanol) as standard free radical scavenger. The extracts (1 ml) were added to 3 ml methanolic solution of DPPH (20 mgl^{-1}) in a test tube. The reaction mixture was kept at 25°C for 1 h in an orbital shaker (BoroLabs, Aldermaston Berkshire, UK). The absorbance of the residual DPPH was determined at 517 nm in UV mini-1240 Single beam Spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan). Methanol (99.8%, 1 ml) was added to 3 ml DPPH solution, incubated at 25°C for 1 h and used as control.. Methanol (99.8%) was used as blank. Each experiment was carried out in triplicates. The percentage radical scavenging capacity was determined using the following formula:

$$\% \text{ DPPH Scavenging} = [(A_0-A_s)/A_0] \times 100$$

where A_0 is the absorbance of control (DPPH in methanol), and A_s is the absorbance of tested samples.

A graph was plotted with concentration along *X*-axis and % DPPH scavenging along *Y*-axis, and IC_{50} value was calculated. IC_{50} value signifies the concentration of tested samples that scavenges 50% of the DPPH radical.

Data Analysis
All experiments were conducted in triplicates, and the data are expressed as Mean ± S.E.M. Concentration responsible for 50% of the maximal effect (EC_{50}/IC_{50}) was determined by using an iterative computer least squares method, with the following non-linear regression (three-parameter logistic) equation

$$Y = \frac{a + (b - a)}{\left(1 + 10^{(LogED_{50} - X)}\right)}$$

Where, *X* is the logarithm of dose and *Y* is the response. *Y* starts at *a* (the bottom) and goes to *b* (the top) with a sigmoid shape.The fitted midpoints (ED_{50}s) of the curves were compared statistically using *F* test (Motulsky & Christopulos., 2003).GraphPad Prism for Windows version 5.0 (GraphPad Software, San Diego, CA, USA) was used for data analysis and EC_{50}/IC_{50}determinations. $P<0.05$ was considered statistically significant.

RESULTS

Phytochemical Analysis

Table 1 shows the phytochemical constituents of the various extracts of *T. monadelpha*. Alkaloids, terpenoids, phytosterols, reducing sugars and coumarins were present in PEE. EAE had tannins, alkaloids, terpenoids, phytosterols, reducing sugars, flavonoids, cardiac glycosides, anthraquinones and saponins whiles EthE contained tannins, alkaloids, reducing sugars, cardiac glycosides, anthraquinones, terpenoids and phytosterols.

Total Phenolic Content

Results of the assay of total phenolics in the extracts are shown in Table 2. There was a concentration-dependent increase in the total phenolics in all the extracts when expressed as tannic acid equivalents. Total phenolics were estimated to be 7.51 ± 0.87 mg tannic acid equivalent/g of petroleum ether extract, 34.14 ± 0.78 mg tannic acid equivalent/g of ethyl acetate extract and 119.30 ± 3.20 mg tannic acid equivalent/g of hydroethanol extract. EAE had the highest phenolic content and PEE the least (Figure 1).

Table 1: Phytochemical constituents of stem bark extract of *T. monadelpha*

TESTS	PEE	EthE	EAE
Tannins and Phenolic compounds			
Lead acetate test	-	+	++
$FeCl_3$ test	-	-	++
Gelatin test	-	+	++
Alkaloids			
Dragendroff's test	+	+	+
Wagner's test	+	+	+
Phytosterols/ triterpenoids			
Salkowski's test	++	++	+
Libermann-Burchard's test	++	++	+
Carbohydrates			
Benedict's test	+	+	+
Fehling's test	+	+	+
Flavonoids			
Shinoda's test	-	-	++
Lead acetate test	-	-	++
Coumarins			
Test for coumarins	+	-	-
Cardiac glycosides			
Keller-Killiani's test	-	+	++
Anthraquinones			
Borntrager's test	-	+	++
Saponins			
Frost test	-	-	++
Foam test	-	-	++

–: Not detected, +: Present in low concentration, ++: Present in moderate concentration.

Reducing power

EAE, EthE and tannic acid exhibited significant concentration-dependent reducing activity with EC_{50} values (in mg ml^{-1}) of 0.87±0.11, 13.63±0.38 and 1.04±0.26 respectively (Figure 2; Table 3). PEE, however, showed very weak reducing power (EC_{50}: 81.06±4.35; Figure 2, Table 3).

DPPH scavenging activity

The DPPH scavenging ability of the extracts are shown in figure 3. All extracts exhibited concentra-

Table 2: Total phenol content of the extracts of *T. monadelpha,* expressed as milligram tannic acid equivalent per gram of extract.

Extract	Total Phenol Content (mg TAE / g of extract)
PEE	7.51 ± 0.87
EthE	34.14 ± 0.78
EAE	119.30 ± 3.20

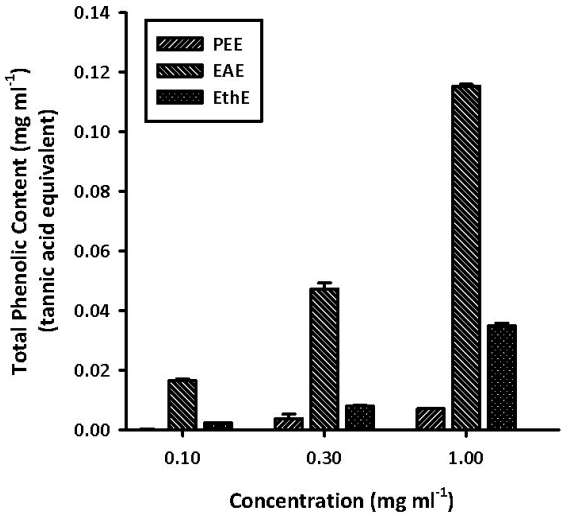

Figure 1: Total phenols (expressed as tannic acid equivalents) present in various concentrations of PEE (0.1-1 mg ml^{-1}), EAE (0.1-1 mg ml^{-1}) and EthE (0.1-1 mg ml^{-1}). Each column represents mean ± S.E.M. (n= 3).

Table 3: EC_{50} values for extracts of *T. monadelpha* and tannic acid in the reducing power assay.

Extract/ standard	Reducing Power	$F_{1,46}$	P value
PEE	81.06 ± 4.35***	317.10	<0.0001
EthE	13.63 ± 0.38***	300.20	<0.0001
EAE	0.87 ± 0.11***	81.21	<0.0001
Tannic acid	1.04 ± 0.26	-	

Values are EC_{50}± S.E.M ***P<0.001 compared to EC_{50} of tannic acid.

Table 4: IC_{50} values for extracts of *T. monadelpha* and *n*-propyl gallate in the DPPH assay.

Extract/ standard	DPPH scavenging	$F_{1,28}$	P value
PEE	0.24 ± 0.04***	50.21	<0.0001
EthE	0.08 ± 0.01***	12.07	0.0017
EAE	0.04 ± 0.04^{ns}	1.69	0.2047
n-propyl gallate	0.02 ± 0.01	-	

Values are IC_{50}± S.E.M ***P<0.0001; ^{ns}P>0.05 compared to IC_{50} of propyl gallate.

tion-dependent scavenging activity in a similar manner to the reference antioxidant, n-propyl gallate (figure 3). The IC_{50} correlates directly with the effectiveness of the substrate/extract to scavenge the DPPH radical. The IC_{50} values (in mg ml^{-1}) obtained (table 4) suggests PEE has the least ability to scavenge free radicals compared to *n*-propyl gallate.

DISCUSSION

Preliminary phytochemical analysis of the various extracts of *T. monadelpha* demonstrated the presence of saponins, tannins, alkaloids, cardiac glycosides, anthraquinones, reducing sugars, flavonoids, coumarins, triterpenoids and steroidal compounds. Of these, PEE indicated the presence of alkaloids, sterols, triterpenoids, reducing sugars and coumarins. EthE contained reducing sugars, sterols, triterpenoids, tannins, alkaloids, cardiac glycosides and anthraquinones while EAE indicated the presence of all the compounds listed except coumarins. The results obtained confirm earlier reports of some of the phytochemical constituents found in extracts of *T. Monadelpha* (Ainooson *et al.*, 2012; Woode *et al.*, 2012). This study, however, reports for the first time, the presence of coumarins in PEE; cardiac glycosides and anthraquinones in EAE and EthE.

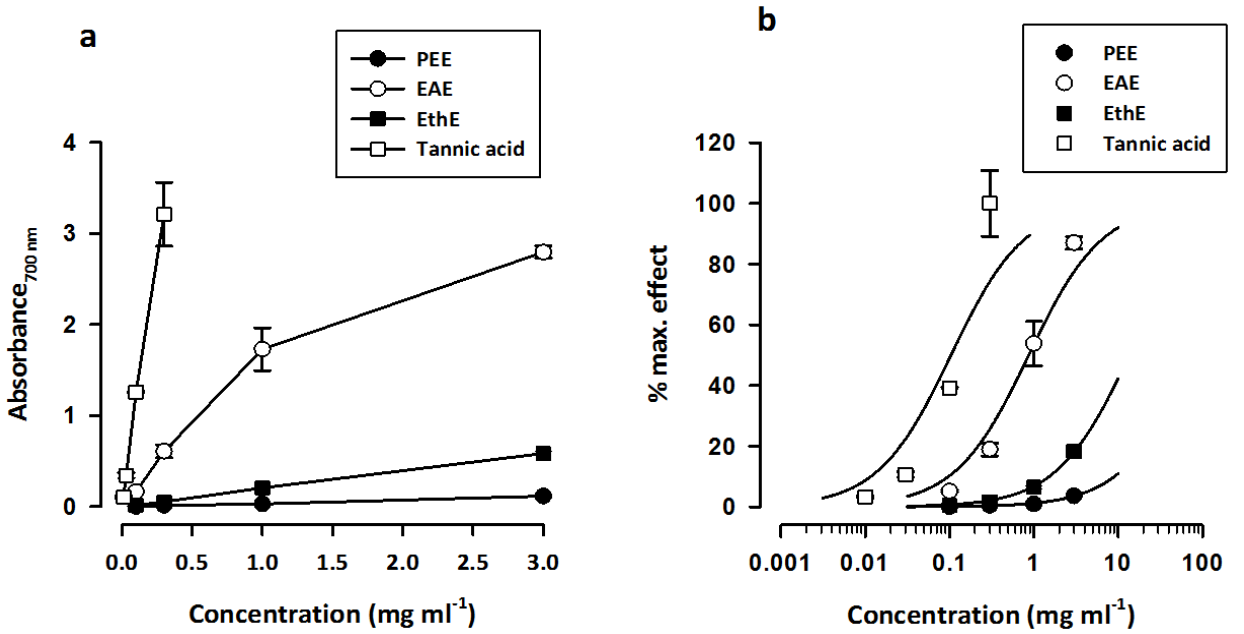

Figure 2: Change in (a) Absorbance and (b) maximal reducing power of the three extracts (0.1-3 mg ml^{-1}) of *Trichilia monadelpha* compared to tannic acid (0.1-3 mg ml^{-1}). Each point represents mean ± S.E.M. (n= 3).

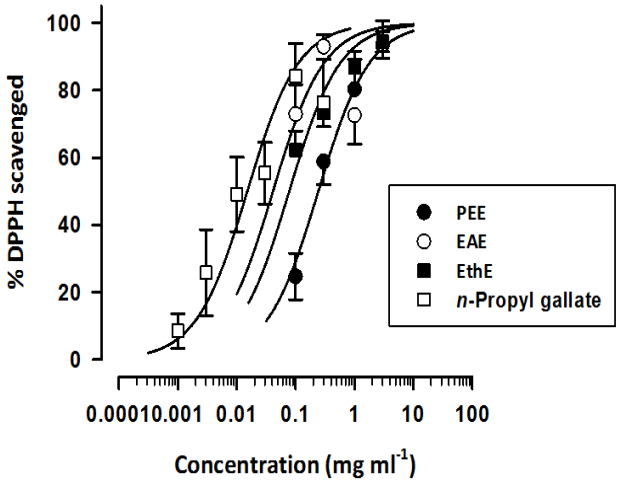

Figure 3: Free radical scavenging ability of the extracts, PEE, EthE, EAE (0.1-3 mg ml^{-1}) compared to *n*-propyl gallate (0.01-0.3 mg ml^{-1}) in the DPPH radical assay. Each point represents the mean ± S.E.M (n = 3).

The phytochemical constituents detected in the three extracts of *T. monadelpha* could contribute to the traditional therapeutic use of the whole stem bark. Phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites and possess diverse biological properties including anti-apotosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection, inhibition of angiogenesis and cell proliferation as well as the improvement of endothelial function (Han *et al.*, 2007). Tannins have also received immense attention in many fields especially in the fields of nutrition, health and medicine due to their physiological activities (e.g. antioxidant, antimicrobial and anti-inflammatory activity)(Mota *et al.*, 1985; Lin *et al.*, 2001; Buzzini *et al.*, 2008; Koleckar *et al.*, 2008). Flavonoids, a group of polyphenolics, are free radical scavengers, super antioxidants which have anti-inflammatory activity, prevent oxidative cell damage through their water soluble property and also possess strong anti-cancer activity (Gurib-Fakim, 2006; Salah *et al.*, 1995). Coumarins are potential antioxidants, according to stud-

ies (Tseng, 1991; Kostova, 2006; Kostova *et al.,* 2011), with the ability of scavenging free radicals and chelating metal ions. Triterpenoids possess analgesic and anti-inflammatory properties (Savithramma *et al.*, 2012). More research is required to determine the specific roles of these phytochemical constituents present in *Trichilia monadelpha*.

The reducing power and DPPH scavenging tests conducted in this study sought to establish the *in vitro* antioxidant properties of the plant extracts. The detection of phenolic compounds, particularly in EAE and EthE, strongly suggests possible antioxidant activity of the extracts. Phenolic antioxidants are potent free radical terminators (Shahidi *et al.*, 1992). The high potential of phenolic compounds to scavenge radicals may be explained by their phenolic hydroxyl groups (Sawa *et al.*, 1999).

Reducing power assay is a convenient and rapid screening method for measuring the antioxidant potential (Meir *et al.*, 1995) of a substance. From the results, there was significant, concentration-dependent Fe^{3+} reducing activity by EAE and EthE compared with tannic acid. The findings further affirm the antioxidant activity of the extracts.

The DPPH test is widely used as measure for the electron donation capacity of the antioxidant under the assay conditions. DPPH (2, 2-diphenyl-1-picryl hydrazyl) is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The DPPH radical scavenging ability of EAE was significantly as potent as that of the standard, *n*-propyl gallate, but PEE and EthE were less effective in scavenging the DPPH radical. The DPPH scavenging ability of EAE shows the extract could serve as a free radical inhibitor or scavenger.

From the activities shown by the extracts, particularly EAE, in the antioxidant tests conducted, it is clear that extracts have antioxidant activity. The mechanism of antioxidant activity may be due to the reduction of free radicals as well as scavenging of reactive oxygen species and other free radicals. EAE had the highest amounts of phenolics and hence it is not surprising that it also exhibited most reducing power and free radical scavenging ability. The antioxidant activities observed in this study could account, partly, for the anti-inflammatory effect observed in an earlier study on *T. monadelpha* (Ainooson *et al.*, 2012) since a large pool of evidence implicates free radicals in the process of inflammation (Closa *et al.*, 2004; Conner *et al.*, 1996; Reuter *et al.*, 2010).Further studies are required to clarify the *in vivo* potential of *T. monadelpha* in the management of human diseases resulting from oxidative stress.

CONCLUSION

The current study has shown that the petroleum ether, ethyl acetate and ethanol extracts of the stem bark of *Trichlia monadelpha* containsaponins, tannins, alkaloids, cardiac glycosides, anthraquinones, reducing sugars, flavonoids, coumarins, triterpenoids and steroidal compounds. The extracts also possess antioxidant and radical scavenging properties *in vitro*.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Visual Impairment and Ocular Findings among Deaf and Hearing Impaired School Children in Central Region, Ghana

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The scourge of global blindness continues to be a concern for eye care professionals, International Non-governmental Development Organizations (INGDOs) and eye care workers. While emphasis has been placed on how to address this scourge in the general population, not much is being done among special needs group such as the deaf and hearing impaired. The study was conducted to investigate the prevalence of visual impairment and ocular findings among hearing impaired children in a school for the deaf in the Cape Coast Municipality of Ghana. A cross-sectional descriptive study design was undertaken amongst children in the school for the deaf who had been previously diagnosed of hearing impairment or deafness. A total of 243 children underwent comprehensive eye examination in the school with prior approval from the school board. The mean age of the 243 children examined was 15.9 ± 4.0 years with a range of 9 – 27 years. Fourteen children (5.8%) had moderate visual impairment (WHO grade 1 visual impairment i.e. VA < 6/18 to 6/60) in the right eye, while 15 (6.2%) had moderate visual impairment in the left eye. Refractive error was present in 75 (31.9%) of the children with astigmatism being the commonest form of refractive error. Anterior segment abnormalities were present in 27 (11.1%) while posterior segment abnormalities were present in 25 (10.3%). The overall prevalence of visual impairment was 5.8% among hearing impaired school children in the Central Region of Ghana. There were ocular abnormalities that were previously undiagnosed among the studied population. There is the need for regular eye examination for children diagnosed of hearing impairment.
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Keywords*:* Disability, eye, ear, refractive error, ocular deviation .

INTRODUCTION

Studies have indicated that the prevalence of ocular abnormalities among the deaf and hearing impaired is higher than the general population of comparable age group (Nikolopoulos *et al.,* 2006). This association has been suggested to be due to the close anatomical relationship of the retina and cochlea which develops from the same embryonic layer (Abah *et al.,* 2011). Of all the sense organs, visual and auditory inputs are responsible for 95% of information acquisition (Fillman *et al.,* 1987). It is also generally claimed that visual input accounts for 75% of information acquisition. Existing co-morbidity of hearing and visual impairment in children predisposes them to many challenges including difficulties in communication, learning and social interaction. Suchman (1967) reported that hearing and visually impaired children are significantly more debilitated, less cooperative, less able to lip read and less capable of manual tasks compared to hearing impaired children with normal vision.

In Ghana, there is a high prevalence of hearing impairment. Recent studies have reported a prevalence of 16 per 1000 persons (Amedofu *et al.,* 1997; Amedofu *et al.,* 2005). Notwithstanding this however, there is a paucity of published data on the prevalence of visual impairment and visual abnormalities among the hearing impaired. The aim of this study was to investigate the prevalence of visual impair-

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ment and ocular abnormalities among hearing impaired children in a school for the deaf in Ghana.

MATERIALS AND METHODS

Study site and participants

A cross-sectional descriptive study was conducted to determine the prevalence of visual impairment and ocular abnormalities among deaf and hearing impaired children in a school for the deaf in the Cape Coast Municipality of Ghana from February to April, 2011. The school is the only school for the deaf in Central Region of Ghana. Before admission, each child is examined by an otolaryngologist to certify their status as either being deaf or hearing impaired. Thus, the basis for considering a study participant as being deaf or hearing impaired was based on the status established upon being admitted into the school. None of the children had had a previous eye examination. Every child present in the school during the eye examination visits was eligible to participate in the study. The school is residential with every child living within the premises.

Eye examinations

All eye examinations were carried out by qualified and registered optometrists. Every participant underwent a comprehensive eye examination comprising presenting visual acuity testing using the Snellen Tumbling E chart, near point of convergence test (NPC), cover test, external and internal ocular health examination using a penlight and direct ophthalmoscope respectively as well as both objective and subjective non-cycloplegic refraction. Communication during the examination process was facilitated by a designated teacher from the school who also had responsibility of reporting the response of each child during the examination.

Visual impairment classification

The World Health Organization category of visual impairment was used to specify the category of visual impairment among the children (WHO, 2005). Refractive error was specified as follows: myopia was defined as spherical power of $\geq$ - 0.50D, hyperopia $\geq$ + 2.00D and astigmatism $\geq$ - 0.50D. Emmetropia was defined as spherical correction of $<$ - 0.50D and $<$ + 2.00D and cylindrical correction of $<$ - 0.50D. Ocular deviation of $\geq 10^{\Delta}$ was considered as significant.

Ethical clearance

The protocol for the study was approved by the research committee of the Department of Optometry, University of Cape Coast. Administrative approval was also obtained from the Metropolitan Education Office as well as the head teacher of the school. Given the challenge of securing parental consent as per the ages of the participants, the head teacher further granted institutional clearance. Notwithstanding the institutional clearance, participants were required to give assent to be examined for the study or opt out if they so whish. The purpose of the eye examination was explained to every child and the study was conducted in accordance with the Declaration of Helsinki (WHO, 2007).

Statistical analysis

The data on eye examination was entered electronically using Microsoft Excel 2007. Data analysis was done using Statistical Package for Social Sciences (SPSS 17) (IBM Boston, USA). For qualitative variables, frequencies, percentage proportion and their 95% confidence intervals were computed. Quantitative variables were expressed as means $\pm$ standard deviation.

RESULTS

Study Participants

There were a total of 243 children in the school at the time of the study comprising 141 (58%) males and 102 (48%) females who were enrolled into the study. The mean age of the participants was 15.9$\pm$4.0, (95% CI = 15.4 – 16.4) years with age range of 9 – 27 years. The mean age of the male participants was 16.3$\pm$4.3 years while the mean age of the females was 15.3$\pm$3.6 years. There was no significant difference between the mean ages of male and female participants ($p = 0.068$). One hundred and eighty-eight (77.4%) of the subjects were aged between 11 – 20 years (Table 1).

Table 1: Age and sex distribution of study participants

Parameters	Gender		Total
	Male (n = 141)	Female (n = 102)	(n = 243)
Age (yrs)	16.3 ± 4.3	15.3 ± 3.6	15.9 ± 4.0
Age grp			
6 – 10	15 (10.6)	10 (9.8)	25 (10.3)
11 – 15	48 (34.0)	42 (41.2)	90 (37.0)
16 – 20	56 (39.7)	42 (41.1)	98 (40.3)
21 – 25	20 (14.2)	7 (6.9)	27 (11.1)
26 – 30	2 (1.4)	1 (1.0)	3 (1.2)

Data are presented as mean ± SD, absolute counts and proportions.

Table 2: Visual acuity distribution among study participants (n = 243)

Visual acuity	Right Eye (%)	Left Eye (%)
≥ 6/6	191 (78.6)	189 (77.8)
6/9	19 (7.8)	20 (8.2)
6/12	6 (2.5)	6 (2.5)
6/18	3 (1.2)	4 (1.6)
6/24	3 (1.2)	2 (0.8)
6/36	5 (2.0)	10 (4.1)
6/60	6 (2.5)	3 (1.2)
HM & LP	1 (0.4)	1 (0.4)
Undetermined	9 (3.7)	8 (3.3)

HM = hand movement, LP = light perception

Visual Acuity for distance

The distribution of visual acuity presentations are is shown in Table 2. One hundred and ninety-one (78.6%) had normal vision (VA≥6/6) in the right eye and 189 (77.8%) had normal vision in the left eye. Another 28 (11.5%) and 30 (12.3%) had mild visual impairment in the right and left eye respectively.

Fifteen (4.9%) participants had severe visual impairment in the right eye while 16 (6.6%) had severe visual impairment in the left eye (Table 3). Thus using WHO classification, 219 (90.1%, 95% CI = 85.9 – 93.4) had WHO grade 0 visual impairment (i.e VA ≤ 6/18). Another 14 (5.8%; 95% CI = 3.3 – 9.3) and 15 (6.2%; 95% CI =3.6 – 9.8) had moderate visual impairment (WHO grade 1 visual impairment i.e. VA < 6/18 to 6/60) (WHO, 2005) in the right and left eye respectively. One participant had vision of 'hand movement' and another one had 'light perception' in one eye. The vision in the other eye was 6/5 and 6/4 respectively. This could not be categorized as visually impaired using the WHO classification which considers vision in the worse eye. Visual acuity could not be determined in right eyes of 9 (3.7%) and left eyes of 8 (3.3%) participants. This was usually due to the fact that the children could not respond to the examination routine.

Refractive Error

Refraction results were unreliable in 8 (3.3%) of the subjects and therefore were not included in the analysis. The results from the right eye were used to identify the refractive status of each participant as described by Dandona, *et al*, (2002). One hundred and sixty (68.1%) participants had emmetropia while 75 (31.9%) had various forms of refractive error which comprised of astigmatism, present in 57 (76.0%) participants, myopia, 13 (17.3%) and

Table 3: Distribution of visual impairment categories among study participants (n = 243)

Type of impairment	Right eye (%)	Left eye (%)
Normal (VA≥6/6)	191 (78.6)	189 (77.8)
Mild visual impairment (VA < 6/6 to ≥6/18)	28 (11.5)	30 (12.3)
Severe visual impairment (VA <6/18 to light perception)	15 (4.9)	16 (6.6)
Visual acuity could not be determined	9 (3.7)	8 (3.3)

hyperopia 5 (6.7%) (Figure 1). Thus the prevalence of astigmatism, myopia and hyperopia among the participants was 24.3%, 5.5% and 2.1% respectively. There were 47 (62.7%) males and 28 (37.3%) females with refractive error. Whether a participant had a refractive error was independent of the sex of the participants ($\chi^2 = 1.59$, $p = 0.207$).

Binocular vision

The cover test performed at 40 cm revealed that 50 (20.6%) participants had various forms of ocular deviations including exophoria, exotropia, esophoria

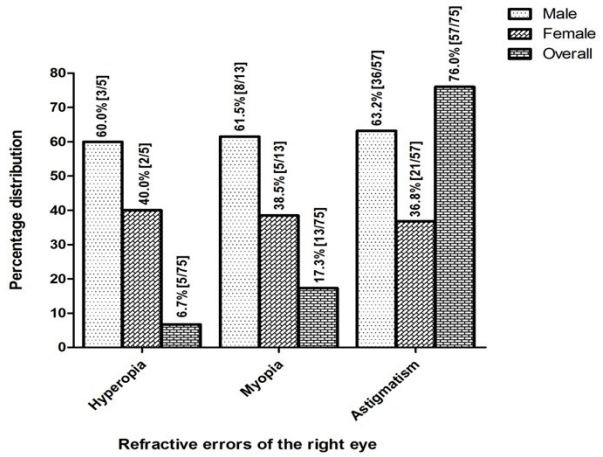

Figure 1: Distribution of refractive errors of the right eye among the participants

and esotropia while 184 (75.7%) had no deviation. Cover test could not be performed in 9 (3.7%) of the participants mainly because they were unable to maintain fixation at the fixation target. The results of the cover test are presented in Figure 2. Exophoria was the commonest ocular deviation being present in 39 participants out of the 50 who had ocular deviation (78%). Respectively, esophoria and exotropia were present in 8% (4/50) of the participants while esotropia was present in 6% (3/50) of the study participants.

Near point of convergence (NPC) could not be determined in 29 participants because they could not maintain fixation and respond to the test procedure. The mean NPC (break/recovery) was 5.6/7.4 ± 3.1/3.5 cm) with range of 1/1 to 25/28. Out of the 214 in which NPC was determined, 26 (12.1%) participants had values ≥ 8 cm for break while 21 (9.8%) had recovery values ≥ 11 cm. This proportion could estimate the proportion of participants who have convergence insufficiency.

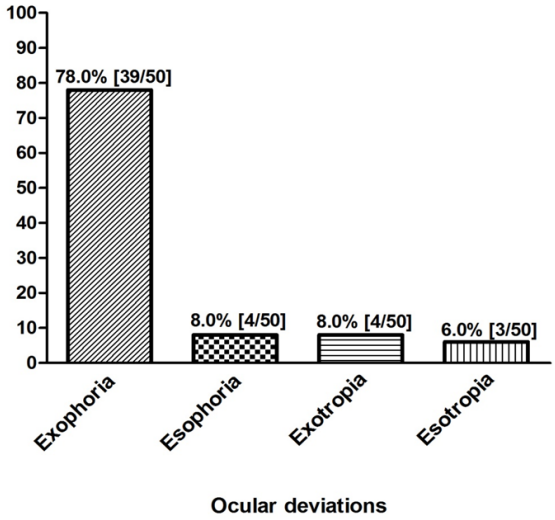

Figure 2: Distribution of ocular deviation among the participants

Ocular health

Ocular health was assessed in all 243 participants with some participants having a co-existence of anterior and posterior segment abnormalities. Anterior segment anomalies were found in 27 (11.1%) of the participants while posterior segment anomalies were found in 25 (10.3%). Table 4 shows the anterior and posterior segment anomalies found in the study.

DISCUSSION

The present study was conducted as an initial exploratory investigation of the ocular findings among the deaf and hearing impaired children in the Cape Coast Municipality of Ghana. Previous studies have reported the frequency of visual impairment and ocular abnormalities among hearing impaired subjects (Regenbogen, 1985; Fillman *et al.,* 1987; Nicoll and House, 1988; Armitage *et al.,* 1995; Mafong *et al.,* 2002; Hanioðlu-Kargý *et al.,* 2003; Nikolopulous *et al.,* 2006; Gogate *et al.,* 2009; Osaiyuwu and Ebeigbe, 2009; Bist *et al.,* 2011). Intepretative analy-

Table 4: Anterior and posterior segment anomalies among participants (N = 243)

Parameters	n (%)
Anterior Segment Abnormality	
Corneal abnormalities	5 (2.1)
Conjunctivitis	6 (2.5)
Lid	7 (2.9)
Nystagmus	1 (0.4)
Dacryocystitis	1 (0.4)
Heterochromia iridis	1 (0.4)
Posterior Segment Abnormality	
Lens	2 (0.8)
Choroid/retinal	13 (5.3)
Retinitis pigmentosa	3 (1.2)
Vitreous	2 (0.8)
Optic nerve/disc	4 (1.6)
Phthisis bulbi	1 (0.4)

sis from results of this study should therefore be done cautiously as a result of the considerable challenge presented in comparative analysis to studies conducted elsewhere due largely to variations in the criteria for reporting findings with typical examples being the cut-off points for visual acuity and the variations in ocular conditions presented.

In Nigeria, the frequency of visual disorders among the hearing impaired has been reported to range between 20.9 – 73.3% (Osaiyuwu and Ebeigbe, 2009; Abah *et al.,* 2011). Some of these studies have also indicated that the frequency of ocular abnormalities are higher among hearing impaired subjects compared to normal hearing subjects in the general population with similar age groups (Gogate *et al.,* 2009; Abah *et al.,* 2011). Recommendations for regular screening for the presence of visual disorders among the hearing impaired have been advanced by several authors so that appropriate remedial measures are taken to address the challenges faced by this cohort of subjects.

Some studies have reported on ocular abnormality findings among hearing impaired children without reporting the level of visual functioning at least as measured by visual acuity (Mafong *et al.,* 2002; Hanioðlu-Kargý *et al.,* 2003; Gogate *et al.,* 2009; Osaiyuwu and Ebeigbe, 2009; Abah *et al.,* 2011). From this study, 10.3% of the children for whom visual acuity was measured had a VA <6/9 with a further 7.3% having category I and II visual impairment. This is more than the 6.4% found by Nicoll and House (1988) in a study conducted in Western Australia but lower than 25.3% reported by Armitage *et al.,* (1995) in the UK. Armitage *et al.,* (1995) in their study had reiterated the fact that a deaf child is dependent on vision for communication and learning. There is no doubt therefore from this study that the estimated visual impairment prevalence rate of 7.3% may lead to this cohort of study participants having certain challenges in acquisition of communication skills.

Refractive error was present in 30.9% of the children for whom refractive error was determined. This is comparable to the estimated 28.9% and 29.8% reported by Armitage *et al.,* (1995) and Hanioðlu-Kargý *et al.,* (2003) in their respective studies. It was much higher compared to values reported by other authors: 7.9% (Abah *et al.,* 2011); 18.5% (Gogate *et al.,* 2009); 16.5% (Bist *et al.,* 2011) and much lower compared to the 73.3% reported by Osaiyuwu and Ebeigbe (2009). In terms of the commonest form of refractive errors, astigmatism was the most prevalent refractive error in this study. While this is consistent with the report of Hanioðlu -Kargý *et al.,* (2003), it was different from other reports. For example, hyperopia was the commonest refractive error reported by Abah *et al.,* (2011) and Mafong *et al.,* (2002) whereas myopia was the commonest refractive error reported by Gogate *et al.,* (2009), Bist *et al.,* (2011) as well as Osaiyuwu and Ebeigbe (2009). Nicoll and House (1988) reported an equal prevalence for myopia and hyperopia. The reason for this variation could be attributed to the differences in the definition and cut off point used by different authors in specifying the types of refractive errors.

A comparison of the prevalence of visual impairment and refractive error in the present study to two earlier studies on refractive error among school children without hearing impairment in Centreal

Region of Ghana by Ovenseri-Ogbomo and Omuemu (2010) and Ovenseri-Ogbomo and Assien (2010) shows that the 10.3% proportion of children with VA< 6/9 in this present study was significantly more than the 2.1% and 4.5% respective prevalence rates reported for school children without hearing impairment. The proportion of refractive error in this study 31.9% was also significantly more than the 25.6% and 13.3% reported by Ovenseri-Ogbomo and Omuemu (2010) and Ovenseri-Ogbomo and Assien (2010) respectively for school children without hearing impairment. This difference could even be more marked when one considers the fact that in the present study, cycloplegic refraction was not incorporated among the list of ocular problems as was the case in earlier studies conducted by Ovenseri-Ogbomo and Omuemu's (2010) or the use of higer cut-off point of +2.00D for hyperopia compared to +0.75D used by Ovenseri-Ogbomo and Assien (2010). Given that these studies were from children without hearing impairment within the same geographical and socio-cultural background, it might be inferred that children with hearing impairment have significantly higher proportion of visual impairment and refractive error compared to those without hearing impairment which finding is consistent with that of Brinks *et al.,* (2001) and Rogers *et al.,* (1988).

The ocular deviation proportion of 20.6% estimated in this study is greater than the 16.5% reported for Ghanaian school children without hearing impairment (Ovenseri-Ogbomo and Assien, 2010). In earlier studies conducted by Ovenseri-Ogbomo and Assien (2010) in children without hearing impairment and this study, exophoria happenened to be the commonest observed ocular deviation. Furthermore, the esophoria proportion of 8.0% estimated from this study is greater than the 2.0% estimated from the study conducted in children without hearing impairment by Ovenseri-Ogbomo and Assien (2010). This study recorded a squint prevalence of 2.5% in the children and this rate is higher than the 1.3% reported by Gogate *et al.,* (2009) for hearing impaired children in India.

The study noted the presence of previously undetected ocular abnormalities among the participants and this underscores the need for regular and more periodic scheduled eye examination for these groups of people as related by Gogate *et al.,*(2009) in their study. The occurrence of ocular abnormalities estimated in this study might have been underreported considering the fact that eye examinations in the school were conducted with direct ophthalmoscope which might have limitations in the diagnosis of some subtle ocular presentation that could have been uncovered with more sophisticated diagnostic procedure.

CONCLUSION

There are high proportions of refractive errors and other ocular abnormalities among the hearing impaired and deaf population of school going age. Given the high proportion of refractive error in this group, it is highly recommended that alongside otolaryngologic examination, children diagnosed of hearing impairment should have scheduled eye examination before placement in the school and during the duration of the schooling period. The relevant authorities should also ensure periodic reexamination of these children to detect any new ocular abnormality and evaluate on-going pathology. This high proportion of retinal/choroidal abnormalities could warrant a detailed retinal evaluation by experienced eye care practitioners. The detection of ocular abnormalities and taking prompt action will have a significant impact in the social interaction of the hearing impaired child.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Comparative Study of the Simulated and Calculation Gantry Angle Methods in Tangential Breast Irradiation

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Breast irradiation involves a complex geometric and field-matching technique. Simulators are used to obtain the best and accurate patient treatment positioning as well as irradiation geometry for radiation portals. However many centers in developing countries lack this important equipment. The study was designed to determine the accuracy and reliability of the gantry angle calculation method for tangential breast irradiation in comparison with the simulated method. This prospective study was conducted at the National Center for Radiotherapy and Nuclear Medicine, Accra, Ghana between June and October, 2012 with a sample size of 50 breast cancer patients. The simulator method was compared with the calculated method to assess if the two methods can be used interchangeably using Bland-Altman analysis. The sensitivity and specificity values of the calculated formula in estimating accurate gantry angle as well as beta gantry angle were also calculated using Receiver Operator Characteristic. The coefficient of variation (CV) of the results generated by the simulation method (65.18%) was similar to the coefficient of variation of the results generated by the proposed formula (65.30%). The CV of the beta angles results from the breast bridge (13.50%) was also consistent with the calculated formula (14.04%). The sensitivity and specificity of the calculated formula for gantry angle are 100% and 100% respectively. The sensitivity and specificity of the calculated formula for beta angle are 100% and 98% respectively. None of the gantry angle values generated by calculated formula was different from the corresponding value from the simulator by 3 degree or more. Within the limit of this study, the two methods can be used interchangeably without significant variation in treatment plan and outcome.
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Keywords*:* Breast cancer; Target-volume, Localization; Simulator; Fluoroscopic; Geometric .

INTRODUCTION

Approximately one and half million new breast cancer cases are reported annually worldwide (ACS, 2010; Globocan, 2008; Parkin and Pisani, 2008). In sub-Sahara African countries, it is very difficult to determine the actual incidence of breast cancer due to the absence of a national cancer registry, as cancer receives low priority for health care services (Tannerberger *et al.,* 2004; Parkin and Fernandez, 2006; Jamison *et al.,* 2006). In Ghana, breast cancer is the leading malignancy accounting for 15.4% of all malignancies, and this number increases annually (Clegg-Lamptey and Hodasi, 2007; Stark *et al.,* 2010). Available statistics from the International Agency for Research on Cancer puts the Ghana's 2008 estimates at 25.8 per 100,000 with 2062 recorded cases in Ghana (Globocan, 2008).

Patients who receive breast irradiation represent a significant proportion of the workload in radiotherapy departments across the globe (Bentel, 1996, Bomford and Kunkler, 2003). The rationale for post-mastectomy chest wall irradiation or lumpectomy followed by radiotherapy is to improve overall survival and prevent local recurrence of cancer (Halperin *et al.,* 2008). Radiotherapy is also indicated for locally advanced and metastatic breast cancer

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for palliative intent (Clifford, 1999).

External beam irradiation of the breast involves a complex geometric and field-matching technique, which consists of a supra-clavicular field, a pair of tangential fields to treat the breast or chest wall and sometimes an internal mammary field (Halperin *et al.,* 2008; Clifford *et al.,* 1999, Washington and Leaver, 2000). The supra-clavicular field is used to treat the involved supra-clavicular and/or axillary nodes.

The technical complexity arises from matching the inferior border of the supraclavicular field with the superior borders of the tangential fields to prevent any overdose to the junction of the fields, as well as choosing the right gantry angles for the tangential fields to minimize the volume of lung being irradiated with reference to the posterior edge of the tangential fields (Clifford *et al.,* 1999; Lichter *et al.,* 1998). Shielding (with either lead or Multileaf Collimators) is used to protect the shoulder joint and the apex of the lung to prevent fibrosis of the joint and minimize radiation to the lung respectively. If irradiation of internal mammary chain is intended, then a direct internal mammary field is used (Lotayef *et al.,* 2005; Bomford and Kunkler, 2003).

Simulation helps in target localization and beam placement in the treatment planning for accurate breast irradiation (Lotayef *et al.,* 2005). However, despite the important role of a simulator, many centers in developing countries do not have this equipment. At the centers where they are available, frequent break down and long periods before repair lead to treatment delays with its associated problems. The problem becomes more serious in the tangential fields irradiation of the breast where the gantry angles need to be determined accurately with minimal error margins to spare the lungs from excessive irradiation. Without fluoroscopic guidance with a simulator, the above procedure becomes cumbersome and complicated.

The gantry angles for tangential breast irradiation can, however be calculated with geometric formulas which are based on measurements of the field length, the horizontal distance between midline and mid-axillary line, the vertical distances from the mid axillary line to the inferior and superior beam border and central axis at midline (Clifford *et al.,* 1999; Lichter and Padikal, 1998; Lotayef *et al.,* 2005; Bomford and Kunkler, 2003; Sillanpa *et al.,* 2005; Lederer and Schwendener, 1997, Khan, 2007). This comparative study was conducted to determine the accuracy and reliability of the gantry angle calculation method based on simple geometric formula when compared with angles obtained with a conventional simulator for breast irradiation technique in use at the National Center for Radiotherapy and Nuclear Medicine, Korle-Bu Teaching Hospital (KBTH), Accra, Ghana.

MATERIALS AND METHODS

This prospective study was carried out at the National Center for Radiotherapy and Nuclear Medicine (NCRNM), Korle-Bu Teaching Hospital (KBTH), Accra, Ghana between June and October, 2012. Fifty (50) patients diagnosed with breast cancer and scheduled to undergo radiotherapy were consecutively enrolled into the study after giving informed consent. The study was approved by the management of the NCRNM, Korle-Bu Teaching Hospital and ethical clearance for the study was given by the Ethics and Protocol Review Committee of the School of Allied Health Sciences, University of Ghana.

Convenient sampling was employed in selecting study participants during their simulation appointments at the centre as part of the radiotherapy process. The inclusion criteria included patients having both supra-clavicular and tangential breast field irradiations as well as those treated with only tangential breast fields if the treatment technique required the use of a couch rotation (Neal and Hoskin, 2000). Patients with very large or flaccid breast were excluded from participating in the study. This was based on the assumption that such breasts could not be measured accurately by the breast bridge and also the fact that the breast bridge could not fit well on patients with a big separation (Krystyna, 2007).

Gantry Angle Calculation Technique

Parameters obtained during simulation such as couch rotation(*a*); separation (*s*); gantry angles obtained with a simulator (β) and that obtained with a breast bridge (β_b) were considered for each of the study participants. Additional parameters, comprising the treatment depth (*H*) on the posterior border (P_1) of the medial tangential field in the transverse plane containing the beam central axis was also determined for each study participant. In determining H, the lateral laser was made to coincide with posterior border of the lateral tangential field in the same transverse plane (Chang et al., 2007). Pilot simulation was done on each study participant and this involved taking specific measurements to determine the gantry angles required for simulation and treatment. Individual patient measurements such as skin to lateral laser distance and separation were taken to determine an angle (β) which was then used to calculate the gantry angle (Chang et al., 2007). A breast bridge (MT-BB01; MED-TEC, Orange city, Iowa, USA) was used to determine the angle (β) in order to find the correlation with the calculated (β) (Clifford *et al.,* 1999; Lichter and Padikal, 1998; Lotayef *et al.,* 2005; Bomford and Kunkler, 2003; Sillanpa *et al.,* 2005; Lederer and Schwendener, 1997, Chang *et al.,* 2007). The calculated gantry angle technique was verified fluoroscopically with the conventional simulator to determine the: accuracy of the gantry angle from calculation, coincidence of the posterior borders of the tangential fields and volume of lung in the beam (Lichter and Padikal, 1998; Lotayef *et al.,* 2005). The simulated gantry angles were determined fluoroscopically with the conventional simulator and were considered as the control data (Chang *et al.,* 2007).

Derivation of Gantry Angles from Geometric Formula

A horizontal line was drawn through point P_1 which made an angle of θ with the posterior border of the tangential field. From simple geometry it implies;

$$\sin\theta = \frac{H}{S} \qquad (1)$$

Hence:

$$\theta = \sin^{-1}\frac{H}{S} \qquad (2)$$

Statistical analysis

The simulator method was compared to the formula using Bland-Altman analysis. It is most unlikely that different methods will agree exactly by giving identical results for all individuals. Hence, the need to know by how much the new method (i.e. using the calculated formula) is likely to differ from the conventional and gold standard method (i.e. using simulator): Bland-Altman analysis first calculates the difference in measurement values obtained by the two methods on the subjects. The mean of such difference in a sample of subjects is the estimated bias (difference between method), and the standard deviation (SD) of the difference measure random fluctuation (precision) around this mean. If the "limit of agreement" (mean difference ± SD) between the two methods is not clinically important, both methods could be used interchangeably.

Another essential feature of the analysis is the graphical representation of the data with between-method difference (y axis) plotted against the average (x axis). This allows for evaluation of existing relationships between the error measurement (*difference*) and the assumed true value (*average*). Accuracy is assessed by analyzing how close data points are to the x-axis and the observed trend as the value on the x-axis increases.

The sensitivity and specificity values of the formula in estimating accurate gantry angle as well as beta gantry angle were calculated using Receiver Operator Characteristic (ROC) analysis. In all statistical tests, a value of $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

A total sample size of 50 breast cancer patients visiting the National Center for Radiotherapy and Nuclear Medicine, Accra, Ghana were enrolled into the study to determine the accuracy and reliability of the gantry angles calculation method for tangential breast irradiation as compared to the simulation method.

Table 1: General characteristic of the studied population

Variables	SGA (°)	CGA (°)	BB (ß) (°)	C (°)	SLLD (cm)	S (cm)	DA (°)
Minimum	52.0	51.0	20.8	20.8	8.0	16.0	1.0
Maximum	309.0	310.0	42.0	42.0	17.0	28.0	5.5
Mean	185.9	185.7	33.0	32.9	11.6	21.3	2.9
Std. Deviation	121.2	121.2	4.4	4.6	2.1	2.8	1.0
SEM	17.1	17.2	0.6	0.7	0.3	0.4	0.1
CV	65.18%	65.30%	13.50%	14.04%	17.80%	13.19%	33.17%

The parameters include; simulated gantry angle (SGA), gantry angle obtained with breast bridge (BB), skin to lateral laser distance (SLLD), calculated angle from the proposed formula (C), couch angle (DA), and calculated gantry angle considering the quadrant of the gantry (CGA).

From the general characteristic of the studied population, the range, mean, SD and SEM of SGA *vs.* CGA as well as BB *vs.* C were comparable as indicated in Table 1. The coefficient of variation of the results generated by the simulation method (65.18%) was also similar to the coefficient of variation of the results generated by the proposed formula (65.30%). The CV of the beta angles results from the breast bridge (13.50%) was also consistent with the CV generated by the beta angles from the proposed formula (Table1).

When the mean values of the simulated gantry angles (SGA) result was compared to the calculated gantry angle from the proposed formula (CGA) using paired *t*-test, there was no significant difference ($p = 0.124$) in the result as shown in Figure 1. There was also no significant difference ($p = 0.581$) when results of the beta angle from the breast bridge (BB) was compared to results of the beta angle from the proposed formula (C) (Figure 1).

From the Bland-Altman analysis in Figure 2, when the reference simulator method was compared to the calculated formula with regards to the gantry angle generated, it indicated that the calculated formula was producing gantry angles very close to the reference method. This finding is in complete agreement with earlier observations made in Table 1 as well as Figure 1. The estimated bias (i.e. mean difference between the two methods) is 0.2; the precision (i.e.

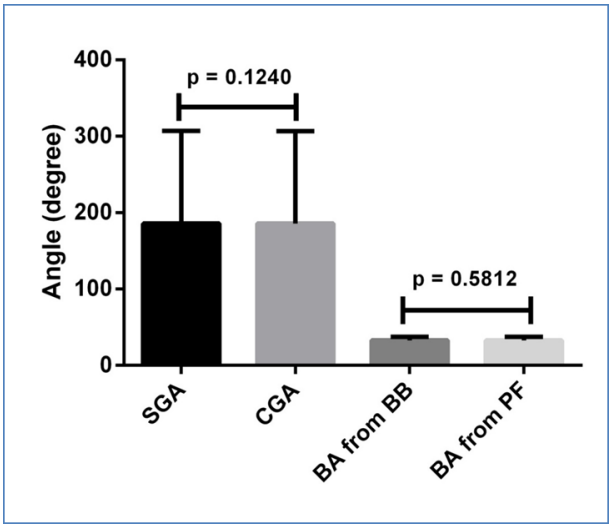

Figure 1: A comparison of SGA *vs.* CGA as well as beta angle (BA) from the breast bridge (BB) *vs.* BA from the proposed formula (PF) using paired *t*-test

standard deviation of the mean difference) for the calculated formula is 0.90 and the 95% limits of agreement ranged from -1.57 to 1.97. From this analysis, only 1 point out of the 50 fell out of the 95% limit of agreement, but this point is however, within the 3° allowable difference between the two methods (Figure 2A).

Method comparison of the beta angle results produced from the breast bridge as well as the calculat-

ed formula also indicate good agreement in line with earlier observations made in Table 1 and Figure 1. The estimated bias (i.e. mean difference between the two methods) is 0.1; the precision (i.e. standard deviation of the mean difference) for the calculated formula is 0.90 and the 95% limits of agreement ranged from -1.70 to 1.90. From this analysis, 3 points out of the 50 fell out of the 95% limit of agreement, 2 out of which are within the 3° allowable difference between the two methods. The other points were 4° above the corresponding results generated by the breast bridge (Figure 2B).

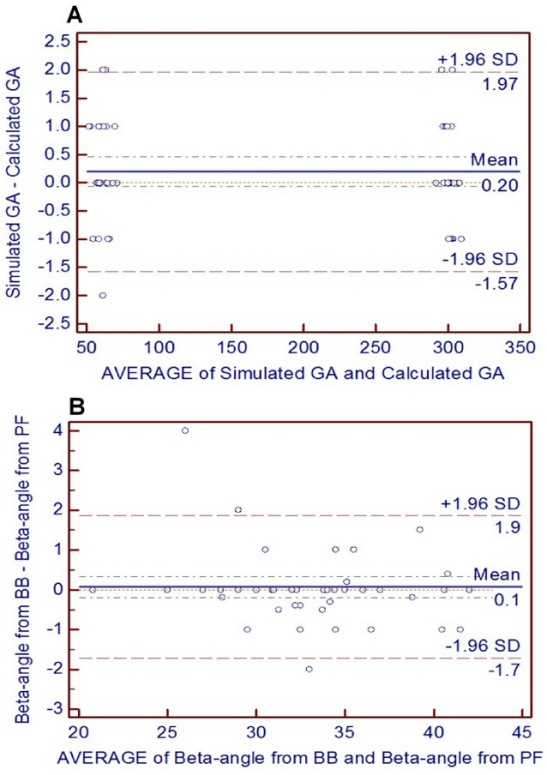

Figure 2: (A) Bland-Altman graph of difference scores for SGA vs. CGA and (B) Bland-Altman graph of beta-angle from Breast Bridge vs. beta-angle from proposed formula

Considering the two methods with regards to gantry angle, none of the gantry angle values generated by the proposed formula was different from the corresponding value from the simulator by 3° or more. With regards to the beta angle results from the proposed formula only 1 point was differed by 3° or more (i.e. 4°) from the beta angle generated by the breast bridge. Further estimations were done on the sensitivity and specificity of the calculated formula with regards to the gantry angle and beta angle at a cut-off of > 3° (Figure 3). At this cut-off, the sensitivity and specificity of the calculated formula for gantry angle were 100% and 100% respectively with the area under the curve (AUC) being 1.00 ($p < 0.0001$) (Figure 3A). At the same determined cut-off, the sensitivity and specificity of the calculated formula for gantry angle are 100% and 98% respectively with the area under the curve (AUC) being 1.00 ($p < 0.0001$) (Figure 3B).

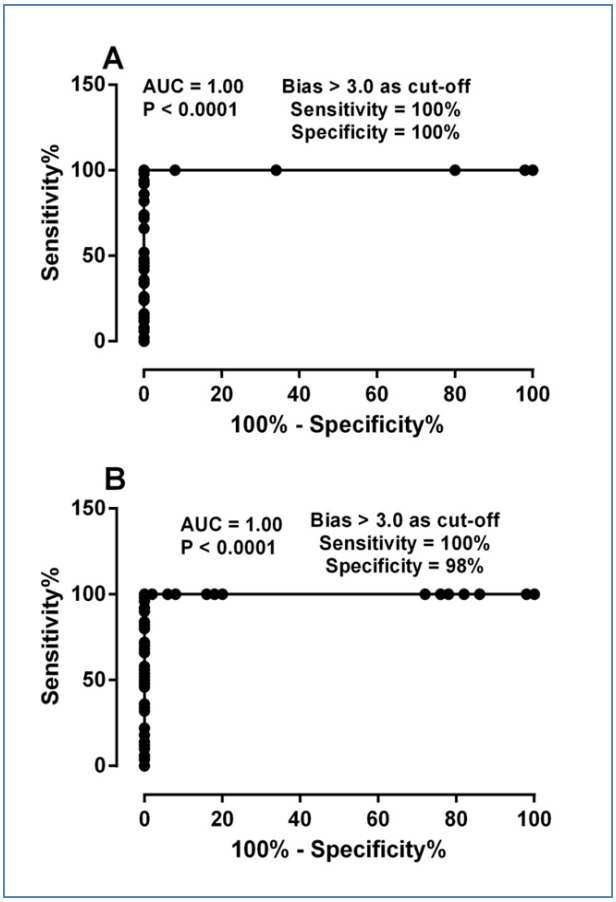

Figure 3: Receiver Operator Characteristic analysis of the proposed formula using the simulator as the reference method with regards to gantry angle (A) and beta angle (B)

CONCLUSION

A fair assessment of the two methods shows that both methods can be utilized interchangeably. Furthermore, none of the gantry angle values generated by the proposed formula was different from the corresponding value from the simulator by 3° or more. In addition the sensitivity and specificity of the proposed formula with regards to the gantry angle and beta angle at a cut-off of > 3° were 100% respectively.

It suffices to note the limitation of the calculated gantry angle method. This limitation is as result of the breast bridge being unsuitable for patients with large or flaccid breasts as it did not fit on them well. Furthermore, patients with a separation of more than 28 centimeters could not be measured accurately with the breast bridge due to the inherent physical limitations of the breast bridge which was utilized for the study (Griffith and Short, 1994, Halperin *et al.,* 2008).

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Anti-ulcerant Activity of an Aqueous Fruit Extract of *Musa x paradisiaca* on Acetic Acid-Induced Gastric Ulceration in ICR Mice

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Peptic ulcer disease has and continues to cause high mortality in Ghana and other countries worldwide. This study investigates the anti-ulcerant effect of an aqueous fruit extract of *Musa x paradisiaca* and its possible receptor site of action to verify and ascertain its traditional use. Phytochemical analyses on the extract revealed the presence of alkaloid, tannins, saponin, glycosides and flavonoids. Thin layer and high performance liquid chromatography analyses performed on the extract to establish fingerprint chromatograms showed four spots and three peaks respectively. Acetic acid–induced (0.2 ml; 8%) gastric ulceration in ICR mice treated with 0.2, 0.4, and 0.8 mg/kg of the extract and 0.3 mg/kg Esomeprazole significantly decreased the ulcerative index ($P \leq 0.001$) and the number of ulcers formed per stomach and increased curative ratio ($P \leq 0.01 - 0.001$). Histopathological studies of gastric mucosa showed corrections in the architectural distortions caused by the acetic acid-induced ulceration. Contractile effect of histamine on the isolated guinea-pig ileum was significantly inhibited ($P \leq 0.001$) by Mepyramine and the extract. The aqueous fruit extract of *Musa x paradisiaca* has anti-ulcerant property in ICR mice and possibly works as an antagonist to histamine receptors.
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Keywords*: Ulcer, traditional medicine,* rodent, Musa x paradisiaca, acetic acid.

INTRODUCTION

Peptic ulcer is a disease of the gastro-intestinal tract characterized by mucosal damage secondary to pepsin and gastric acid secretion and it usually occurs in the stomach and proximal duodenum (Ramakrishnan and Salinas, 2007). Peptic ulcer may also result from *Helicobacter pylori* which survive the acidic environment of the stomach (Baako and Darko, 1996; Goodman *et al.,* 1997; Peach *et al.,* 1997). Infections and co-morbidities that are associated with peptic ulcer disease e.g., cytomegalovirus, tuberculosis, Crohn's disease, hepatic cirrhosis, chronic renal failure, sarcoidosis and myeloproliferative disorders increases the risk of patients having more complications (Ramakrishnan and Salinas, 2007; Huang *et al.,* 2012). The use of non-steroidal anti-inflammatory drugs (NSAIDs) could also lead to peptic ulcer development (Bytzer and Teglbjaerg, 2001; Hamid *et al.,* 2006) while burns and trauma, acute illness, multi-organ failure and ventilator support can cause physiologic stress ulcers (Ramakrishnan and Salinas, 2007; Wachirawat *et al.,* 2003). The lifetime risk for developing peptic ulcer is approximately 10% (Snowden, 2008).

Peptic ulcers are associated with several signs and symptoms which include, abdominal and epigastric pain, indigestion, loss of appetite, weight loss, vomiting, heart burns and reflux disease (Spiegelhalter *et al.,* 1987). In very severe conditions, certain complications like gastrointestinal bleeding, perforation and gastric outlet obstruction may set in (Hilton *et al.,* 2001).

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A number of drugs including proton pump inhibitors, prostaglandin analogues, histamine receptor antagonists and cyto-protective agents are available for the treatment of peptic ulcer. Most of these drugs produce several adverse reactions including toxicities and may even alter biochemical mechanisms of the body upon chronic usage (Ariyphisi *et al.,* 1986).Although there are several effective orthodox medications for the treatment of ulcer on the market, research into plant extracts with potent medicinal activities have been on the rise as they are readily available and reported to be comparatively safer,. Most African societies depend on medicinal activities of plants as their orthodox congeners are expensive and not readily accessible. Ethno-medicinal remedies are being adopted in disease treatment as herbs are staging a comeback and herbal renaissance is happening world-wide (Chinthana and Ananthi, 2012).

In the Ashanti Region of Ghana, the freshly chopped fruits of *Musa x paradisiaca* are steeped in hot water and the decoction taken orally for the treatment of peptic ulcer. The aim of the study was to determine the anti-ulcerant activity of the aqueous fruit extract of *Musa x paradisiaca* (Family Musaceae) as used traditionally in the management of peptic ulcer and the possible receptor site(s) of action.

MATERIALS AND METHODS

Collection, Identification and Authentication of Plant Material

The unripe fruit of *Musa x paradisiaca* (Family Musaceae) was obtained in January, 2013, at Ayeduase, a suburb in the Kumasi Metropolis of the Ashanti Region. It was authenticated at the Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. A specimen of the plant sample (voucher number: KNUST/HM1/2012/S010) has been kept in the Department of Herbal Medicine, KNUST, Herbarium.

Preparation of Aqueous Fruit Extract of *Musa x paradisiaca*

The unripe fruit of *Musa x paradisiaca* was washed thoroughly and chopped into small pieces. Eight kilograms of the chopped pieces was soaked in 11 litres of distilled water and allowed to stand for at least 8 hours and strained. The strained solution was then dried in a hot air oven (Gallenkamp Oven 300 Plus series, Weiss Technik, UK) at 40°C to obtain 15 g of a solid mass which was labeled aqueous fruit extract of *Musa x paradisiaca* (AEMP) for use in this study. AEMP was then reconstituted in distilled water and administered at different doses to experimental animals.

Phytochemical Screening

Phytochemical analysis was done to ascertain the presence of secondary metabolites such as flavonoids, phytosterols, alkaloids, glycosides (saponin glycosides, anthracene glycosides, cyanogenetic glycosides), tannins and terpenoids on AEMP using standard procedure as described by Wagner and Bladt, (1996); Harborne (1998) and Kujur *et al.* (2010).

Thin Layer Chromatography

Aluminium pre-coated silica gel plates 60 F_{254} (0.25 mm thick) was cut to an appropriate size so as to fit in a chromatank. AEMP (5 mg) was constituted in ethanol (95%) and applied onto the TLC plates as spots with the aid of capillary tubes at one end of the plate in a straight line of about 2 cm above the edge and 1.5 cm away from the margins. Using the one way ascending technique of TLC development, the plates bearing the dried spots were placed in a chromatank saturated with a chloroform, ethanol and water (ratio: 7:3:0.5) solvent system as the mobile phase (Praha *et al.,* 2011). The zones on the TLC plates corresponding to separated components were detected under UV light 254 nm and 366 nm by spraying with anisaldehyde (0.5 % w/v) in an acetic acid/sulphuric acid/methanol mixture (ratios: 10:5:85) and heating for 5-10 minutes at 105°C.

High Performance Liquid Chromatography (Qualitative Analysis)

Approximately 2 ml of a 0.1% w/v ethanol solution of AEMP was transferred into 1cm square cuvette and placed in a double beam UV machine (T90 +

UV/Visible Spectrophotometer, PG Instruments Ltd., UK). A quantity (20 µl) of the sample was analyzed isocratically at a wavelength of 230 nm (flow rate of 1 ml/min) to obtain a chromatogram.

Ethical and Biosafety Considerations
Laboratory studies were carried out in a level 2 biosafety laboratory. Protocols for the study were approved by the Departmental Ethics Committee. All activities during the studies conformed to accepted principles for laboratory animal use and care (EU directive of 1986: 86/609/EEC). Biosafety guidelines for protection of personnel in the laboratory were observed.

Experimental Animals
ICR mice (22-26 g) were obtained and maintained in the animal house of the Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi. The animals were housed in plastic cages with soft wood shavings as bedding. They were fed with normal commercial animal food pellet from Ghana Agro Food Company Limited (GHAFCO), Tema, Ghana (with water *ad libitum*) and kept under ambient conditions of temperature, relative humidity, and light/dark cycle throughout the experiment.

Drugs and Chemicals Used
Acetic acid (BDH Limited, Poole England) was used to induce ulcer in the mice while Esomeprazole (AstraZeneca Pharmaceuticals, USA), a proton pump inhibitor, was used as the reference antiulcerant.

Induction of Gastric Ulceration
The protocol for the induction of gastric ulceration was as described by Wang *et al.,* (1989). Experimental animals were starved for 24 hours but were provided with water *ad libitum*. Mice were given 0.2 ml of 8% acetic acid orally. After 5 hours they were sacrificed, dissected and their stomachs removed. The stomachs were dissected along the greater curvature, the contents removed and washed with normal saline to enable observation for ulcerative lesions.

Experimental Procedure
Gastric ulceration was induced in 50 ICR mice. After 6 hours, the animals were put into five groups (A-E) of 10 animals each. Group A was treated with 1 ml/kg distilled water only (vehicle group). Groups B, C, and D were treated with 0.2, 0.4, and 0.8 mg/kg AEMP respectively and Group E was treated with 0.3 mg/kg Esomeprazole. Another group, F, in which ulcerations were not induced were also kept and given 1 ml/kg distilled water. All treatments were conducted for 7 days after which the mice were sacrificed and their stomach removed and examined by count for ulcerative lesions. The maximum length of each lesion in millimetres was determined and the sum of the lengths of all lesions in each stomach expressed as the Ulcerative Index (UI) (Sivaraman and Muralidharan, 2010). The Curative Ratio (CR), expressed in percentage, was determined for each group using the formula:

$$CR\,\% = \frac{(UI\ of\ Control - UI\ of\ treatment)}{UI\ of\ Control}\ x\ 100$$

The stomachs were then fixed in 10% buffered paraformaldehyde for histopathological evaluation.

Determination of Site of Action of AEMP
A 2 cm long guinea-pig ileum was mounted in 20 ml of Tyrode solution in a Harvard tissue bath (Harvard Apparatus Ltd, Kent, UK) maintained at 32^{o}C as described by Koffuor *et al.,* (2012). The tissue was constantly aerated and allowed to stabilize in the bath for 15 minutes. With a contact time of 30 seconds, a time cycle of 1 minutes and a Harvard kymograph (Harvard Apparatus Ltd, Kent, UK) speed of 4 mm/min, a complete dose-response tracing was generated for Acetylcholine ($2.0 \times 10^{-3} - 2.56 \times 10^{-1}$ µg/ml). A sub-maximal response of about 75% of the maximum response given by a dose of 6.4×10^{-2} µg/ml of Acetylcholine, was selected. Equipotent doses (doses that gave similar responses to the submaximal response selected for Acetylcholine) of Nicotine (9.2×10^{-2} µg/ml) and Histamine (1.28×10^{-1} µg/ml) were obtained and responses matched.

A dose of Hexamethonium (0.05 mg/ml) was added to the organ bath and left in contact with the tissue for 30 seconds after which the equipotent dose of Nicotine was added to the bath and response recorded. The tissue was then washed free of the drugs and the step was repeated for AEMP (0.16 mg/ml) and the matched dose of Nicotine. The procedures were performed for Atropine (5.0×10^{-6} mg/ml)/ Acetylcholine, AEMP/Acetylcholine, Mepyramine (0.2 mg/ml)/Histamine and AEMP/Histamine.

Data analysis

All graphs and statistical evaluations were done using GraphPad Prism version 5 (GraphPad Software, San Diego, CA, USA). Data were presented as mean ± SD. Significant differences between percentage inhibitions of agonists (comparing to "zero inhibition") were determined using One-Way Analysis of Variance followed by Dunnett's Multiple Comparisons Test *post hoc*. $P \leq 0.05$ was considered statistically significant.

RESULTS

Phytochemical Screening

The phytochemical screening of the aqueous extract showed the presence of alkaloid, tannins, saponins, glycosides and flavonoids.

Thin Layer Chromatography

The developed TLC plate showed the presence of four different components with the following retention factors (Table 1).

High Performance Liquid Chromatography

HPLC analysis showed the development of 4 peaks which represent the presence of four groups of compounds absorbing ultraviolet radiation at 280 nm in the AEMP (Figure 1).

The Effect of AEMP on Acetic Acid-Induced Gastric Ulcer

It was observed that the vehicle-treated group had the highest lesion lengths. AEMP-treated groups (0.2-0.8 mg/kg) showed significant ($P \leq 0.001$) reduction in gastric ulceration similar to the esomeprazole-treated group (Table 2). Photographs taken after acetic-acid treatment of ICR mice showed gastric erosion and ulceration (Plate 1B) which were healed on treatment with 0.3 mg/kg esomeprazole and AEMP (Plate 1C and 1D). Histopathological studies showed gross appearances of hemorrhagic gastric mucosal lesions during the ulceration (Plate 2F), which was healed and regenerated during treatment with 0.3 mg/kg esomeprazole and 0.4 mg/kg

Table 1: The number of spots obtained on the

Spots	Retention Factor
1	0.725
2	0.875
3	0.925
4	0.975

Developed plate was viewed under ultraviolet light at 254 nm and 366 nm.

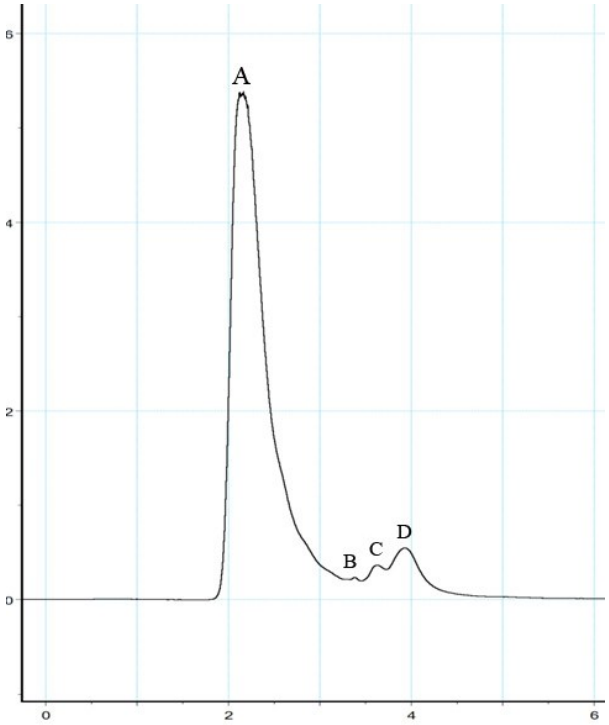

Figure 1: A chromatogram of AEMP showing peaks A, B, C, and D of different heights and area.

Table 2: The number of ulcers formed, the Ulcerative Index (UI) and the Curative Ratio (CR) for 7-day AEMP and Esomeprazole (ESO) treated ulcerated mice and ulcerated but untreated mice

Parameters	Control	ESO	AEMP		
		0.3 mg/kg	**0.2 mg/kg**	**0.4 mg/kg**	**0.8 mg/kg**
Number of ulcers/ stomachs/group	1.50 ± 0.4	1.05 ± 0.33 ns	1.00 ± 0.41 ns	1.00 ± 0.41 ns	0.75 ± 0.48 *
UI (mm)	7.50 ± 0.87	2.25 ± 1.32***	1.50 ± 0.50***	1.25 ± 0.48***	1.00 ± 0.58***
CR (%)	0.00 ± 0.00	65.47 ± 19.96**	79.52 ± 7.36***	80.95 ± 7.53***	85.71 ± 8.25***

Values are mean ± SD (n = 10), ulceration was induced in all groups. Significant differences between the ulcerated but treated groups and the control were determined using One-Way Analysis of variance followed by Dunnet's Multiple Comparison's Test. ns implies P > 0.05; * implies P ≤ 0.05** implies P ≤ 0.01; *** implies P≤ 0.001.

AEMP as seen in photomicrographs in Plate 2G and 2H.

Determination of Site of Action of AEMP

Acetylcholine, nicotine, histamine showed contractions of the guinea-pig ileum. AEMP inhibited significantly the responses of acetylcholine, nicotine and histamine by 4.7 ± 1.56 %, 12.44 ± 4.9 % and 54.2 ± 7.2 % respectively compared to zero inhibition (Figure 2). This shows that the extract has significant (P ≤ 0.001) anti-histaminic activity with minimal anti-nicotinic and anti-muscarinic activities.

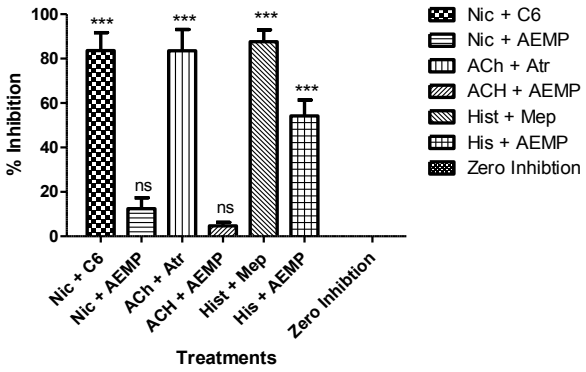

Figure 2: Percentage inhibition of Nicotine (Nic), Acetylcholine (Ach), and Histamine (Hist) by Hexamethonium (C6), Atropine (Atr), Mepyramine (Mep), and AEMP. Values plotted are means SD, n=3. Significant differences between percentage inhibitions were determined (comparing to zero inhibition) using One-Way Analysis of Variance followed by Dunnett's Multiple Comparisons Test *post hoc*. ** implies P≤ 0.01; * implies P≤ 0.001.**

DISCUSSION

Acetic acid-induced gastric ulceration has been noted to be a suitable model for investigating anti-ulcerant effect as it causes round haemorrhagic lesions which resemble human ulcers. The ulcers do not heal spontaneously and are simple and reproducible (Okabe *et al.,* 2010). Acetic acid ulcer induction is due to the embolization of blood vessels in the gastric mucosa leading to a blockade in mucosal blood flow. The imbalance between mucosal oxygen supply and demand leads to ischemia and necrosis of the mucosal tissue (Okabe *et al.,* 2010).

AEMP increased curative ratio and significantly decreased the number of ulcers formed per stomach and ulcerative index thus indicating anti-ulcerant effects. The anti-ulcerant activity could have been due to the collective effects of glycosides, flavonoids, alkaloid, tannins and saponins which are phytochemicals present in the extract. These classes of phytochemicals conform to the presence of more specific phytochemicals reported by Ahlborn

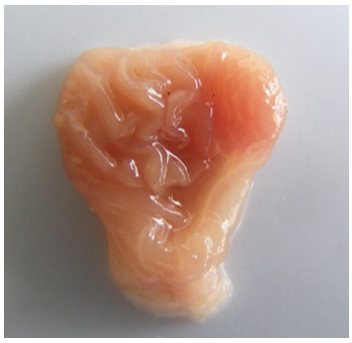

Plate 1A: Normal stomach of an ICR mouse

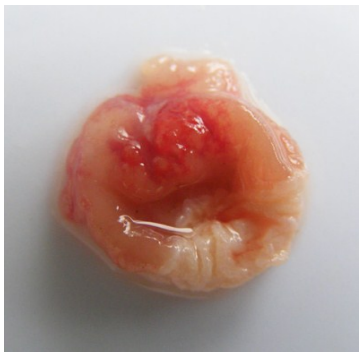

Plate 1B: Acetic acid-induced gastric ulceration in an ICR mouse

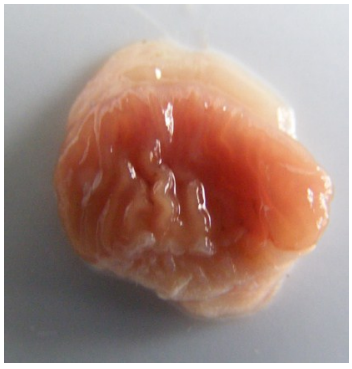

Plate 1C: 0.3 mg/kg Esomeprazole treatment after acetic acid-induced gastric ulceration in an ICR mouse

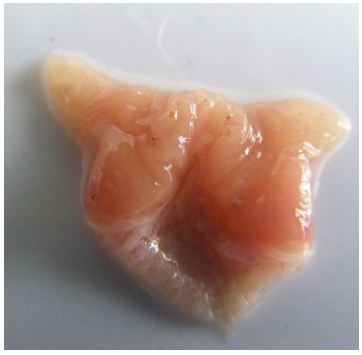

Plate 1D: A 0.4 mg/kg AEMP treatment after acetic acid-induced gastric ulceration in an ICR mouse

Plate 1: Photographs of the gastric mucosa of normal, ulcerated and ulcerated but treated with AEMP and Esomeprazole for 7 days in ICR mice

(2013) in *Musa x paradisiaca*. *Musa x paradisiaca* contains a glycoside named aucubin which has anti-histaminic activity (Ahlborn, 2013) thus supporting the anti-histaminic activity observed. Research by Lewis and Shawb, (2001) reported the presence of leucocyanidin, a flavonoid, which has the ability to increase mucus and mucosal protein production. Lectin, a protein with a high affinity for carbohydrates, is said to be present (Ahlborn, 2013) and this binds a mannose oligosaccharide in the cell wall of normal commensals to the gastric and intestinal lining. The presence of these normal commensals prevents the colonization of the gastric lining by *H. pylori* hence reducing their ulcerative potency.

Tannins, specifically allantoin, present in *Musa x paradisiaca* (Ahlborn, 2013), has astringent and protein coagulation properties. Baicalein (a flavonoid) and iridoid (a glycoside) have anti-inflammatory activity (Ahlborn, 2013). The characteristics of these phytochemicals can be associated with the wound healing (anti-ulcerant) and mucosal healing ability of AEMP. Gastritis is one of the signs of gastric ulceration and may be caused by erosion of the mucosal defenses. *H. pylori* have the ability to cause mucosal damage by immune/inflammatory response alteration in the host (Suerbaum and Michetti, 2002).

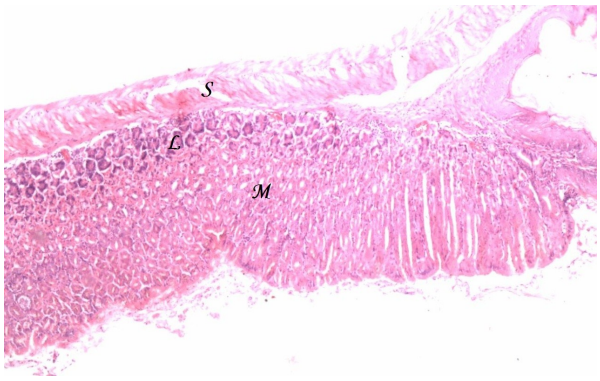

Plate 2E: Normal arrangement of cells in the gastric mucosa and sub-mucosa of an ICR mouse

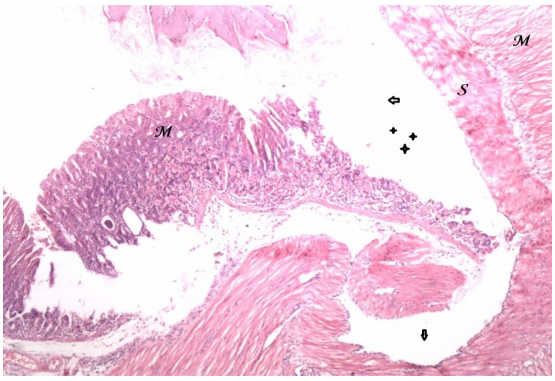

Plate 2F: Gross appearances of hemorrhagic gastric mucosal lesions (shown by arrows) in an ulcerated ICR mouse

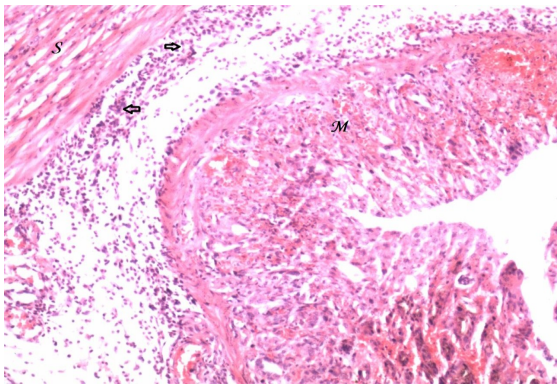

Plate 2G: Effect of the esomeprazole (0.3mg/kg) showing mild mucosal healing

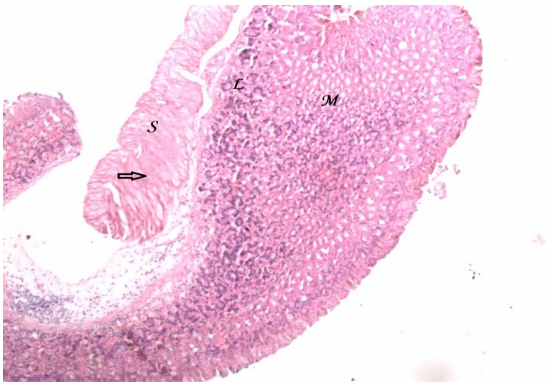

Plate 2H: Gastric mucosa of a 0.4 mg/kg AEMP-treated mouse showing mucosal regeneration

Plate 2: Photomicrographs of the gastric mucosa of normal, ulcerated and ulcerated with AEMP and Esomeprazole treatment for 7 days in ICR mice

In general, mucosal defense and repair mechanisms are important in protecting the integrity of the mucosal layer and resultant inhibition of these mechanisms could lead to necrosis. Examples of such defense mechanisms include pre-epithelial factors (mucus-bicarbonate-phospholipid barrier), surface epithelial cells connected by tight junctions, bicarbonate and mucus production, prostaglandins, heat shock proteins and blood flow through the mucosal vessels (Laine *et al.,* 2008).. . AEMP in effect, may directly protect the mucosal layer from noxious substances such as NSAIDs, acids and alcohol and enhances mucosal regeneration. In ulcer healing it is important that the distorted architecture of the mucosal and submucosal layers regain their normal arrangement through cell regrowth and protein coagulation. The findings of histopathological studies conducted from this study confirmed the mucosal cell regenerative property of the AEMP.

Parietal cells of the stomach bear receptors for three stimulators of acid secretion: Acetylcholine

(muscarinic type receptor), Gastrin and Histamine (H2 type receptor). Gastric acid is produced via the M2 receptors through the parasympathetic stimulation of neurons (e.g. vagus) which stimulates acetylcholine production and hence binding on the M2 receptors for the release of the gastric acid by the parietal cells. Gastrin, synthesized in the endocrine cells of the gastric mucosa also stimulates the parietal cell to produce acid (Rang *et al.,* 2007). Histamine from enterochromaffin-like cells is however thought to represent the final mediator of acid secretion but the magnitude of the stimulus appears to result from a complex additive or multiplicative interaction of signals of each type (Furutani *et al.,* 2003; Yao and Forte, 2003; Samuelson and Hinkle Forte, 2003; Zhu, 2010). From the results of this study, the AEMP extract had significant anti-histaminic effect, with minimal anti-nicotinic and anti-muscarinic effects. This reveals the possible suppressor effects of the AEMP on gastric acid secretion hence its subsequent healing of gastric ulcers as excessive gastric acid secretion is one of the factors promoting mucosal damage. Excess acid in the stomach, affects the integrity of the mucus membrane thereby leading to erosions and subsequent ulcerations.

The HPLC chromatogram run at a wavelength of 230 nm showed three peaks with one distinct peak. Evidence of these peaks signifies the presence of UV absorbing secondary metabolites. Some of these secondary metabolites have aromatic structure and show varying polarity according to their retention times. The most prominent peak if determined to be monocomponent may be used as a biomarker in the standardization of the extract.

CONCLUSION

The aqueous fruit extract of *Musa x paradisiaca* has anti-ulcerant effect in acetic acid-induced peptic ulcers in ICR mice. This fruit extract had mainly antihistaminic and gastric mucosal cell regenerative property. The chromatogram obtained should serve as a fingerprint or a standard to which another sample prepared under the same conditions may be compared to.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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