Original Research Article

Comparative analysis of phytochemical constituents and antibacterial activity of crude and purified ethanol and ethyl-acetate extracts of *Euphorbia hirta* L. whole plant

ABSTRACT

Aims: To study the phytochemical constituents and antibacterial efficacy of crude and purified *Euphorbia hirta* whole plant extract on Gram-positive bacteria isolated from otitis media.

Study design: Experimental Research design.

Place and Duration of Study: Sample: Department of Microbiology (Mtech Laboratory) and Department of Chemistry(Organic Chemistry Laboratory), School of Sciences, Federal University of Technology, Akure, Ondo State, Nigeria. Between November 2018 and March 2019.

Methods: The streak plate method was used for bacterial isolation, maceration method for *Euphorbia hirta* whole plant extraction using ethanol and ethyl-acetate as solvents. The *E. hirta* whole plant extracts were purified using column chromatography method. The extracts were assayed on the test bacterial isolates by agar diffusion technique. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts were carried out by agar dilution and agar diffusion techniques, respectively.

Results: The ethanolic extract had the highest extraction yield (19%). The *Staphylococcus aureus* was resistant to multiple antibiotics: amoxicillin (30µg), gentamycin (10µg), and streptomycin (30µg).The phytochemical screening of crude plant extracts showed presence of flavonoids, glycosides, saponins, tannins and terpenoids. At 100mg/ml, crude and purified ethanol extracts showed antibacterial activity with 18±0.57mm and 14±0.57mm respectively on *Streptococcus pyogenes*. The MIC and MBC of purified ethanol extract ranged between 6.25-50mg/ml and 25mg/ml-100mg/ml respectively.

Conclusion: This research showed that *E. hirta* whole plant extract possesses antibacterial activity. The purified *E. hirta* whole plant extract showed higher inhibitory effect compared to crude extracts. This is an indication that purified *E. hirta* whole plant extract can be used in the development of novel therapeutic drugs in the treatment of otitis media.

Key words: Antibacterial activity, antibiotics resistance, ethanol extract, Euphorbia hirta whole plant, phytochemical constituents, otitis media.

INTRODUCTION

Otitis media is the inflammation of the mucous membrane of the middle ear cleft. It is one of the most common infectious diseases of childhood worldwide (1). It is a leading cause of healthcare visits and the sequalae are responsible for cases of preventable hearing loss (2). Bacteria have remained the most important etiological agents in otitis media (1).

In recent years, drug resistance in bacterial pathogens has developed due to indiscriminate use of conventional antibiotics. This situation, coupled with the undesirable side effects of certain antibiotics is of serious health concern (3). The urgent need for alternative treatment methods to combat the rise in antibiotics resistance has led to search for new antimicrobial compounds with different chemical structures and new mechanisms of action, for emerging and re-emerging infections (4).Medicinal plants have curing actions, due to the presence of complex chemical components (5).

The WHO reported that over 80% of the world's population rely on traditional medicine for therapy. *Euphorbia hirta L.* belongs to family Euphorbiaceae, commonly known as asthma herb and it is known in Nigeria as Emi-ile,Kadanya, Itasin Uloko, OgbunaIzu by the Yoruba, Hausa, Edo and Igbo ethnic groups (3). It is an annual hairy plant, common in waste sites, over the roadsides and also available open grasslands. It can grow to a height of 50 cm. It has ared, slender stem covered with yellowish bristly hairs specifically in the younger parts with abundant milk sap (6). Traditionally, *E. hirta* is believed to be effective in the treatment of asthma, bronchitis, athlete's foot, dysentery, enteritis, and skin conditions (7), the stem sap is used in the treatment of eyelid styes, otitis and in wound healing (8).Study reported that the plant exhibited antipyretic, anti-helmintic, antispasmodic, antibacterial, antifertility, antifungal and anti-inflammatory activities (7).The *E. hirta* have been documented to contain saponins, alkaloids, flavonoids, tannins and phenolic acids. Therefore *E. hirta* is said to have potential for the development of novel therapeutic agents in the disease treatments (6, 5). However, there is limited study comparing the antibacterial effect of crude and purified extract of *E. hirta* whole plant on bacterial pathogens associated with otitis media. Therefore, this study was undertaken to investigate the antibacterial properties of crude and purified ethanol and ethyl-acetate extract of *E. hirta* whole plant against Gram-positive bacterial isolates associated with otitis media.

MATERIALS AND METHODS

Plant Collection and Identification

The whole plant of *E. hirta* L. was used as the sample under investigation. The plant was collected at Federal University of Technology, Akure (FUTA), Nigeria. The plant was identified and authenticated at the Department of Crop, Soil and Pest Management, FUTA.

Extraction of *E. hirta* whole plant

The *E. hirta* whole plant was washed in distilled water, air dried and pulverized using mortar and pestle. The solid constituents in the *E. hirta* plant were extracted using two solvents: 100% ethanol and 100% ethyl-acetate (BDH England) as extraction solvent. The crude extracts were obtained by extracting 100 grams each of pulverized plant in 500ml of respective solvents. The mixture was left to stand for 24 h in a shaking water bath maintained at 40°C. The mixture was then filtered using a clean double layered muslin cloth and then with Whatman No. 1 filter paper (UK). The filtrate was then evaporated to dryness using a rotary evaporator (RE-52A Union laboratories England) (3). The percentage yield of the crude extract was determined for each solvent.

$$\text{The percentage extract yield was estimated as: } \frac{\text{dry weight} \times 100\%}{\text{dry material weight}}$$

The extract was aseptically streaked on sterilized nutrient agar plates and incubated at 37^0C for 24h for sterility check. The extracts that showed no growth was reconstituted by dissolving in 5% Dimethylsulphoside (DMSO) to obtain200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.56 mg/ml concentration and kept at 4^oC prior use as stock crude extract.

Qualitative and quantitative phytochemical screening of ethanol and ethyl-acetate extracts of *Euphorbia hirta* whole plant

Phytochemical screening was carried out on the powdered plant material for the presence and quantity of bioactive constituents such as tannins, phenols, alkaloids, glycosides, anthroquinones, saponins and flavonoids (12).

Purification of plant extracts of ethanol and ethyl-acetate extracts of *Euphorbia hirta* whole plant

The crude ethanol and ethyl-acetate extracts of *E. hirta* whole plant was chromatographed on silica gel (60-120 mesh size) matrix packed into a glass column and eluted successively with 100% petroleum ether, 100% chloroform, 100% ethyl acetate and 100% methanol. The sample was mixed with a little gel to form powder, and was then carefully poured on top of the packed silica gel in the column. It was then covered with glass wool to avoid spattering of the eluant on the extract which may affect the separation process. The solvent system was gently poured on the sample by the side wall of the inside column with the help of glass funnel. The column tap was gently opened to allow the

eluant to flow at the rate of 30 drops per minute. The eluted fractions were collected in 100ml conical flasks (13,14).

Fractions of purified extracts of same solvents were pooled together and reconstituted by dissolving in 5% DMSO to obtain 200, 100, 50, 25, 12.5, 6.25, 3.13 and 1.56 mg/ml concentration and kept at 4^{o}C as stock purified extracts prior to use.

Collection of bacterial isolates

Clinical Gram-positive bacterial isolates for this study were obtained from Microbiology Department culture collection. The clinical bacterial isolates (*Staphylococcus aureus* and *Streptococcus pyogenes*) were locally isolated from ear swab samples of otitis media patients at University of Medical Sciences Teaching Hospital, Akure, Nigeria between the period of November, 2018 and March, 2019. Typed bacterial isolates were obtained from Federal Institute of Industrial Research, Oshodi, Nigeria. Thetyped bacterial isolates were(*Staphylococcus aureus* NCTC 6571and *Streptococcus pyogenes* ATCC 12384).These organisms were confirmed by morphological identification and biochemical tests. The stock cultures were maintained at 4°C on slopes of Nutrient agar (Hi-media) and sub cultured for 24 h before use (9).

Antibiotics sensitivity pattern of bacterial isolates

Antibiotic susceptibility testing was performed using the Kirby Bauer disk diffusion method (10). The Gram-positive antibiotic discs used for these bacterial isolates were manufactured by MAXICARE MEDICAL LAB. These antibiotics include pefloxacin 10 µg (PEF), gentamycin 10 µg (CN), ampiclox 30 µg (APX), zinnacef 20 µg (Z), amoxacillin 30 µg (AM), rocephin 25 µg (R), ciprofloxacin 10 µg (CPX), streptomycin 30 µg (S), septrin 30 µg (SXT) and erythromycin 10 µg (E). 18-24 h old broth cultures of the bacterial isolates were standardized to 0.5 McFarland standard (10^{8} cfu/mL). A sterile swab stick was inserted into the standardized isolate inocula, drained to remove excess load of inoculum and inoculated by spreading on the surface of prepared Mueller-Hinton agar plate. Then, the inoculated Mueller-Hinton agar plate was allowed to dry for 15 minutes with the lid closed. The antibiotic impregnated discs of known concentration were carefully applied on the inoculated Mueller-Hinton agar plates using flame-sterilized forceps. The plates were then incubatedat37^{0}C for 24h. The zones of inhibition were measured using a transparent calibrated ruler to the nearest millimetre and recorded. The recorded values indicated the inhibitory effect of the antibiotics on the test bacterial isolates (11)**.**

Determination of the antimicrobial activity of ethanol and ethyl-acetate extracts of *Euphorbia hirta* whole plant

Antibacterial activity of ethanol and ethyl-acetate extracts of *E. hirta* whole plant against test bacterial isolates was carried out using agar-well diffusion method (14). 18-24 h old broth culturesof the bacterial isolates were standardized to 0.5 McFarland standard (10^{8} cfu/mL) and inoculated on the solidified Mueller Hinton agar plates using sterilized cotton swabs and allowed to set for 15 minutes. Wells of 6 mm diameter and 3 mm depth were made in the solidified agar using a sterile borer. About 1ml of test samples which are the crude and partially purified ethanol and ethyl-acetate extracts (100 mg/ml) were aseptically dispensed into the wells and allowed to stand for 15 minutes for pre-diffusion of samples. The remaining two wells were filled with 1ml of ciprofloxacin at a concentration of 5 mg/ml (positive control) and distilled water (negative control). The plates were allowed to stand upright for 1h for proper dilution of the solutions into the medium then incubated at 37ºC for 24 hours. The sensitivity of the test bacteria to the extracts were determined by measuring the diameters of the zone of inhibition surrounding the wells with a transparent calibrated ruler in millimetre (mm). The effects of the crude and purified *E.hirta* extract on bacterial isolates were compared with standard antibiotic (ciprofloxacin) at a concentration of 5 mg/mL which served as a positive control. All the tests were performed in triplicates.

Determination of minimum inhibitory concentration and minimum bactericidal concentration of ethanol extracts of *Euphorbia hirta* whole plant

Determination of the minimum inhibitory concentration (MIC) was carried out using the Broth dilution method (15). Stock solutions of crude and purified ethanol extract prepared was used, 1ml each of the extracts of concentration 200, 100, 50, 25, 12.5, 6.25, 3.13 and 1.56 mg/mL was dispensed in

different test tubes with sterile broth. Control tubes without extract were constituted similarly. Ciprofloxacin was included as positive control and distilled water as negative control in different tubes. Then 1 ml of an 18 h old culture of each bacterial isolate earlier adjusted at 0.5 McFarland standard was dispensed into each tube and thoroughly mixed. The tubes were incubated at 37°C for 24 h and observed for growth in form of turbidity. The test tube with the lowest dilution with no detectable growth by visual inspection was considered the MIC. 0.1 ml of bacterial suspension from the MIC tubes that did not show any growth was streaked on solidified Mueller Hinton agar plates and incubated at 37°C for 24 h. After incubation, the concentration at which no visible growth was seen was recorded as the MBC (17).

Statistical analysis

All the experiments were carried out in triplicate and data obtained was analysed by two-way analysis of variance using SPSS 20.0. Means were compared by Duncan's new multiple range test and considered statistically significant at $P \leq 0.05$.

RESULTS

Percentage yield of ethanol and ethyl-acetate *E. hirta* whole plant extract

The percentage yield of extract with respect to the extraction solvent used is presented in Figure 1. The ethanol extract had the highest extraction yield of 7.1% while ethyl-acetate had the least yield of 5%.

The qualitative phytochemical constituents of the extract

Table 1 shows the phytochemical properties of the ethanol and ethyl-acetate extracts of *E. hirta* whole plant. Saponins, tannins and glycosides were seen in all the extracts of the plant. Phlobatanins and alkaloids was absent in both plant extracts. Steroid was present in only ethanol extract but absent in ethyl-acetate extract.

The quantitative phytochemicals constituents of the extract

Table 2 shows the quantity of phytochemicals present in the ethanol and ethyl-acetate extract of *E. hirta* whole plant. Saponin had the highest quantity in both extracts, while glycosides was lowest in ethanol extract (0.48 ± 0.09^{a}), while tannin had the lowest quantity in ethyl-acetate extract (0.50 ± 0.06^{b}).

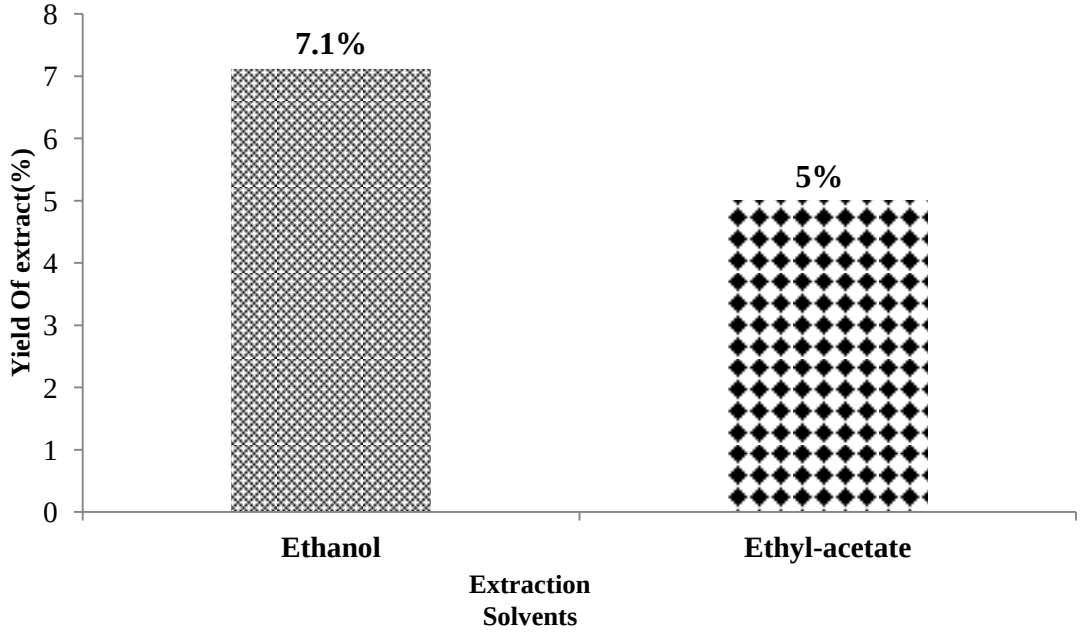

Figure 1: Percentage yield (%) of the crude ethanol and ethyl-acetate extracts of *E. hirta* whole plant.

Table 1: Qualitative phytochemical constituent of *E. hirta* whole plant extract

	Extraction solvents	
Phytochemical constituents	**Ethanol**	**Ethyl-acetate**
Saponins	+	+
Glycosides	+	+
Tannins	+	+
Phlobatanins	_	_
Steroids	+	_
Terpenoids	+	+
Alkaloids	_	_
Phenols	+	+

Key: + Present, - Negative

Table 2: Quantitative constituents of phytochemical *Euphorbia hirta*whole plant extract

	Extraction solvents	
Phytochemical constituents	**Ethanol**	**Ethyl-acetate**
Saponins	**63.68±0.37^{e}**	**60.68±0.37^{d}**
Flavonoids	**1.57±0.34^{b}**	**3.03±0.34^{c}**
Glycosides	**0.48±0.09^{a}**	**2.15±0.09^{c}**
Tannins	**1.88±0.06^{f}**	**0.50±0.06^{b}**
Steroids	**2.25±0.03^{c}**	**0.00±0.03^{a}**
Terpenoids	**27.39±0.17^{b}**	**37.75±0.17^{f}**
Phenols	**13.34±0.50^{c}**	**32.66±0.50^{b}**

Data are represented as mean ± standard error (n=3) with the same superscript across the row are not significantly different (*P*<0.05).

Antibiotic Sensitivity Patterns of Bacterial Isolates

Figure 2 shows the antibiotics sensitivity pattern of the Gram-positive bacterial isolates from otitis media and their respective typed cultures. The *S. aureus* and *S. aureus* NCTC 6571 bacterial isolates showed total resistance to streptomycin and gentamycin, while their highest susceptibility was recorded for ciprofloxacin with 25±0.37mm and 30±0.37mm respectively. The *S. pyogenes* and *S. pyogenes* ATCC 12384 showed resistant to rocephin, while highest susceptibility was recorded in ciprofloxacin with 28±0.37mm and 29±0.373mm respectively.

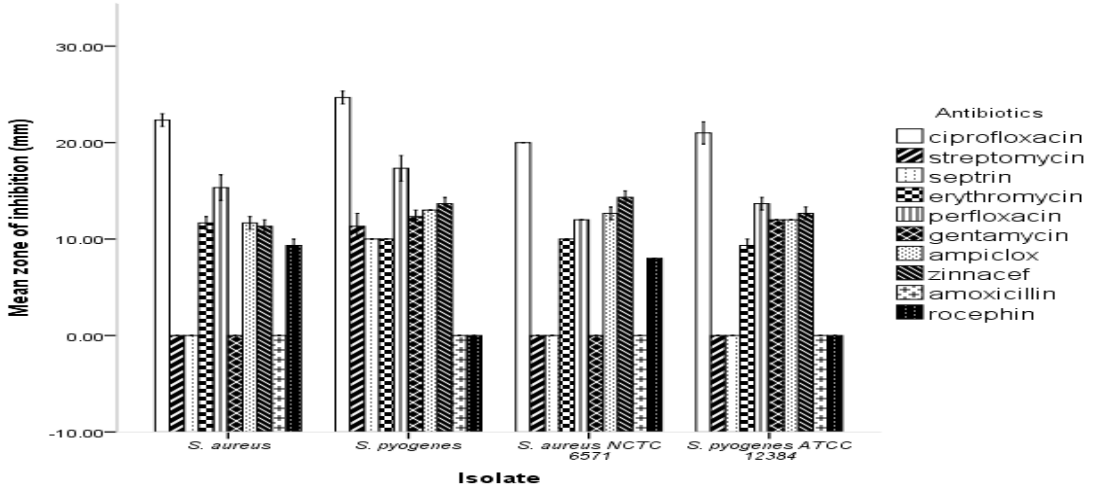

Figure 2: Antibiotics sensitivity pattern of bacterial isolates.

Antibacterial effect of extracts of *Euphorbia hirta* whole plant

Figure 3 shows the effect of ethanol extract on Gram-positive bacterial isolates at concentration of 100mg/ml. The crude extract and purified extract showed inhibitory effect against all isolates. The purified and crude extract showed highest inhibitory effect against *S. pyogenes* with zones of 18±0.57mm and 14±0.57mm respectively.

Figure 4 shows the effect of ethyl-acetate extract (100 mg/ml) on the bacteria isolates. The crude and purified extracts showed no inhibitory effect against *S. aureus* NCTC 6575and *S. pyogenes* ATCC 12384. The purified extract showed highest inhibitory effect against *S. aureus* and *S. pyogenes* with 12±0.667mm on both bacterial isolates. The crude ethyl-acetate extract showed inhibitory effect against only *S. pyogenes* with zone of 11±0.667mm.

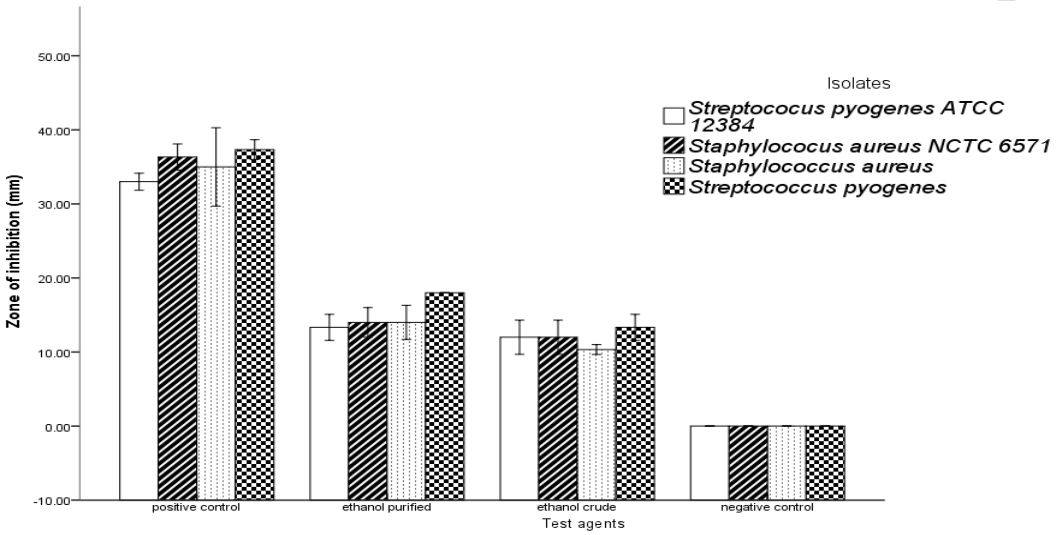

Figure 3: Antibacterial effect of *Euphorbia hirta* ethanol extract (100mg/ml) on Bacterial isolates

Key: Positive control=ciprofloxacin (0.1mg/ml), Negative control=Distilled water

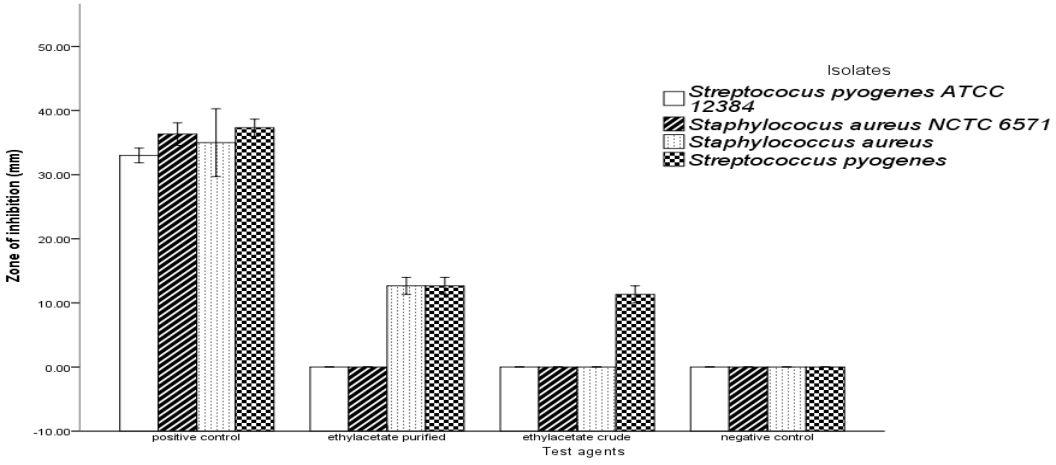

Figure 4: Antibacterial effect of *Euphorbia hirta* ethyl-acetate extract (100mg/ml) on Bacterial isolates

Key: Positive control=ciprofloxacin (0.1mg/ml), Negative control=Distilled water

Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of *E. hirta* whole plant ethanol extract.

Figure 5 showed the MIC of extracts on bacterial isolates. The purified ethanol extract of *E. hirta* plant displayed MIC ranging between 6.25-25mg/ml. The lowest MIC recorded in *S. pyogenes* (6.25 mg/ml). The crude ethanol extract of *E. hirta* plant displayed MIC ranging between 25- 50mg/ml. The lowest MIC recorded in *S. pyogenes* (25 mg/ml).

Figure 6 showed the MBC of extracts on bacterial isolates. The purified ethanol extract of *E. hirta* plant displayed MBC ranging between 12.5- 100mg/ml. The lowest MBC recorded in *S. pyogenes*. The crude ethanol extract of *E. hirta* plant displayed MBC ranging between 100- 200mg/ml. The lowest MBC recorded in *S. pyogenes*.

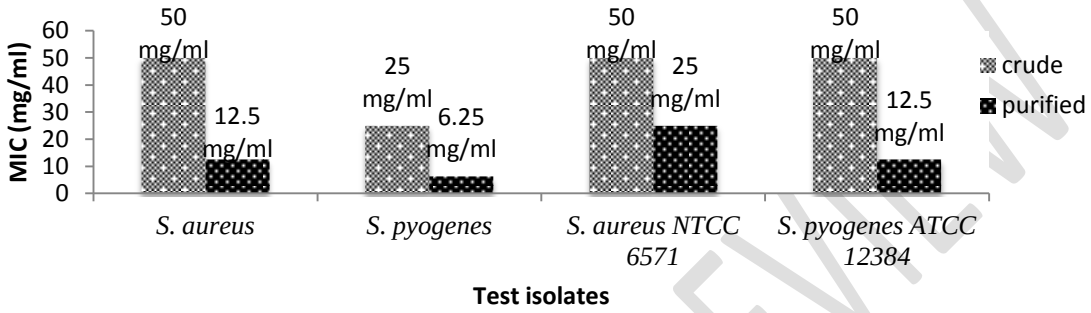

Figure 5: Minimum Inhibitory Concentration (mg/ml) of crude and purified ethanol extracts on gram positive bacterial isolates

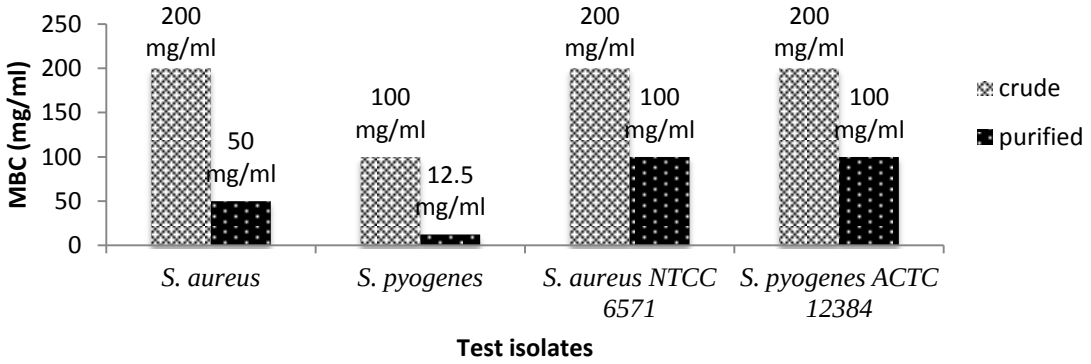

Figure 6: Minimum Bactericidal Concentration (mg/ml) of crude and purified ethanol extracts on gram positive bacterial isolates.

Discussion

This work offers a guide to the extraction, phytochemical screening, purification and antibacterial activity of *E. hirta* whole plant ethanol and ethyl-acetate extracts.Ethanol had the highest extraction yield (7.1%). This is not in line with El-Mahmood (18) who reported highest yield in cold water and Patel and Patel (6) who reported highestyield in acetone compared to other solvent employed in extraction of *E. hirta* plant collected from the Federal University of Technology Yola, Nigeria and Gujarat College, Ahmedabad respectively. A study stated that factors like the age of the plant, geographical location and the polarity of the solvent used affects the yield (18). The location and higher polarity of ethanol compared to ethyl-acetate may explain the higher extraction yield recorded in this study.

Phytochemical screening of the crude extracts of *E. hirta* whole plant revealed the presence of some bioactive components such as tannins, phenolics, terpernoids, glycosides, saponins and flavonoids. This is in line with a report that showed the presence of tannins, flavonoids and glycosides in crude ethanolic extract of *E. hirta* (19). These compounds have potentially significant application against

human pathogens, including those that are infectious (20). Several authors have linked the presence of these bioactive compounds to the antimicrobial properties of crude plant extracts (21, 22). Tannins are known to possess inhibitory effect on bacteria by deactivating the bacterial enzymes and proteins (23). Terpenoids are lipophilic compounds with bacterial cell memebrane disruption potential (17), it possesses anti-inflammatory properties, these compounds induce both antibacterial and antifungal effects (24). Phenolic compounds have medicinal properties such as anti-inflammatory, antioxidant, anti-allergic, antibacterial and antiviral activity as a result of their possible influence on intracellular redox status (5).

All tested bacterial isolates were susceptible to ciprofloxacin, which is similar to report by Muluye(25). *S. aureus* was resistant to multiple antibiotics (amoxicillin, streptomycin and gentamycin). This is similar study reported multiple drug resistance to isolates from otitis media (26). The *E. hirta* whole plant extracts had antibacterial effect against tested bacterial isolates, which is in agreement with previous work which showed antibacterial potential of *E. hirta* plant extract against bacteria isolates from urinary tract infection (6).The antibacterial potency of *E. hirta* plant extract shown in this study may be linked to the secondary bioactive components present in the plant, with the ability to disrupt the cell membrane and inhibit protein synthesis in these gram positive isolates (17).The purified *E. hirta* extracts showed significantly higher antibacterial effect on tested bacterial isolates compared to the crude extracts; this may be because inert impure substances are present in the crude extracts which could have inhibited its antibacterial activity (27).Ethanol extract (100 mg/ml) showed higher antibacterial effect on *S. aureus* (14 ± 0.667mm) compared to complete resistance recorded in ethyl-acetate extract by *S. aureus*. This is similar to report on ethanol extract of *E.hirta* (100 mg/ml) against *S. aureus* (14.33 mm) from Federal Medical centre, Abeokuta (28). The phyto-constituents present could explain the antibacterial effect shown in this extract, the presence of steroid which is absent in ethyl-acetate and the higher quantity of saponin in the ethanol extract (63.68 ± 0.37) compared to the ethyl-acetate (60.68 ± 0.374). Saponin is said to be a detergent-like substance with antibacterial potential (29). Sterol (a subgroup of steroid) of *E. hirta* stem was reported to have antibacterial activity against *S. aureus* with zone of 19.5mm (30).

The MIC and MBC assay were used to evaluate the efficacies of antibacterial agents. In this study, the ethanol extract used gave varying MIC and MBC values in bacterial isolates. According to Patel and Patel (6) the purified ethanol extract of *E. hirta* plant displayed an excellent antibacterial activity against *S. aureus* with MIC of 12.5mg/ml, compared to the crude ethanol extract of *E. hirta* plant which displayed an antibacterial activity against *S. aureus* with MIC of 50 mg/ml. A previous study revealed crude ethanol *E. hirta* extract showed a low MIC of 8.42 mg/ml against *S. aureus* (28). The low MIC of ethanol extract on *S. aureus* is an indication of the extract's use in treating antibiotics resistant *S. aureus* infections (21) implicated in otitis media. This may help minimize side effect associated with the use of antibiotics. Agents with high antibacterial activity gave low MIC and MBC values. Antibacterial agents are considered bacteriostatic when the ratio MBC/MIC >4 and bactericidal when MBC/MIC ≤4 (31). This study shows that purified ethanol extract showed potential of a bactericidal agent because of its cidal effect against most tested isolates.

CONCLUSION

The study revealed the presence of saponin, glycoside, tannin, flavonoid, terpenoid and phenols in the ethanol and ethyl-acetate extracts of *E. hirta* plant. Saponin had the highest quantity in ethanol extract. Ciprofloxacin had inhibitory effect on the tested bacterial isolates. *E. hirta* plant whole plant extracts possess antibacterial activity which compared positively with ciprofloxacin. *E. hirta* whole plant ethanol extract had higher antibacterial activity compared to the ethyl-acetate extract. The purified ethanol extract of *E. hirta* plant had higher antibacterial activity against *S. aureus* and *S. pyogenes* in comparison to the ethyl-acetate extract. The tested bacteria were more susceptible to purified *E. hirta* plant ethanol extract than the crude *E. hirta* plant ethanol extract. These findings showed that purified ethanol extract of *E. hirta* L. whole plant may be employed as an alternative in treatment of otitis media. Thus, there is need to investigate the cost effectiveness of using these *E. hirta* extracts in chemotherapy and also further studies should be carried out *in-vivo*.

COMPETING INTERESTS

Authors declare no competing interest exists.

REFERENCES

1. Ilechukwu GC, Ilechukwu CGA, Ubesie AC, Ojinnaka CN, Emechebe GO, Iloh KK. Otitis Media in Children: Review Article. *Open Journal of paediatrics*. 2014; 4: 47-53.
2. Ashik AA. Otitis Media in Children: A Review. *Journal of Pharmaceutical Sciences and Research*. 2016; 8(8): 844-849.
3. Adjeroh LA, AjuruchiVC, Nnokwe JC, Azuwike CO, Mgbemena IC. Preliminary phytochemical screening and antimicrobial/antifungal activities of leaf extracts of Euphorbia hirta. *Journal of Natural Sciences Research*. 2015; 5(16).
4. Beegum BN, Devi GT. Antibacterial activity of selected Seaweads from Kovalan South West coast of India. *Asian Journal of Microbiological Biotechnology Environmental Science*. 2003; 5:319-22.
5. Karanga Y1, Ilboudo O1, Bonzi S. Phytochemical and Antifungal Properties of *Euphorbia hirta* L against *Fusarium moliniforme* and *Phomasorghina*. *Nat Prod Ind J*. 2017; 13(1):105.
6. Patel NB, Patel KC. Antibacterial Activity of *Euphorbia hirta* L. Ethanomedicinal Plant against Gram Positive UTI Pathogens. *The World Journal of Engineering and Applied Sciences*. 2014; 1(1): 1-5
7. Kader J, Noor HM, Radzi SM, Abdul Wahab NA. Antibacterial activities and phytochemical screening of the acetone extract from *Euphorbia hirta*. *International Journal of Medicinal Plant Research*. 2013; 2 (4): 209-214.
8. Igoli JO, Ogaji TA, Tor-Anyii, Igoli, NP. Traditional Medicine Practice Amongst the Igede People of Nigeria. *African Journal of Traditional CAM*. 2005; 2(2): 134-152.
9. Association of Analytical Chemist. Official methods of analysis, Association of official analytical chemist 19th edition, Washington D.C., USA. 2012.
10. Igwe KK, Ijeh II, Okafor PN Anika SM. Effect of *VernoniaAmygdalina*.DelEthanolic Extract Fraction on Serum Prolactin in Lactating and Non Lactating Female Albino Wistar*IOSR Journal of Agriculture and Veterinary Science*.2015; 8(1): 82-88.
11. Bob IM, Terungwa AK, Flora OU, Patrick EE. Effect of Phytochemical Fractions of *Vernoniaamygdalina*on Liver of STZ Induced Diabetic Male Wistar Rats *International Journal of Curr.Microbiology and Applied Science*. 2016; 5(11): 656-667.
12. Sundaram S, Dwivedi P, Purwar S Antibacterial Activities of Crude Extracts of Chlorophytumborivilianum to Bacterial Pathogens. *Research Journal of Medicinal Plant*. 2011; 5:343-347.
13. Vadhana P, Singh BR, Bharadwaj M, Singh SV. Emergence of Herbal Antimicrobial Drug Resistance in Clinical Bacterial Isolates. *Pharm Anal Acta*. 2015; 6:434.
14. Cheesbourgh M. Medical laboratory manual for tropical countries Part 2: second edition. England: ButterworthrHeineman LTD: 45-70. 2010.
15. Adegoke AA, Tom M, Okoh Anthonyl, Jacob S. Studies on multiple antibiotic resistant bacterial isolated from surgical site infection. *Scientific Research and essay*. 2010; 5(24): 3876-3881.
16. Obi OJ, Ike AC. Prevalence and antibiogram profile of Salmonellae in intensively reared and backyard chickens in Nsukka area, Nigeria. *Nigeria Journal of Biotechnology*. 2015. 30:18-25.
17. Abegunde MT, Akinpelu DA, Omololu-AsoJ, Otusanya OO, Akinlolu JT. Determination of Antimicrobial, Antioxidant and Phytochemical Properties of *Cocos nucifera*linn Endocarp Extract on Bacteria Associated with Human Infection. *Journal of Pharmaceutical Microbiology*. 2018; 4(1): 4.
18. El-Mahmood AM. Antibacterial activity of crude extracts of *Euphorbia hirta* against some bacteria associated with enteric infections. *Journal of Medicinal Plants Research*. 2009; 3(7) 498- 505.
19. Srilaskshimi M, Saravanan R, Dhachinamoorthi D, Senthilkumar K, Sri TD. Antibacterial activity of *Euphorbia hirta* Extracts. *IJRAP*. 2012; 3(3).
20. El-Mahmood AM, Doughari JH, Chanji FJ. In-vitro antibacterial activities of crude extracts of Nauclealatifolia and Daniella oliveri. *Science. Res. Essay*. 2008;3(3):102-105.
21. Ogbolie JN, Ogeke CC, Okoli IC, Anyanwu BN. Antibacterial activities and toxological potentials of crude ethanolic extracts of *Euphorbia hirta*. *African Journal of Biotechnology*. 2007; 6(13): 1544-1548.

22. Owolabi OJ, Omogbai EKI, Obasuyi O. Antifungal and antibacterial activities of the ethanolic and aqueous extracts of *Kigelia Africana* (Bignoniaceae) stem bark. *African Journal of Biotechnology*. 2007;6(14):1677- 1680.
23. Frutose P, Hervas G, Giraldez FJ, Mantecon AR. Review. Tannins and ruminant nutrition. *Spanish Journal of Agricultural Research*. 2004; 2(2): 191-202.
24. Asha S, Deevika B, Sadiq M. A *Euphorbia hirta* Linn - A review on traditional uses, phytochemistry and pharmacology. *World Journal of Pharmaceutical Research*. 2014. 3(4); 180-205.
25. Muluye D, Wondimeneh Y, Ferede G, Moges F, Nega T. Bacterial isolates and drug susceptibility patterns of ear discharge from patients with ear infection at Gondar University Hospital, Northwest Ethiopia. *BMC Ear Nose and Throat Disorder*. 2013; 13:10.
26. Seid A, Deribe F, Ali K, Kibru G. Bacterial otitis media in all age group of patients seen at Dessie referral hospital, North East Ethiopia. *Egypt Journal of Ear Nose Throat Allied Sciences*. 2013; 14:73–78.
27. Abubakar EM. Antibacterial activity of crude extracts of Euphorbia hirta against some bacteria associated with enteric infections. *Journal of Medicinal Plants Research*. 2009; 3(7):498-505.
28. Titilope KK, Rashidat EA, Christiana OC , Kehinde ER, Omobolaji JN, Olajide OJ. In- vitro antimicrobial activities of Euphorbia hirta against some clinical isolates. *Agric. Biol. J. N. Am*. 2012; 3(4):169-174.
29. Arabski M, Wegierek-Cuik A, Czerwonka G, Lankoff A, Kaca W. Effects of Saponins against Clinical *E. coli* strains and Eukaryotic cell line. *Journal of Biomedicine and Biotechnology.* 2012. 6.
30. Singh G, Kumar P, Jindal A. Antibacterial effect of sterols of some medicinal plants. *International Journal of Pharmaceutical Sciences*. 2012. 4(3); 159-162.
31. Gatsing D, Nkeugouapi C, Nkah B, Kuiate J, Tchouanguep F. Antibacterial activity, bioactivity and acute toxicity evaluation of the leaf extract of *Alchorneacordifolia* (Euphobiaceae). *International journal of Pharmacology*. 2010; 6:173-182.