The activity of commercial antimicrobials, and essential oils and ethanolic extracts of *Olea europaea* on *Streptococcus agalactiae* isolated from pregnant women

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**Abstract**

**Background:** *Streptococcus agalactiae* also known as Group B Streptococcus (GBS) is a major cause of disease in pregnant women and new born babies where it causes early and late onset disease characterised by sepsis, pneumonia and meningitis. Ten to 37% of pregnant women in the world are colonised with GBS while intrapartum antibiotic prophylaxis has led to significant reduction in early onset disease. The increase in drug resistant microorganisms has become a major threat. Development of vaccines is still in progress so there is need for new and safer alternatives to treatment.

**Methods:** Benzyl penicillin, Ampicillin, Cefotaxime, Ceftriaxone, Levofloxacin, Erythromycin, Clindamycin, Linezolid, Vancomycin, Tetracycline and Cotrimoxazole, *Olea europaea* leaf extracts and essential oil were tested against GBS isolates from South Africa and Namibia.

**Results:** The isolates showed 100% sensitivity to benzyl penicillin, ampicillin, ceftriaxone, levofloxacin, erythromycin, clindamycin, linezolid, vancomycin, Tetracycline and Cotrimoxazole, *Olea europaea* leaf extracts and essential oils. Only one isolate (0.6%) was resistant to cefotaxime and 23.4 and 10.4% were resistant to clindamycin and erythromycin respectively.

**Conclusion:** GBS isolates showed sensitivity to *O. europaea* extracts at low minimum inhibitory concentrations. *B lactams* are still the drugs of choice for treatment of GBS disease but *O. europaea* extracts potent as an alternative source of antimicrobials.

**Keywords:** *Olea europaea*, *Streptococcus agalactiae*, Antimicrobial, *β* Lactams

**Background**

Antibiotic resistance has existed since the beginning of the antibiotic era. The increase in the frequency of drug resistant and invasive bacteria, a reduction in the development and approval of new drugs and projections of an imminent post antibiotic era threaten human health worldwide. Current practices of Intrapartum Antibiotic prophylaxis (IAP) against *S. agalactiae* involve the administration of *β*-lactam antibiotics penicillin and ampicillin. The alternatives to penicillin include: cefazolin, clindamycin, erythromycin and vancomycin however, these alternative drugs have not been assessed in clinical trials and data on their concentration levels in amniotic fluid, foetal blood and tissue are limited [1, 2].

There is an increasing prevalence of multidrug resistant microorganisms and these bacterial pathogens are a major threat to existing antibacterial therapeutic options [3]. GBS is generally sensitive to *β*-lactams antibiotics but isolates with reduced minimum inhibitory concentrations (MICs) have been described from as far back as 1995 [1] while MIC changes of cefazolin to GBS have been described from 1999 [2]. In a recent study in Italy, *
outright resistance to GBS was noted for the first time where out of 65 GBS isolates recovered in the study, 12.07% were resistant to benzyl penicillin [4].

Antibiotic resistance can arise due to the expression of genes that confer resistance to drugs in an organism. The most clinically significant genes are those that code for enzymes responsible for hydrolysis of β-lactams (bla genes). These genes confer high level bacterial resistance to β-lactam antibiotics like cephalosporins (including 4th generation cephalosporins), penicillins and monobactams (except cefamycins) [5]. The bla genes can be borne on plasmids or transposons which are transferable and this facilitates horizontal spread of antibiotic resistance [6].

While erythromycin and clindamycin were indicated as alternative therapy to GBS IAP, there has been increasing resistance of GBS to these drugs and in the United States of America (USA), clindamycin and erythromycin resistance have been recorded at 13–20% and 25–35% respectively [2]. Across the world, resistance has been estimated at 14.5–70% and 8.2–70% for erythromycin and clindamycin respectively [1]. The CDC guidelines removed erythromycin as a second line prophylactic antibiotic in women with penicillin allergy due to the high resistance of GBS to it while clindamycin is still acceptable but only after susceptibility testing [7].

The increasing resistance of GBS to empiric antibiotics has led to the administration of expensive drugs and those previously considered as very toxic [3]. While the development of vaccines is still in progress, there is need for new and safe alternatives to GBS treatment [1]. The World Health Organization (WHO) suggests the use of safe and effective medicinal plants; and in both developing and developed countries, herbal medicine has become an integral part of primary health care due to its low cost and availability [8]. The antimicrobial activities of several natural plant products against GBS that have been documented indicate them as potential alternatives to IAP. Essential oils from various plants have been shown to have antibacterial, antiviral, anti-leishmanial, antioxidant, analgesic and anti-inflammatory properties [1, 3]. One such plant which is widely used for its medicinal properties is *Olea europaea* species of the genus *Olea* and subspecies *Africana* which is known as umNquma in the Eastern Cape, South Africa.

*O. europaea* is known to contain a wide range of phytochemical components with medicinal properties from as far back as 1854 and a number of phytochemical components have been isolated from different parts of *O. europaea*. The bark, fruits, seeds, leaves, olive oil and wood contain phenolic compounds, flavonoids and secoridoids. Due to the presence of many compounds in different parts of the plant, it has an array of uses including control of plasma concentrations of glucose, cholesterol and urate [9].

Leaf extracts and essential oils from *O. europaea* have been reported to have antimicrobial activities against many microorganisms including bacteria, viruses, fungi and parasites. While research has shown that the plant does not have broad spectrum antimicrobial activity it has been shown to be potent against some Gram positive and Gram negative bacteria such as *Staphylococcus aureus* and *Escherichia coli* respectively [10]. Antimicrobial activity has also been shown against fungi such as *Candida albicans* and *Cryptococcus neoformans* while other researches have reported the antimicrobial activity of the plant against *Helicobacter pylori*, *Campylobacter jejuni*, *Bacillus subtilis* and *Pseudomonas aeruginosa* [9].

This study therefore documents the antibiotic susceptibility profiles of *S. agalactiae* isolates. It also seeks to determine the antimicrobial susceptibility of these isolates to leaf extracts and essential oils from *O. europaea* collected from the Cala community in the Eastern Cape, South Africa.

**Methods**

**GBS culture, isolation and identification**

A rectal and a vaginal swab were collected from each participant who were between 35 and 37 weeks gestation by a registered nurse. In South Africa (Eastern Cape), 100 participants were included in the study while in Namibia (Windhoek), 588 participants took part in the study. All swabs collected in South Africa were processed at the AEMREG laboratory, University of Fort Hare, Alice while samples in Namibia were processed at the Namibia University of Science and Technology, Microbiology Laboratory.

Samples were inoculated onto Columbia blood agar and Todd Hewitt broth (Rochelle Chemicals and Laboratory Equipment, Johannesburg, South Africa) and incubated at 37 °C for 24 h. Inoculums in Todd Hewitt broth were subcultured onto blood agar and incubated at 37 °C for 24 h and the cultures were checked for colonies exhibiting β-haemolysis on the blood agar. The colonies were tested for the catalase reaction and catalase negative colonies were further screened using the Lancefield grouping antisera (Becton Dickinson, New Jersey, USA). Colonies which tested positive for the latex agglutination assay were presumptively identified as GBS isolates and were confirmed using the Vitek 2 and by molecular techniques. All presumptive isolates were stored in glycerol stocks at −80 °C until further analyses.

**Molecular confirmation of isolates**

Glycerol stocks were inoculated in Muller Hinton broth for 24 h at 37 °C and streaked on Columbia blood agar. GBS isolates were confirmed by molecular techniques using a pair of primers specific for the *scpB* gene.
single colony of GBS was picked from Columbia blood agar containing 5% horse blood and suspended into 160 μL of nucleic acid free water in a 2 mL conical tube that was placed on a heating block and allowed to boil at 100 °C for 15 min. The mixture was centrifuged and the supernatant containing GBS DNA was transferred in a clean 1.5 mL microcentrifuge tube and stored at −80 °C. The amplification reactions were performed using the One Taq Master Mix (New England Biolabs, United Kingdom) according to the manufacturer’s recommendations. Thus, the reactions were performed in a final volume of 25 μL containing 12 μL of Master mix, 6 μL of purified DNA, 10 pmol of scpBF (5’ACAACGGAA GGCCTACTGTTC3’) and scpBR (5’ACCTGGTGT TTGACCTGAACTA3’) primers as adapted from Elbaradi et al., 2009 [11] and deionized water to adjust the final volume.

The cycling conditions were as follows: an initial denaturation at 94 °C for 4 min followed by 35 cycles of: denaturation at 93 °C for 1 min, annealing at 57 °C for 1 min and extension at 72 °C for 1 min. A final elongation step of 72 °C for 7 min was followed by a hold at 4 °C.

S. agalactiae ATCC reference strain BAA-2670 was used as positive control. Amplicons were loaded on 1% agarose gel stained with 10 μL ethidium bromide and electrophoresed for 45 mins at 110 V in 0.5X Tris/Borate/EDTA (TBE) buffer and viewed under transilluminator and photographed.

**Sensitivity testing using Vitek**

Antimicrobial sensitivity testing was performed using Vitek Sensitivity Card (AST-ST01) on the following antibiotics: Benzyl penicillin, Ampicillin, Cefotaxime, Ceftriaxone, Levofloxacin, Erythromycin, Clindamycin, Linezolid, Vancomycin, Tetracycline, Cotrimoxazole and Inducible Clindamycin Resistance (ICR). MICs were determined for each antibiotic on all the isolates and the results were interpreted according to the Clinical Laboratory Standards Institute (CLSI, 2015) guidelines. The results were classified as sensitive (S), intermediate (I) or resistant (R). All isolates were evaluated for inducible clindamycin resistance.

**Plant material**

*O. europaea* plant parts were collected in the Cala community, geographical coordinates 31.52.30°S, 27.69.80°E, Eastern Cape Province, South Africa during the winter of 2016. The plant was identified by Cala Traditional Health Practitioners using the vernacular name umNquma and was authenticated by Mr. Tony Dold, a Plant Taxonomist at Selmar Schönland Herbarium, Rhodes University, South Africa. The Voucher (No ADE 2016/2) was deposited in the Giffen Herbarium, University of Fort Hare.

**Preparation of plant extracts**

Leaves were harvested from the plants and washed in water to remove dust. The leaves were rinsed in distilled water to cleanse them of impurities and were dried in air for 3 days. The leaves were pulverized as previously described by Darsanaki et al., 2012. Fifty grams of leaf powder was mixed with one litre of ethanol and placed on a shaker to dissolve and thereafter, the solution was filtered and the solvent dried to obtain a crude ethanol extract of the *O. europaea*. For the extraction of essential oils, 200 g of dried pulverized was mixed with 200 mL of water and the mixture placed in a modified hydro distiller or a Clevenger's type apparatus and allowed to hydro distillate for 3 h for complete extraction of the essential oils from the leaves. The procedure was repeated thrice to obtain adequate essential oil for experimental assays and the extracted essential oil was dried using anhydrous sodium sulphate and extracts stored at −20 °C in actinic bottles from which working solutions were prepared with Dimethyl Sulfoxide (DMSO) for use in experiments.

**Gas chromatography-mass spectrometry (GC-MS) analysis**

The GCMS analysis of essential oil was performed on the Agilent 5977A MSD and 7890B GC system, Chemetrix; Agilent Technologies, DE (Germany) with a Zibron-5MS column. GC grade helium at 2 mL/min flow rate and splitless 1 mL injection was used and temperatures were set at 280 °C (Injector), 280 °C (Source) and 70 °C (oven) while the ramp settings were set as follows and held for 3 min; 15 °C/min to 120 °C, followed by 10 °C/min to 180 °C and finally 20 °C/min to 270 °C.

**Preparation of plant extracts for antimicrobial activity**

Plant extract and essential oils were diluted with 10% DMSO using serial dilution methods to make 300 mg/mL of essential oil and 0.5 mg/mL of leaf extract. These solutions were used as stock solutions for all antimicrobial testing.

**Agar well diffusion**

One hundred microliters of 0.5 McFarland standard of each isolate was prepared using sterile normal saline (0.9% w/v). Twenty microliters of each 0.5 McFarland preparation was inoculated onto Mueller Hinton agar and spread for a lawn growth of the bacteria on the agar. Standard bores of eight millimetres in diameter were made in the agar plates using a sterile cork-borer. Ten percent DMSO was used as a negative control while 5 μg of ciprofloxacin served as a positive control. One hundred microliters each of the ethanolic leaf extracts (0.5 mg/ml), essential oils (300 mg/ml), positive control and negative control were put in labelled wells and
allowed to stand for 1 h and culture plates incubated at 37 °C for 24 h. The test extracts were compared to the negative and positive control to determine the antimicrobial activity of the extracts on the isolates. Ten isolates each from both South Africa and Namibia were selected by systematic sampling and used in the experiment.

**Minimum inhibitory concentration (MIC) determination**

Four hundred microliters of broth was put in each of four sterile tubes and serial dilutions were made using 100 μL of extract creating four dilutions of each extract. The final extract concentration in each tube for the extracts is presented in Table 1.

Broth was used as a negative control while 5 μg of ciprofloxacin was used as a positive control. One hundred microliters of broth was placed in six labelled tubes for each experiment. One hundred microliters each of broth (negative control), 5 μg ciprofloxacin (positive control) and each of the dilutions for both essential oil and plant extract were put in a respective labelled tube and 20 μL of 0.5 McFarland of the test isolate was inoculated in each tube and incubated at 37 °C for 24 h. Thereafter, the test tubes were checked for turbidity and compared to the negative and positive control to establish the antimicrobial activity of the different concentrations of the extracts. Tubes without apparent growth were regarded as concentrations where the extract had growth inhibitory activities.

For the concentrations of the extract which showed antimicrobial effect against the bacteria it was determined if the extract had bactericidal or bacteriostatic effect. This was performed by inoculating 20 μL from each of the tubes above onto Mueller Hinton agar and the culture plates were incubated at 37 °C for 24 h. Absence of growth in the broth and agar plate was assumed as the extract having bactericidal effect while absence of growth in broth and bacterial growth on solid agar plates, indicates bacteriostatic effect of the extract.

**Results**

Results of antimicrobial sensitivity testing of antibiotics against GBS and susceptibility to plant extracts are presented below. Twenty GBS isolates (10 from South Africa and 10 from Namibia) were employed in antibacterial activities of testing of plant extracts using the Minimum Inhibitory Concentration (MIC) approach.

**Table 1 Concentrations of plant extracts after dilution**

| Tube number | Concentration of essential oil (mg/ml) | Concentration of leaf extract (mg/ml) |
|-------------|----------------------------------------|--------------------------------------|
| 1           | 33.3                                   | 0.1                                  |
| 2           | 3.7                                    | 0.02                                 |
| 3           | 0.4                                    | 0.004                                |
| 4           | 0.05                                   | 0.0008                               |

The antibiotic sensitivity patterns of the GBS isolates are presented in Table 2.

*S. agalactiae* isolates were sensitive to β-lactam antibiotics but some showed resistance to other antibiotics such as tetracycline, cotrimoxazole, erythromycin and clindamycin.

Table 3 shows the chemical composition of the essential oils of *Olea europaea* obtained after GC-MS analysis.

The GC-MS analysis of the plant extract showed that the plant contained flavonoids, flavonones, biophenols, benzoic acid derivatives, sterols and secoiridoids.

Essential oils and leaf extracts exhibited antimicrobial activity against GBS isolates and leaf extracts showed bigger zones of inhibition compared to essential oils.

**Discussion**

In this study 117 isolates from Namibia and 37 isolates from South Africa were analyzed and they were confirmed as GBS based on the *scpB* gene. All the isolates showed absolute sensitivity to benzyl penicillin, ampicillin, ceftriaxone, levofloxacin, linezolid and vancomycin with only one isolate (0.6%) showing resistance to cefotaxime as shown in Table 2. In reports from other studies done in other African countries, such as in Malawi [12], Ethiopia [13], Zimbabwe [14], South Africa [15] and Nigeria [16], GBS has not shown resistance to the β-lactams. In a study in Brazil, GBS also exhibited no resistance to β-lactams [17]. However, resistance to β-lactam group of antibiotics has been evolving as noted in reduced MICs for more than a decade and recently an Italian study recorded outright resistance by some GBS isolates to penicillin [4].

Thirty six isolates (23.4%) and sixteen isolates (10.4%) were resistant to clindamycin and erythromycin.

**Table 2 Antibiotic sensitivity patterns of GBS isolates**

| Antibiotic       | MIC (μg/mL) | Susceptible | Intermediate | Resistant |
|------------------|-------------|-------------|--------------|----------|
| Benzyl penicillin (P) | ≤ 0.12     | 154 (100.0) | 0 (0.0)     | 0 (0.0)  |
| Ampicillin (Amp)  | ≤ 0.25     | 154 (100.0) | 0 (0.0)     | 0 (0.0)  |
| Cefotaxime (CTX)  | ≤ 0.5      | 153 (99.4)  | 0 (0.0)     | 1 (0.6)  |
| Ceftriaxone (CRO) | ≤ 0.5      | 154 (100.0) | 0 (0.0)     | 0 (0.0)  |
| Levofloxacin (Lev) | ≤ 2        | 154 (100.0) | 0 (0.0)     | 0 (0.0)  |
| Erythromycin (E)  | ≤ 0.25     | 138 (89.6)  | 0 (0.0)     | 16 (10.4) |
| Clindamycin (CD)  | ≤ 0.25     | 118 (76.6)  | 0 (0.0)     | 36 (23.4) |
| Linezolid (Li)    | ≤ 2        | 154 (100.0) | 0 (0.0)     | 0 (0.0)  |
| Vancomycin (Va)   | ≤ 1         | 154 (100.0) | 0 (0.0)     | 0 (0.0)  |
| Tetracycline (T)  | ≤ 2        | 6 (3.9)     | 0 (0.0)     | 148 (96.1) |
| Cotrimoxazole (SXT)| ≤ 2       | 137 (89.0)  | 0 (0.0)     | 17 (11.0) |
| Inducible clindamycin | 7 (4.5)    |             |              |          |
| S/N | Chemical constituents | Chemical formula | RT  | Area % |
|-----|-----------------------|------------------|-----|--------|
| 1   | 2-Butenal, 2-ethenyl-  | C6H8O             | 3.255 | 1.56   |
| 2   | 2-Hexenal, (E)-       | C6H10O            | 3.292 | 2.95   |
| 3   | 1-Hexanol             | C6H14O            | 3.365 | 0.75   |
| 4   | Heptanal              | C7H14O            | 3.638 | 4.00   |
| 5   | (1S)-2,6,6-trimethylbicyclo[3.1.1] hept-2-ene | C10H16 | 3.975 | 1.89   |
| 6   | Pyridine, 3-ethenyl-  | C7H7N             | 4.204 | 2.21   |
| 7   | 2,4-Heptadienal, (E,E)- | C8H14O         | 4.412 | 1.58   |
| 8   | Octanal               | C8H16O            | 4.450 | 0.77   |
| 9   | alpha-Phellandrene    | C10H16            | 4.530 | 1.72   |
| 10  | 1,6-heptadiene,2,5,5-trimethyl | C10H18  | 5.674 | 0.73   |
| 11  | Acetaldehyde, (3,3dimethylcyclohexylidene),(E) | C10H16O | 5.720 | 1.07   |
| 12  | 1-Methylpentyl cyclopropane | C9H18O    | 6.059 | 2.23   |
| 13  | Decanal               | C10H20O          | 6.278 | 0.99   |
| 14  | Octane, 1-iodo-       | C8H17I           | 6.347 | 0.57   |
| 15  | 2,6-Octadien-1-ol, 3,7-dimethyl-(Z)- | C10H18O | 6.418 | 0.39   |
| 16  | 1,6,10-Dodecatrien-3-ol,3711,trimethyl-, [S-(Z)] | C15H26O | 8.508 | 1.82   |
| 17  | Caryophyllene         | C15H24O          | 7.206 | 0.78   |
| 18  | cis-beta-Farnesene    | C13H24           | 7.422 | 0.60   |
| 19  | 1-H-Inden-1-one, 2,4,5,6,7,7a-hexahydro-4,4,7a-trimethyl- | C12H18O | 7.931 | 0.46   |
| 20  | 1,4,7-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z- | C13H22O | 7.974 | 0.44   |
| 21  | trans-beta-lonone     | C13H2O           | 8.093 | 2.39   |
| 22  | 21-ri-methyl-17-isocholest-16-en-3beta-ol  | C23H46O | 8.150 | 0.47   |
| 23  | Naphthalene, 1,2,3,4a,5,6,8a-octahydro-4a,8-dimethyl-2- (1-methylethenyl) 1,2R-(2 alpha, 4a alpha, 8a beta)  | C30H34 | 8.232 | 0.69   |
| 24  | 1,6,10-Dodecatrien-3-ol,3711,trimethyl-, [S-(Z)] | C15H26O | 8.323 | 2.91   |
| 25  | Benzocic acid, nonadecyl ester | C29H44O2 | 8.631 | 0.79   |
| 26  | Naphthalene, 1,2,3,4a,5,6,8a-octahydro-4a,8-dimethyl-1-(1-methylthene)  | C13H34 | 8.232 | 0.69   |
| 27  | 1H-Cyclopropene azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene, [1ar-{1alpha, 4a alpha, 7 beta, 7a alpha, 7b alpha}] | C13H2O | 8.750 | 0.80   |
| 28  | Caryophyllene         | C13H2O           | 8.809 | 1.49   |
| 29  | 4,2,8-Ethylidenediene-2H-1-benzopyran, octahydro-4-methyl- | C24H32O | 8.923 | 0.55   |
respectively and the results of this study are comparable to a report from a recent study in Pretoria, South Africa where GBS isolates had 17.2 and 21.16% resistance to clindamycin and erythromycin respectively [15]. The results also fall within world statistics of clindamycin resistance (8.2–70%) and erythromycin resistance (14.5–70%) [1]. However, studies in Brazil have recorded low GBS resistance to clindamycin (3.0%) and erythromycin (4.1%) [17].

There was high resistance of GBS to tetracycline (96.1%) and this appears consistent with other studies which have reported high GBS resistance to tetracycline (86.7%) in South Africa [15] and in Brazil [17] and this could be due to the indiscriminate use of tetracycline and its availability as a non-prescription drug. Tetracycline is commonly used at farms as a growth promoter and for treatment purposes. Resistance to the drug could spread through the environment due to agricultural runoff and contaminated effluent while antibiotic residue in farm produce like meat is another serious concern which perpetuates drug resistance as low levels of drugs in farm produce exert persistent selection pressure on bacteria thus giving rise to the emergence of resistant strains [18].

Resistance to cotrimoxazole was low in this study (11.0%) contrary to its reported high resistance level among GBS isolates from other studies in other African countries like Ethiopia (29.0%) [19]. In Tanzania, just like many other sub Saharan African countries burdened by human immunodeficiency virus (HIV) infection, cotrimoxazole is used for prophylactic purposes in all acquired immunodeficiency syndrome (AIDS) patients including pregnant women before 36 weeks gestation. Resistance to cotrimoxazole in Tanzania ranges from 56.0% in Escherichia coli to 87.5% in Pseudomonas aeruginosa with no difference in prevalence among patients on cotrimoxazole prophylaxis and those not on it. The high resistance to cotrimoxazole could be attributed to the excessive use of the drug for prophylactic purposes in HIV positive patients. In a South African study, 85.7%
of *E. coli* isolated from HIV infected children was resistant to cotrimoxazole [20] while another South African study showed a significant increase in resistance to cotrimoxazole by organisms from 1999 to 2002 [21]. The World Health Organization noted that although cotrimoxazole is a broad spectrum antibiotic, increased use of the drug for prophylactic purposes would spur development of drug resistance which could ultimately render the drug ineffective when applied to common community pathogens [22, 23].

This current study found that the essential oils were made up of 68 compounds and the main components were nonanal (10.57%), phytol (6.40%), bicyclo[4.4.0]deca-1,ene, 2-isopropyl-5-methyl-9-methylene (5.65%), 4-tert-Butylcatechol, dimethyl ether (4.63%), 2-hexanol (2.95%), 5,9-Undecadien-2-one, 6,10-dimethyl- (2.91%) and naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-di-methyl-1-(1-methylethyl)- (2.91%) as shown in Table 3. The findings of this study are different from those of a similar study done by Boukhebti et al., (2015) in Algeria in which the essential oils were composed of 38 components. In the study palmitic acid (14.71%), z-nerolidol (9.45%), octacosane (6.32%), ceryphylene oxide (4.77%), tetracosane (4.06%) and 4-hydroxy-4-methy-2-pentane (4.04%) were the main components of the essential oils [24].

A study by Upadhyay (2014) in India reported *O. europaea* plants to contain 24 compounds with the main components being 2-propanone (8.80%), 2,4imidaizolidinedione (14.92%) and z- (13,14- Epox) tetra dec-11-en-1-ol acetate (7.77%) [25]. Different studies in Tunisia reported differences in phytochemical composition of *Olea* extracts. Plant extracts have variations in total phenols, colour, composition of polysaturated fatty acids and water content [26–28]. The variation in chemical composition is attributed to differences in climatic conditions under which the plant grew or cultivation conditions such as irrigation and the ripening stage of the plant [26, 29]. Water content affects activity of enzymes responsible for synthesis of phytochemicals like phenols and increased water supply to plants increases the content of phenolic components, alcohols and esters such as (E)-2-hexanol [26].

### Table 4 Screening for antimicrobial activities of plant extracts against GBS isolates

| Extract (Concentration) | Zone of inhibition on MH (Mean diameter) |
|-------------------------|------------------------------------------|
| Essential oil (300 mg/ml) | Present 25 mm |
| Leaf extract (0.5 mg/ml) | Present 28 mm |
| 5 µg Ciprofloxacin (Positive control) | Present 35 mm |
| 10% DMSO (Negative control) | Not present 0 mm |

### Table 5 MIC for essential oils against GBS isolates

| Concentration (mg/mL) | 0.05 | 0.4 | 3.7 | 33.3 | MIC |
|-----------------------|------|-----|-----|------|-----|
| Isolates              | A    | B   | C   | A    | B   | C   | A   | B   | C   | Essential oils |
| GBS in broth          | ++   | +   | +   | ++   | +   | +   | ++   | +   | +   | 33.3 |
| GBS on MH             | ++   | +   | +   | +    | +   | +   | +    | +   | +   | 33.3 |

Key: A = Essential oil, B = 10% DMSO, C = Ciprofloxacin, + = Growth, – = no growth

Essential oils showed antibacterial activity to GBS at an MIC of 33.3 mg/mL and had bactericidal effect on the isolates at that concentration.

Extracts from *O. europaea* had antimicrobial activity against GBS isolates from both South Africa and Namibia as shown in Table 4 and other studies have shown that *O. europaea* has antimicrobial activity against other bacteria although the extracts do not have broad spectrum antimicrobial activity [9]. While *Olea* has been shown to have antimicrobial properties in several countries including Spain, Israel, Palestine, plants in different geographical locations exhibit varying phytochemical constitutions therefore resulting in different properties and thus ethno botanical uses of the same plant [9, 30].

*O. europaea* essential oils showed bactericidal activity against GBS at 33.3 mg/mL as shown in Table 5 and studies have shown that essential oils have antimicrobial activity against a wide range of bacteria [9, 10]. Extracts from the leaves exhibited bactericidal activity at very low concentration (0.02 mg/mL) as presented in Table 6 and the antimicrobial activity could be attributed to phytochemical components present in the *Olea* plant extract such as flavonoids, iridoids, secoiridoids, biophenols and benzoic acid derivatives [1, 3, 9].

### Conclusions

GBS isolates from South Africa and Namibia did not exhibit a lot of resistance to antibiotics and they showed sensitivity to *Olea europaea* essential oils and plant extracts. With the increasing resistance of bacteria to antibiotics in the world, *Olea* plant extracts and essential oils could potent as alternative therapy to GBS infection.

### Table 6 MIC for leaf extracts against GBS isolates

| Concentration (mg/mL) | 0.0008 | 0.004 | 0.02 | 0.1 | MIC |
|-----------------------|--------|-------|------|-----|-----|
| Isolates              | A      | B     | C    | A   | B   | C   | A   | B   | C   | Leaf extracts |
| GBS in broth          | ++     | +     | +    | ++   | +   | +   | ++   | +   | +   | 0.02 |
| GBS on MH             | ++     | +     | +    | +    | +   | +   | +    | +   | +   | 0.02 |

Key: A = Leaf extracts, B = 10% DMSO, C = Ciprofloxacin, + = Growth, – = no growth

Leaf extracts showed antibacterial activity to GBS at an MIC of 0.02 mg/mL and had bactericidal effect on the isolates at that concentration.
The authors declare that they have no competing interests.

Consent for publication
prior to sample collection which was carried out by qualified midwives. (Namibia) and the Department of Health, Eastern Cape (South Africa). The research was granted by the Ministry of Health and Social Services Centre (GMRDC), University of Fort Hare (UFH) and consent was

Ethical approval was granted by Govan Mbeki Research & Development Ethics approval and consent to participate

Authors' contributions
MM conceptualized the research, carried experimental work and wrote

Ethics approval and consent to participate

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Consent for publication
Not applicable.

Competing interests
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20. Madhi SA, Petersen K, Madhi A, Khoosal M, Klugman KP. Increased disease burden and antibiotic resistance of bacteria causing severe community-acquired lower respiratory tract infections in human immunodeficiency virus type 1-infected children. Clin Infect Dis. 2000;31(1):170–6.

21. Crewe-Brown HH, Reyneke MP, Khoosal M, Becker PJ, Karstaedt AS. Increase in trimethoprim-sulphamethoxazole (co-trimoxazole) resistance at Chris Hani Baragwanath hospital, Soweto, in the AIDS era. SAMJ. 2000;94 No. 6.

22. Coutoukdis A, Coovadia HM, Kinda G. Time for new recommendations on cotrimoxazole prophylaxis for HIV-exposed infants in developing countries. Bull World Health Organ. 2010;88:49–50.

23. Marwa KJ, Mushi MF, Konje E, Alile PE, Kidola J, Mirambo MM. Resistance to cotrimoxazole and other antimicrobials among isolates from HIV/AIDS and non-HIV/AIDS patients at Bugando medical Centre, Mwanza, Tanzania. AIDS Res Treat. 2015. https://doi.org/10.1155/2015/103874.

24. Boukhebti H, Chaker AN, Lograda T, Ramdani M. Chemical and antimicrobial properties of essential oils of Olea europaea L. Int J Pharmacol Toxicol. 2015;5(1):42–6.

25. Upadhyay RK. Evaluation of Antibacterial and Antifungal Activities of Olive (Olea Europaea) Essential Oil. International Journal of Green Pharmacy. 2014; 8(3). doi.org/10.22377/gpov8338.

26. Chehab H, Issaoui M, Flamini G, Mechri B, Attia F, Luigi CP, et al. Oil quality and aroma composition of ‘Chemlali’ olive trees (Olea europaea L.) under three irrigation regimes. Afr J Agric Res. 2013;8(48):6291–9. https://doi.org/10.5897/AJAR2013.7972.

27. Biaimi F, Flamini G, Mechri B, Dhibi M, Hammond M. Antioxidant activity of the leaf volatile oil and extracts of Olea europaea Lcv. Chetoui from Northern Tunisia. J Food Nutr Disord. 2015;4. https://doi.org/10.4172/2324-9323.1000171.

28. Dabbou S, Dabbou S, Selvaggio R, Urbani S, Taticchi A, Servili M, Hammami M. Comparison of the chemical composition and the organoleptic profile of virgin olive oil from two wild and two cultivated Tunisian Olea europaea. Chem Biodivers. 2011;8:189–202. https://doi.org/10.1002/cbdv.201000086.

29. Gómez-Rico A, Salvador MD, La Greca M, Freppapane G. Phenolic and volatile compounds of extra virgin olive oil (Olea europaea L. cv. Cornicabra) with regard to fruit ripening and irrigation management. J Agric Food Chem. 2006;54(19):7130–6. https://doi.org/10.1021/jf060798b.

30. Wadood A, Ghufran M, Jamal SB, Naeem M, Khan A, Ghaffar R, et al. Phytochemical Analysis of Medicinal Plants Occurring in Local Area of Mardan. Biochem Anal Biochem. 2013;24. https://doi.org/10.4172/2161-1009.1000144.