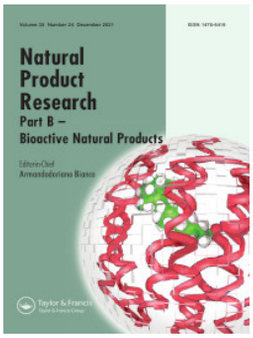

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Antibacterial and antioxidant activities and phytochemical composition of *Stereospermum kunthianum* root bark

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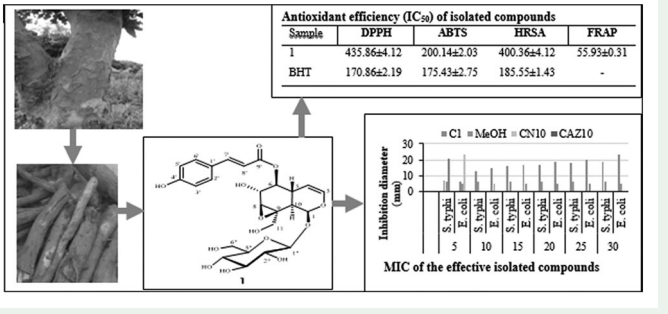
ABSTRACT

A new glycoiridoid (**1**) together with seven (7) known compounds were isolated from the methanol crude extract of the root bark of *Stereospermum kunthianum* using chromatography methods. Their structures were elucidated using HR-ESI-MS, 1 D- & 2 D-NMR spectroscopies in comparison with previous literature. The antioxidant activity was investigated by using FRAP, DPPH, ABTS and HRSA methods while the antibacterial activity was assays on *Escherichia coli* (ATCC25922) and *Salmonella typhimurium* (ATCC14028) strains. The results showed that the isolated compounds had significantly ($p < 0.01$) high radical scavenging (IC_{50}) and reducing power activity. All bacteria strains showed important minimal inhibitory concentration activity against isolated compounds started at 5 mg/mL with an inhibition zone of 6 mm. Thus, the isolated compounds in *S. kunthianum* justify the use of the plant in traditional medicine for the treatment of various diseases in humans. These isolated compounds can be used for formulation of new drug discovery to treat infectious diseases.

Graphical Abstract



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1. Introduction

Plants were used in old tradition across the world for food and medicinal purposes. Nowadays, population of Africa mostly refer to traditional healers concerning their health issues. In addition, the World Health Organization encourages the use of herbal medicines for their safety and efficacy in the healthcare programs to combat various diseases such as viruses, multi-resistant bacteria, etc. *S. kunthianum* is called in the Far-North Region of Cameroon in fulfuldé '*Golombi*' in Moudan '*Təvon Zimé*', in Toupouri '*fo'opir*' and in Mada '*Havaal*'. It belongs to the Bignoniaceae family and is used as a medicinal plant by traditional medical practitioners for the treatment of various diseases (Aliyu et al. 2009). The pods, stem and root barks are used to treat ulcers, skin eruption, bronchitis, pneumonia, cough, rheumatic arthritis, dysentery, erectile dysfunction, syphilis and toothache (Ching et al. 2013). In previous phytochemical studies, several classes of compounds have been reported such as glycoside iridoids (Falodun et al. 2009), flavonoids (Ching et al. 2013), naphthoquinones (Onegi et al. 2002) and their biological activities were also evaluated such as antiplasmodial, antibacterial and antioxidant activities (Onegi et al. 2002, Aliyu et al. 2009, Compaoré et al. 2011, Sarr et al. 2021). In our outgoing investigations of secondary metabolites from the *Stereospermum* genus reported in Cameroon (Lenta et al. 2007, Sob et al. 2011, Ramsay et al. 2012, Leutcha et al. 2021) we suggest the root barks of *S. kunthianum* to a chemical study. Indeed, this study led to the isolation and characterization of a new glycoside iridoid derivative: glycosides 6-*O*-*trans*-*p*-coumaroyl-undecinnamoyl-globularimin (**1**), together with seven (7) known compounds (Figure 1). The antibacterial and the antioxidant activities of the crude extract, main fractions and the isolated compounds were also evaluated.

2. Results and discussion

The MeOH extract of the root bark of *Stereospermum kunthianum* was fractionated using EtOAc. The EtOAc fraction was subjected to repeated column chromatography over silica gel with MeOH, EtOAc and hexane as eluents; which led to the isolation of eight compounds. All the known compounds were identified after the interpretation of their NMR and SM spectra follow by the comparison of their data to the previous reported as: a mixture of stigmasterol (**2**) and β-sitosterol (**8**) (Falodun et al. 2009, Soodabeh et al. 2014), lupeol (**3**) (Sawale et al. 2017), 6-*O*-*trans*-*p*-coumaroyl-decinnamoyl-globularimin (**4**) (Kanchanapoom et al. 2005), 6,6′-*O*-di-*trans*-feruloyl catapal (5) (Kil et al. 2017), 6,10-*O*-di-*trans*-feruloyl catapal (**6**) (Kil et al. 2017) and α-D-Galactosyl-(1→6)-α-D-galactosyl-(1→6)-α-D-glucosyl-(1→2)-β-D-frutoside or stachyose (**7**) (Ben Youssef et al. 2016).

Compound (**1**) was isolated as a white amorphous powder. Its molecular formula was deduced from its positive-ion mode HR-ESI-MS spectrum as $C_{25}H_{30}O_{13}Na^+$ $[M+Na]^+$, *m/z* 561.1584 (calcd. *m/z* 561.1584) (Figure S1) having eleven double bond equivalents. The IR spectrum showed absorption bands of hydroxyl (3425 cm^{-1}), carbonyl (1654 cm^{-1}) functional groups and enol ether system (1600 cm^{-1}) (Kim et al. 2014). The ^{13}C-NMR spectrum (Figure S2) shows the presence of a glucopyranosyl moiety with signals at δ_C 99.8 (C-1″, anomer), 78.7 (C-5″), 77.8 (C-3″), 74.9 (C-2″), 71.8 (C-

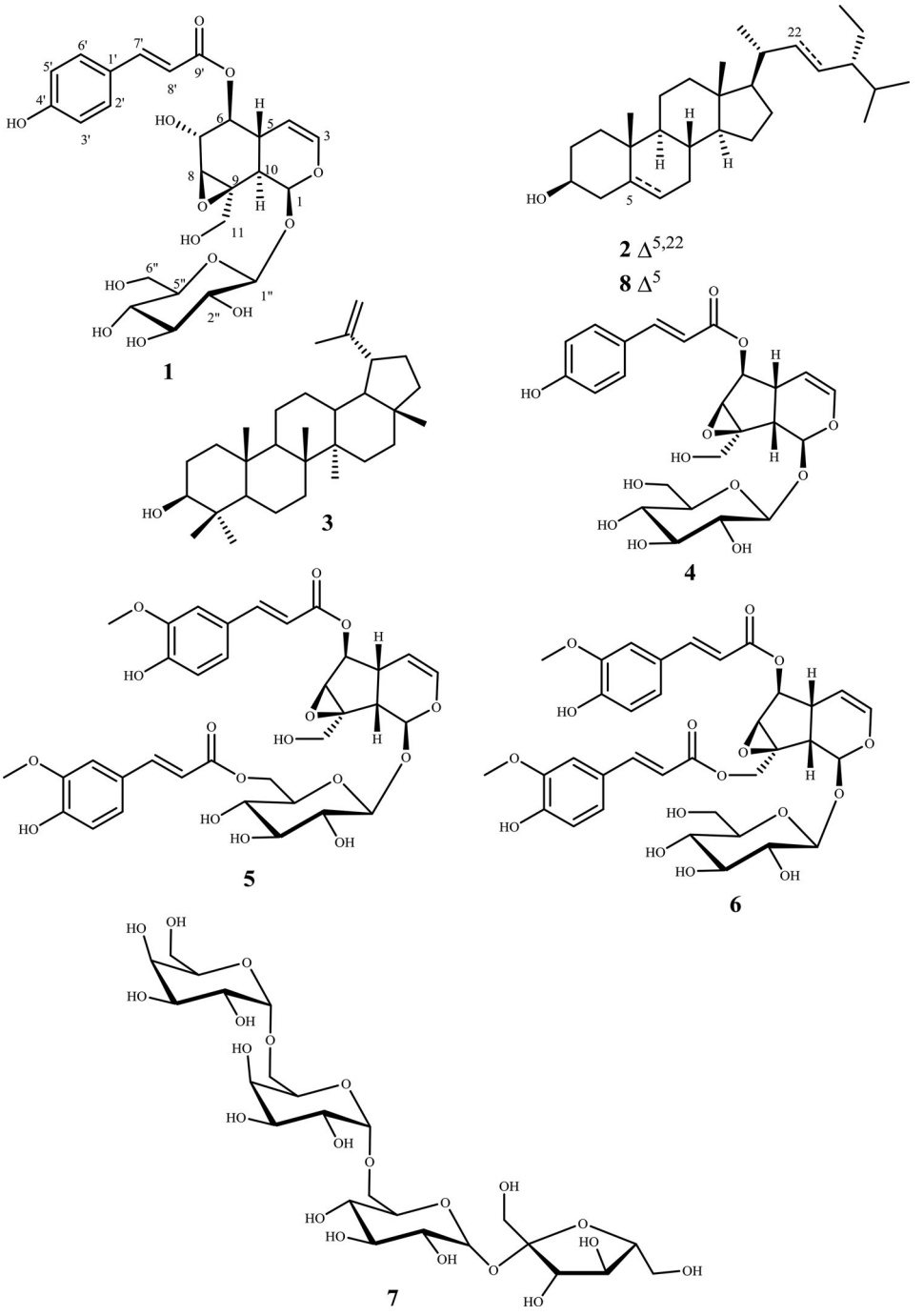

Figure 1. Structures of the isolated compounds 1-8.

4″) and 63.0 (C-6″) (Gololo et al. 2017). This was supported by its ^{1}H-NMR spectrum (Figure S3) which revealed that the sugar unit is a β-D-glucopyranoside according to the coupling constant of the anomeric proton at δ_H 4.82 (d, $J = 7.9$ Hz, 1H-1″) (Nzowa et al. 2010; Assefa et al. 2016;). Ones more, the combination of ^{13}C-NMR, DEPT-135

together with HSQC (Figures S4–S9) led to the identification of a coumaroyl unit at δ_C 169.0 (C-9′), 161.5 (C-4′), 147.3 (C-7′), 131.4 (C-2′/6′), 127.1 (C-1′), 116.9 (C-3′/5′), 114.7 (C-8′) which is *trans* and having an AA′ and BB′ coupling system according to the coupling constant at δ_H 7.70 (d, $J = 15.9$ Hz, 1H-7′), 7.51 (d, $J = 7.3$ Hz, 2H-2′/6′), 6.84 (dd, $J = 8.6$, 1.3 Hz, 2H-3′/5′) respectively (Kil et al. 2017). Moreover, the signals of a quaternary carbinolic carbon (δ_C 66.9/C-9) together with the signals of eight methines among which four are sp^3 oxygen bearing (δ_C 95.1/C-1, 81.4/C-6, 60.3/C-7 and 56.5/C-8), two sp^3 (δ_C 43.2/C-10 and 36.8/C-5) and two sp^2 (δ_C 142.4/C-3 and 103.0/C-4), and a methylene (δ_C 61.4/C-11) which is characteristic of a cyclohexanopyran ring of an iridoid skeleton (Kanchanapoom et al. 2005). In addition, signals at δ_C 142.4, 103.0, 95.1, 66.9 and 56.5 are characteristic of iridoid derivative type catalpol (Kanchanapoom et al. 2005; Kil et al. 2017). From the aforementioned information, it appears that compound **1** is closely related to 6-O-*trans-p*-coumaroyl-decinnamoyl-globularimin except the cyclopentanopyran which become cyclohexanopyran ring. This is more supported by its HMBC spectrum (Figure S8) through correlations between H-6 (δ_H 5.06) with C-9′ (δ_C 169.0), C-4 (δ_C 103.0) and C-7 (δ_C 60.3) then between H-10 (δ_H 2.66) with C-6 (δ_C 81.4), C-4 (δ_C 103.0), C-8 (δ_C 56.5), C-11 (δ_C 61.4) and C-9 (δ_C 66.9) and between H-1 (δ_H 5.20) with C-1″ (δ_C 99.8), C-3 (δ_C 142.4) and C-9 (δ_C 66.9) which support the connection between the coumaroyl unit with the cyclohexanopyran ring at C-6 and the connection between the sugar moiety and the aglycone at C-1 respectively. The COSY and NOESY (Figures S6 and S7) spectra led to the position of the vicinal and the α/β protons. So, the homonuclear correlation between H-5 (δ_H 2.64) with H-6 (δ_H 5.06) and H-4 (δ_H 5.04) led to support the cyclohexanopyran ring and the position of the double bond in the catalpol skeleton while protons H-6 (δ_H 5.06) with H-7 (δ_H 3.74) and H-1 (δ_H 5.20) with H-10 (δ_H 2.66) are α/β orientated (Figure S15) (Kanchanapoom et al. 2005; Falodun et al. 2009; Kil et al. 2017). Thus, compound **1** was characterized as a new glycoiridoid derivative named 6-*O-trans-p*-coumaroyl-undecinnamoyl-globularimin and trivially named wangstereospermoside.

The anti-oxidation mechanisms of polyphenolics are various such as electron and hydrogen transferring, creating chelations with oxidant metal ions suggesting different antioxidant assays needed in order to have a comprehensive view of the antioxidant capacity of extracts and isolated compounds from plants. The antioxidant activities of some isolated compounds and extracts are presented in Table S1. The percentage of scavenging activity evaluated by DPPH method varied significantly ($p < 0.01$) from 35.44 to 69.79% for compound **3** and **EB**, respectively. However, the scavenging activity of compound **4** (65.56%) and **2** were statistically similar with BHT (74.12%) used as a positive control (Figures S8–S12). The results indicate that the extract has statistically similar capacity with BHT to inhibit DPPH radical. The results are confirmed by the IC_{50} values for DPPH scavenging activity of the samples which were significantly ($p < 0.01$) varied between 725.21 and 192.32 μg/mL in compounds **3** and **5** respectively. The IC_{50} values of BHT were 74.12 μg/mL. The lowest IC_{50} value was obtained in compound **5**, indicating that it has the highest potent antioxidant capacity compared to that of other isolated compounds. This result was in agreement with the previous study conducted by Ma et al. (2016). The DPPH radical scavenging assay demonstrated the efficiency of extract and isolated compounds of *S. kunthianum* as hydrogen donors.

The scavenging activity evaluated by the ABTS method varied significantly from 41.65 to 66.27% for compounds **7** and **6**, while the BHT used as positive control had a high inhibition value (74.12%). The IC_{50} values for ABTS scavenging activity of the samples were significantly ($p < 0.01$) similar with the values varied between 180.01 and 205.02 μg/mL in compound **3** and **EB**, respectively. Except, compounds **2**, **5** and **6** which the values were not statistically different with the IC_{50} value of BHT 175.43 μg/mL. Moreover, this result indicated that the isolated compounds were compared with the pure compound BHT used as the positive control to scavenge radicals, suggesting that compound **2** shows potent antioxidant capacity as BHT.

The scavenging activity assessed by the HRSA method varied also significantly ($p < 0.01$) from 44.29 to 67.96% in compound **2** and **EB**, respectively. However, the scavenging activity of compound **1** and compound **4** are significantly similar, while the BHT (75.05%) used as positive control showed high inhibition value (Table S1). Figure S13 presents the scavenging activities of different samples compared to control (BHT). The IC_{50} values for HRSA scavenging activity of the samples were varied with a significant ($p < 0.01$) difference with the values ranged from 210.03 to 409.41 μg/mL in **EB** and compound **7**, respectively, while the value of compound 5 was not different with the IC_{50} value of compound 6 (Table S1). The IC_{50} value of BHT used as a positive control was 185.55 μg/mL which did not differ with significant difference with those of compounds **5** and **6** (Table S1). A lower IC_{50} value represents a stronger free radical inhibitor which means strong free radical inhibitors are active at low concentrations. The various levels of secondary metabolites in **EB** compared to those of isolated compounds might be responsible for the slight high antioxidant activity. Laya and Koubala (2020) have reported that the concentration of phenolics acids and flavonoids in cassava leaves positively correlated with the antioxidant activity assessed by different methods.

The FRAP method measures the reducing capacity of antioxidants based only on the electron transfer mechanism (Table S1). The values ranged significantly from 29.04 to 62.55 mMTE in compounds **7** and **4**, respectively, while **EB** and compound **4** show statistically similar reducing power. The highest antioxidant activity showed by **EB** might be linked by the levels of secondary metabolites in the extract. The present study suggests that, the antioxidants in medicinal plants are natural agents known as responsible for inhibiting oxidants and so reducing oxidative damages.

The antibacterial activity of extract and isolated compounds (Tables S2 and S3) revealed that almost all samples have the potential effect to suppress microbial growth bacteria (*S. typhi* and *E. coli*) with variable potency at a concentration of 1 mg/mL except compounds **1** and **4** on *E. coli*. On the other hand, **EB** was most effective against *S. typhi* at 0.3 mg/mL followed by compound **4** with **0.4 mg**/mL, while compound **3** didn't show any effect against *S. typhi*. Results of antimicrobial activity of the **EB** extract and isolated compounds suggest that *E. coli* was the most resistant strain and *S. typhi* was the most susceptible strain to the isolated compounds.

The antibacterial activity of crude extract and isolated compounds were qualitatively determined by the inhibition zone diameter, and ceftazidim and gentamicin were used as positive references and methanol was used as a negative control. Samples of extract and isolated compounds showed a significant variation of

inhibitory activity against all strains of the tested bacteria as summarized in Figures S13 and S14. *E. coli* (ATCC-25922) strain was the most sensitive against compound **1** (20 mm) at 30 mg/mL among isolated compounds. However, other samples have up to 18 mm inhibition zone diameter at 30 mg/mL (Figure S13) except compound **3**, which the value was 11 mm. At the same concentration, isolated 4 and **EB** showed the same value of inhibition zone (19 mm). Importantly, all the tested samples show high antibacterial activity in all concentrations used in the study. The study indicated that **EB** and isolated compounds of *S. kunthianum* have inhibitory activity against the two bacteria strains mostly pathogens and thus, this confirms the traditional medical practice of the population of the Far North region of Cameroon. Regarding *S. typhi*, the highest inhibition zones of 19 and 20 mm were shown for compounds **1** and compound **4**, respectively, against *S. typhi (*ATCC14028) followed by extract **EB** (19 mm) and compound **1** (19 mm), while the lowest was obtained by compound **3** and **7** which their values were 16 mm at 30 mg/mL (Figures S13 and S14). The diameter of the zone inhibition increased as the concentration of the extract or isolated compounds increased. These results were consistent with those found by Mostafa et al. (2018). The samples showed varying degrees of inhibitory effect against all different bacteria strains human pathogens; hence these results were consistent with the results found by Raja et al. (2011) in medicinal plants in India.

The MIC of the effective extract and isolated compounds against *E. coli and S. typhi* was done by the disc diffusion method to assess their bacteriostatic and bactericidal properties. The concentration effect of the effective extract and isolated compounds were shown in Table S3. The inhibitory activity of extract varied from 5 mg/mL with an inhibition zone of 6 mm (compound **1**) which varied dependent-concentration to reach 30 mg/mL with an inhibition zone of 23 mm (compound **2**) against *E. coli*. Similarly, the antibacterial activity of the leave extract of *S. kunthianum* has demonstrated significant antibacterial activity against *E. coli* (Aliyu et al. 2009) with the MIC value of 8 mg/mL, while, the effect was lower compared to the results obtained in the present study. However, the inhibitory activity of extract and isolated compounds against *S. typhi* started at 5 mg/mL with inhibition zones of 7 in compound **1** to reach the inhibition zone of 21 mm (compound **4**) followed by 20 mm (compound **4**) for 30 mg/mL. Mostafa et al. (2018) reported also a variation of MIC of the effective extract plants against bacterial strains studied.

The present results suggest that the crude extract of *S. kunthianum* can be used as natural antibacterial agents. The isolated compounds can be used for the formulation of new drug discovery to treat various infectious diseases.

3. Experimental

3.1. General

The UV spectrum ann NMR spectra (^{1}H, ^{13}C, DEPT, HSQC, COSY, HMBC and NOESY) were recorded on a Bruker DRX 500 spectrometer machine at different frequencies, running gradients and using TMS (TetraMethylSilane) as internal standard. DPPH (2,2-diphenyl-1-picrylhydrazyl radical), 2,6-di-terbutyl-4-methylphenol (BHT), 6-hydroxy-2,5,7,8-tetramethyl chromane-2-carboxylic acid (98% Trolox), 2,2-azinobis(ethylbenzothiazoline-6-sulfonic acid) diammonium salt (98%, ABTS) and 2,4,6-tris(2-pyridyl)-s-

triazine(98%, TPTZ) were purchased through Sigma-Aldrich Chemical Company (Mumbai, India). *Escherichia coli* (ATCC25922) and *Salmonella typhimurium* (ATCC14028) were obtained from the Microbiology and Food Products of Montreal, Canada.

3.2. Plant material

The root bark of *Stereospermum kunthianum* was collected during the dry season at Moulvoudaye, Diamaré Division from the Far North Region of Cameroun. The plant was identified by Pr. Tchobssala from the Herbarium of the University of Maroua, Cameroon. A voucher specimen is deposited at the Maroua University Herbarium (No. 0019DBUM).

3.3. Extraction and isolation

The shade dried root bark (5.5 kg) of *S. kunthianum* was powdered and extracted for 72 hours with MeOH. The macerate was filtered using Wattmann paper and the filtrate was concentrated under reduce pressure at about 68 °C to obtain a crude MeOH extract (500.2 g). The crude extract was dissolved into distilled water and partitioned with EtOAc to obtain 76.0 g of EtOAc main fraction. 45.0 g of EtOAc fraction was fractionated on a silica gel (60–120 Mesh type analysis) column which led to four (4) fractions indexed from F1 to F4. F1 (6.2 g) was separated on RP-C18 silica gel column chromatographic using Hexane/EtOAc gradient which led to three (3) compounds: a mixture of **2** and **8** (202 mg, 19:1-Hexane/EtOAc) and **3** (122 mg, 9:1-Hexane/EtOAc). Fraction F4 (12.1 g) was chramatographied using EtOAc/MeOH gradient which led to compounds **1** (142 mg, 7:3- EtOAc/MeOH) and **4** (64 mg, 13:7-EtOAc/MeOH). The aqueous phase was dried in an oven at 50 °C, where we obtained 400 g of extract among which 42.0 g of this was fractionated using a column of silica gel and MeOH-EtOAc gradient. This led to four (4) major fractions indexed from A1 to A4. Fraction A2 was chromatographed on RP-C18 silica gel column to afford compound **5** (95 mg, 13:7-EtOAc/MeOH) and **6** (72 mg, 3:2-EtOAc/MeOH). And finally, fraction A2 (8.4 g) was purified as above to afford compound **7** (260 mg, 1:1- EtOAc/MeOH).

3.4. Spectroscopic data of compound 1

wangstereospermoside (**1**) or 6-*O*-*trans*-*p*-coumaroyl-undecinnamoyl-globularimin (**1**): white powder, IR (KBr) υ_{max} 3425, 3340, 2900, 1654, 1634, 1600, 1514, 1204, 1175 cm^{-1}. ^{1}H-NMR (500 MHz, Methanol-d_4) δ_H: 7.70 (d, $J = 15.9$ Hz, 1H-7′), 7.51 (d, $J = 7.3$ Hz, 2H-2′/6′), 6.84 (dd, $J = 8.6$, 1.3 Hz, 2H-3′/5′), 6.40 (m, 1H-3), 6.40 (m, 1H-8′), 5.20 (d, J = 9.0 Hz, 1H-1), 5.06 (d, $J = 7.6$ Hz, 1H-6), 5.02 (dd, J = 5.9, 4.3 Hz, 1H-4), 4.83 (d, J = 7.9 Hz, 1H-1″), 4.20 (d, J = 13.2 Hz, 1H-11), 3.96 (dd, J = 12.0, 3.3 Hz, 1H-6″), 3.92 (d, J = 1.3 Hz, 1H-8), 3.87 (d, J = 13.2 Hz, 1H-11), 3.73 (brs, 1H-7), 3.68 (dd, $J = 12.0$, 6.6 Hz, 1H-6″), 3.45 (m, 1H-3″), 3.36 (m, 1H-5″), 3.34 (m, 1H-4″), 3.31 (m, 1H-2″), 2.66 (m, 1H-10) and 2.64 (m, 1H-5). ^{13}C-NMR (125 MHz, Methanol-d_4) δ_C: 169.0 (C-9′), 161.5 (C-4′), 147.3 (C-7′), 142.4 (C-3), 131.4 (C-2′/6′), 127.1 (C-1′), 116.9 (C-3′/5′), 114.7 (C-8′), 103.0 (C-4), 99.8 (C-1″), 95.1 (C-1), 81.4 (C-6), 78.7 (C-5″), 77.8 (C-3″), 74.9 (C-2″), 71.8 (C-4″), 66.9 (C-9), 63.0 (C-6″), 61.4 (C-11), 60.3 (C-7), 56.5 (C-8), 43.2 (C-10), 36.8 (C-5). HR-ESI$^+$-MS: *m/z* 561.1584 $[M + Na]^+$ (calcd. $C_{25}H_{30}O_{13}Na^+$, 561.1584).

3.5. Antioxidant activity of extracts and isolated molecules

DPPH (2.2-diphenyl-1-picyhydrazyl) radical scavenging, ABTS (2,2-azino-bis(3-ethylbenzylthiozoline-6-sulphonic acid)) radical scavenging and FRAP (Ferric Reducing Antioxidant Potential) activities were evaluated as described by Sun et al. (2002), Re et al. (1999) and Benzie and Strain (1996) respectively. HRSA (Hydroxyl Radical Scavenging Activity) was evaluated as previously described by Gan et al. (2017) with some modifications. For this, 0.5 mL of $FeSO_4$ (9 mM), 0.5 mL of H_2O_2 (0.03%, v/v), 1.5 mL of salicylic acid-ethanol solution (9 mM) and 1.5 mL of sample were mixed. All analyses were done in dim light. Then the reaction was incubated for 40 min at room temperature in the dark place. The absorbance was taken at 510 nm. The HRSA was calculated from the Trolox standard curve plotted with seven concentrations at a range of 5–1000 μg/mL. The percentage of inhibition of $ABTS^{\cdot+}$, DPPH and HRSA by crude extract or isolated compounds was calculated according to the following equation:

$$\text{Inhibition}(\%) = (1 - A_{\text{test sample}}/A_{\text{blank}}) \times 100$$

where A_{blank} is the absorbance of the methanolic blank and $A_{\text{test sample}}$ is the absorbance of the extracts. Trolox were used as a reference and the results were expressed as % or μg trolox equivalent/g dw.

3.6. Antibacterial activity of extract and isolated compounds

3.6.1. Test microbial and condition of growth

Two bacterial strains including *Escherichia coli* (ATCC25922) and *Salmonella typhimurium* (ATCC14028) were used in the present study. These strains were obtained from University of Alberta, Canada. All bacterial cultures were maintained in BHI broth mixed with 20% glycerol and stored at −20 °C until use. All different cultures were grown at 37 °C in test tubes containing 10 mL of BHI broth for 24 hours. Then, the microbial strain was diluted (2:1) in a fresh BHI medium in order to obtain a final cell concentration of approximately 10^8 UFC/mL, equivalent to the McFarland 0.5 standard.

3.6.2. Determination of antibacterial effect of extract and isolated compounds

The antibacterial effect was evaluated by using the well diffusion technique as described by Zhang et al. (2017) with slight modifications. For each bacterium, 100 μL of the inoculum at 10^8 UFC/mL was transferred and spread equally along the surface of Petri dishes containing Mueller Hinton agar. Then, 7 mm diameter wells were put on the surface of the agar with 50 μL of extract or isolated compounds prepared in five different concentrations (10, 15, 20, 25 and 30 mg/mL). All plates were incubated at 4 °C for 3 h in order to maximize the diffusion of samples into the culture media before keeping at 37 °C for 24 h at room temperature. The antibacterial activity was evaluated by measuring the diameter of the inhibition zones induced by samples. In the present experiment, negative controls consisting of 50 μL of methanol and sterile distilled water were also included. Ceftazidim and gentamicin were used as the positive controls for *E. coli and S. typhi,* respectively.

3.6.3. Determination of minimal inhibitory concentrations (MIC) of extract and compounds

Determination of MIC of extract and isolated compounds were assessed as described by Tian et al. (2020) with some slight modifications. The extract and isolated compounds were solubilized in their corresponding solvents before subjecting to a serial dilution ranged from 0 to 3 mg/mL in MHB. A volume of 180 µL of each concentration was introduced into wells of a 96-well micro-titration plate with 20 µL of bacterial suspensions to a final cell density of 10^6 UFC/mL. After incubation of 24 h at 37 °C, 10 µL of sterile rezasurin solution (0.01%) was added to each well and incubated for 30 min. The MIC corresponds to the lowest concentration of extract or isolated compounds that does not produce any discoloration of the rezasurin dye.

4. Statistical analysis

The results obtained data were analyzed using Statgraphics software (version. 16). One-way variation analysis (ANOVA) on the statistical significance level 0.01 was performed. The significance of the differences between mean values was examined using Tukey's test ($p < 0.01$). Analysis of each sample was done in three replicates and the results were expressed as Mean ± Standard deviation.

5. Conclusion

This study revealed that *S. kunthianum* contains high amount of phenolic compounds with a significant antioxidant and bacteria activities, which can be used as a source of natural antioxidants and in pharmaceutical purposes. Our results support the use of this plant in traditional medicine. However, *in vivo* study of isolated compounds in novel drug discovery will be done in the future.

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Disclosure statement

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References

Aliyu MS, Hanwa UA, Tijjani MB, Aliyu Ab YB. 2009. Phytochemical and Antibacterial properties of leaf extract of *Stereospermum kunthianum* (Bignoniaceae). Nig J Basic and Appl Sci. 17(2): 235–239.

Assefa E, Alemayhu I, Endale M, Mammo F. 2016. Iridoid glycosides from the root of *Acanthus senni*. J Pharm Pharmacogn Res. 4:231–237.

Ben Youssef S, Fakhfakh J, Tchoumtchoua J, Halabalaki M, Allouche N. 2016. Efficient purification and complete NMR characterization of galactinol, sucrose, raffinose, and stachyose isolated from *Pinus halepensis* (Aleppo pine) seed using acetylation procedure. J Carbohydr Chem. 35: 224–237.

Benzie IF, Strain JJ. 1996. Ferric reducing ability of plasma (FRAP), a measure of antioxidant power: the FRAP assay. Anal Biochem. 239:70–76.

Ching FP, Otokiti IO, Egert-Omoneukanrin B. 2013. Dimethoxyflavone isolated from the stem bark of *Stereospermum kunthianum* possesses antidiarrhoeal activity in rodents. Afr J Tradit Complement Alter Med. 10(4):47–51.

Compaoré M, Lamien-Meda A, Mogoşan C, Lamien CE, Kiendrebeogo M, Voştinaru O, Vlase L, Ionescu C, Nacoulma OG. 2011. Antioxidant, diuretic activities and polyphenol content of *Stereospermum kunthianum* Cham. (Bignoniaceae). Nat Prod Res. 25(19):1777–1788.

Falodun A, Qadir IM, Poh CF, Omogbai EKI, Choudhary MI. 2009. Bioactive chemical constituents of *Stereospermum kunthianum* (Bignoniaceae). Res J Phytochem. 3(2):35–43.

Gan J, Feng Y, He Z, Li X, Zhang H. 2017. Correlations between antioxidant activity and alkaloids and phenols of Maca (*Lepidium meyenii)*. J Food Qual. 2017:1–10..

Gololo SS, Bassey K, Olivier MT, Agyei NM, Shai LJ, Masoko P, Gamedze M, Mogale MA. 2017. Isolation of an Iridoid glycoside compound from the leaves of *Barleria dinteri* collected from Zebediela sub-region in Limpopo province. South Africa J Pharm Res. 9:1368–1372.

Kanchanapoom T, Noiarsa P, Otsuka H, Ruchirawat S. 2005. Lignan, phenolic and iridoid glycosides from *Stereospermum cylindricum*. Phytochemical. 67:516–520.

Kil Y-S, Kim SM, Kang U, Chung HY, Seo EK. 2017. Peroxynitrite-Scavenging Glycosides from the Bark of Catalpa ovata. J Nat Prod. 80(8):2240–2251.

Kim KH, Park YJ, Chung KH, Yip MR, Clardy J, Senger D, Cao S. 2014. Iridoid Glycosides from *Barleria lupulina*. J Nat Prod. 78(2):320–324.

Laya A, Koubala BB. 2020. Polyphenols in cassava leaves (*Manihot esculenta* Crantz) and their stability in antioxidant potential after in vitro gastrointestinal digestion. Heliyon. 6(3):e03567.

Lenta BN, Weniger B, Antheaume C, Noungoue DT, Ngouela S, Assob JC, Vonthron-Sénécheau C, Fokou AP, Devkota KP, Tsamo E, et al. 2007. Anthraquinones from the stem bark of *Stereospermum zenkeri* with antimicrobial activity. Phytochemistry 68(11):1595–1599.

Leutcha BP, Sema DK, Dzoyem JP, Ayimele GA, Nyongbela KD, Delie F, Alléman E, Sewald N, Lannang AM. 2021. Cytotoxicity of a new tirucallane derivative isolated from *Stereospermum acuminatissimum* K. Schum stem bark. Nat Prod Res. 35(22):4417–4422..

Ma Q-G, Xu K, Sang Z-P, Wei R-R, Liu W-M, Su Y-L, Yang J-B, Wang A-G, Ji T-F, Li L-J. 2016. Alkenes with antioxidative activities from *Murraya koenigii* (L.) Spreng. Bioorg Med Chem Lett. 26(3):799–803.

Mostafa AA, Al-Askar AA, Almaary KS, Dawoud TM, Sholkamy EN, Bakri MM. 2018. Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. Saudi J Biol Sci. 25:361–366.

Nzowa LK, Barboni L, Teponno RB, Ricciutelli M, Lupidi G, Quassinti L, Bramucci M, Tapondjou LA. 2010. Rheediinosides A and B, two antiproliferative and antioxidant triterpene saponins from *Entada rheedii*. Phytochemistry. 71(2–3):254–261.

Onegi B, Kraft C, Köhler I, Freund M, Jenett-Siems K, Siems K, Beyer G, Melzig MF, Bienzle U, Eich E. 2002. Antiplasmodial activity of naphthoquinones and one anthraquinone from *Stereospermum kunthianum*. Phytochemistry. 60(1):39–44.

Raja RDA, Jeeva S, Prakash JW, Antonisamy M, Irudayaraj V. 2011. Antibacterial activity of selected ethnomedicinal plants from South India. Asian Pac J Trop Med. 375–378.

Ramsay KST, Wafo P, Ali Z, Khan A, Oluyemisi OO, Marasini BP, Khan IA, Ngadjui B, Choudhary MI. 2012. Chemical constituents of *Stereospermum acuminatissimum* and their urease and α-chymotrypsin inhibitions. Fitoterapia. 83(1):204–208.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. 1999. Antioxidant activity applying an improved ABT S radical cation decolorization assay. Free Radic Biol Med. 26(9–10):1231–1237.

Sarr A, Deing SIM, Diatta-Badji D, Mbaye AI, Diatta W, Ka A, Fall AD, Bassène E. 2021. Phytochemical screening and determination of polyphenols in the hydro-ethanolic extract of tunk bark and its fractions of *Stereospermum kunthianum* Cham (Bignoniaceae). Asian Plant Res J. 7(1):1–9.

Sawale JA, Patel JR, Kori ML. 2017. Isolation and caracterization of oleonolic acid and lupeol from Vitex negundo leaves. J Pharmacogn Phytochem. 6:936–938.

Sob SVT, Wabo HK, Tang CP, Tane P, Ngadjui BT, Ye Y. 2011. Phenol esters and other constituents from the stem barks of *Stereospermum acuminatissimum*. J Asian Nat Prod Res. 13(12): 1128–1134.

Soodabeh S, Azadeh M, Ahmad RG, Mohammad A. 2014. The story of betasitosterol - a review. Eur J Med Plants 4(5):590–609.

Sun W, Li N, He S. 2002. Large-scale morphological survey of mouse retinal ganglion cells. J Comp Neurol. 451(2):115–126.

Tian M, Wu X, Lu T, Zhao X, Wei F, Deng G, Zhou Y. 2020. Phytochemical analysis, antioxidant, antibacterial, cytotoxic, and enzyme inhibitory activities of Hedychium flavum rhizome. Front Pharmacol. 11(572659):572659.

Zhang HY, Gao Y, Lai PX. 2017. Chemical composition, antioxidant, antimicrobial and cytotoxic activities of essential oil from *Premna microphylla* Turczaninow. Molecules. 22(3):381.