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Biological Activities of Extracts and Isolated Calotropin from *Gomphocarpus purpurascens* Leaves: A Computational and Experimental Study

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Abstract: *Gomphocarpus purpurascens* A.Richs. (sub-family: Asclepiadaceae, family: Apocynaceae) is an endemic plant in Ethiopia and Eritrea. The chromatographic fractionation of the chloroform:methanol (1:1) leaf extract resulted in the isolation of a cardenolide-type compound named calotropin. Its structure was confirmed based on the 1D-NMR (^{1}H, ^{13}C, and DEPT-135) spectral data along with reported work. This is the first report on the isolation of calotropin from the leaves of *G. purpurascens*. The *n*-hexane extract displayed better antibacterial activity against *E. coli* and *P. aeruginosa* (8.1 ± 0.0 to 10.8 ± 0.1 mm) up to 12,500 µg/mL concentration. Generally, all the extracts and the isolated compound, calotropin, showed better antibacterial activity against the *P. aeruginosa* strain than chloramphenicol (7.1 ± 0.2 to 7.3 ± 0.5 mm). The tested chloroform: methanol (1:1) extract indicated a weak antifungal activity against *C. albicans* compared to ketoconazole 10 µg/disc (17.67 ± 2.52 mm). On the contrary, all the extracts were found to have a good DPPH radical scavenging activity (IC_{50} values, 1.1-2.7 µg/mL) comparable to ascorbic acid (1.0 µg/mL). The isolated calotropin exhibited a stronger binding capacity (-10.3 kcal/mol) against the *P. aeruginosa* PqsA (5OE3) protein model than chloramphenicol (-7.0 kcal/mol). This compound was also found to violate Lipinski's rules of five (with a molecular weight > 500 g/mol) and showed immunotoxicity and cytotoxicity properties. Given that this is a preliminary report, further biochemical investigations would be needed on this *G. purpurascens* plant to identify additional phytoconstituents with superior efficacy.

Keywords: *Gomphocarpus purpurascens*, Asclepiadaceae, Apocynaceae, Calotropin, Biological activities, Docking analysis.

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1. INTRODUCTION

Gomphocarpus purpurascens A.Richs. (Syn. *Asclepias purpurascens* and *Gomphocarpus fruticosus*) is a member of the sub-family Asclepiadaceae of the family Apocynaceae. It is locally known as "*Tefreina*" (in Amharic) (1) and "*Ari-Yuyo*" (in Oromifa) (2). *G. purpurascens* grows as an annual shrub on open, rocky ground and in disturbed areas. It occurs in most Ethiopian regions and is endemic to the highland regions of Ethiopia and Eritrea (1). In the prehistoric Harla town of eastern Ethiopia, the leaves of *G. purpurascens* are used for the treatment of itching skin and evil eyes (2). Currently, there is a claim that the itchy skin has potentially been caused by the methicillin-resistant *Staphylococcus aureus* (3). In Ethiopia, people also use *G. purpurascens* for the treatment of wounds and wart diseases (4). Besides, extracts prepared from the roots and leaves of the plant have traditionally been used as anti-inflammatory and analgesic agents in the Tigray, Amhara, and southern regions of Ethiopia (5).

Regarding the experimental-based biological activity of the plant, to our knowledge, only a few reports were found in the literature. For example, to our knowledge, only one study (6) was reported about the antimicrobial activity of the leaves of *G. purpurascens*. According to this report, three concentrations (150, 300, and 600 mg/mL) of the ethanol and methanol leaf extracts of *G. purpurascens* were tested against some bacterial pathogens and the *C. albicans* fungal strain. These extracts scored respective inhibition zone values of 6.09 ± 0.18 to 6.90 ± 0.40 mm and 9.19 ± 0.07 to 13.20 ± 0.1 mm against *E. coli*; 9.51 ± 0.1 to 12.7 ± 0.15 mm and 7.1 ± 0.01 to 8.2 ± 0.01 mm against *S. aureus*; 9.01 ± 0.01 to 9.77 ± 0.15 mm and 9.8 ± 0.01 to 13.8 ± 0.10 mm against *P. aeruginosa*; and 6.34 ± 0.04 to 6.64 ± 0.04 mm and 8.01 ± 0.01 to 13.79 ± 0.01 mm against *C. albicans*. The reported result was compared with the tetracycline (13.09-25.65 mm at 0.025 mg/mL) and ketoconazole (7.87 ± 2.18 mm at 25 mg/mL) standard drugs. Asfaw et al. (7) also reported the antibacterial activity, against only the *E. coli bacterium*, of acetone and methanol extracts of the leaves and stems of *G. purpurascens* with inhibition values of 55 mm and 60 mm, respectively (concentration was not mentioned). Another study reported by Ayanaw et al. (5) indicated that 80% of methanol extracts from the leaves and roots of this plant showed a dose-reliant anti-inflammatory and analgesic effect. The same authors (5) also reported the presence of some classes of phytochemicals, such as terpenoids, flavonoids, alkaloids, and phenolics. Different plant species of the family Asclepiadaceae were claimed for their anticancer and antioxidant potentials (8). However, according to our information, no reports were found on the antioxidant activity of the *G. purpurascens* species. Few compounds were previously reported from some species of the genus *Gomphocarpus*. Three triterpenoids (3*β*-taraxerol acetate, 13*α*-methyl,27-norolean-14-en-3*β*-ol(3*β*-taraxerol and betulinic acid), a pregnane glycoside ((lineolon-3-*O*-*β*-D-cymaropyranosyl-(1-4)-*β*-D-cymaropyranosyl-(1-4)-*β*-D-oleandropyranose)) and a cardenolide glycoside (gomphoside) were reported from the aerial parts of *Gomphocarpus fruticosus* (9). Besides, two other cardenolide compounds named uzarigenin and calotropin were isolated both from *G. fruticosus* and *G. sinaicus* (9, 10). However, based on our best search, no chemical constituents were reported from the species *G. purpurascens*. The limited antimicrobial activity reports, the absence of any report on the antioxidant activity study and isolated chemical constituents, and the claimed traditional medicinal uses against itchy skin and wound diseases (caused by common bacteria like *Staphylococcus aureus, Pseudomonas aeruginosa*, and *Escherichia coli*) of *G. purpurascens* leaves initiated us to do the present work. If not, no biological and phytochemical studies conducted on this *G. purpurascens* species have initiated us to do the present work. This study reported, for the first time, the isolation of the cardenolide compound, calotropin, and its *in vitro* and *in silico* antibacterial and antioxidant activities from the leaves of *G. purpurascens*. Also, the *in vitro* antimicrobial and antioxidant effects of crude leaf extracts of this plant were reported herein.

2. MATERIALS AND METHODS

2.1. Plant Material

The leaves of *Gomphocarpus purpurascens* (Figure 1) were collected from the prehistoric Harla town and surrounding villages, Dire Dawa, Eastern Ethiopia (latitude 9°27′ and 9°39′N, longitude 41°38′ and 42°20′E, 950-2260 m asl) during October 2022. The plant was identified by Dr. Anteneh Belayneh, Haramaya University, Ethiopia, and a voucher specimen (voucher number AHU126) was deposited in the herbarium of the mentioned University. Collected fresh samples were washed with tap water and then dried at room temperature under shade. The dried samples were coarsely powdered and sieved using an electrical blender. Powdered samples were packed in a tightly sealed glass bottle and stored in a refrigerator (4 °C) for later experimental work.

Figure 1: Photo of *Gomphocarpus purpurascens* plant (photo by Tsegu K., 2022).

2.2. Test Microbial Cultures

Three human standard bacterial pathogens, viz., *Staphylococcus aureus* (*S. aureus,* ATCC 25923), *Escherichia coli* (*E. coli,* ATCC 25922), and *Pseudomonas aeruginosa* (*P. aeruginosa,* ATCC 27853), and a fungal strain, *Candida albicans* (*C. albicans*, ATCC 10231), were obtained from the Ethiopian Public Health Institute (EPHI).

2.3. Chemicals, Apparatuses and Instruments

Chemicals

Chemicals such as methanol, ethanol, *n*-hexane, ethyl acetate, chloroform, dichloromethane, acetic acid, acetone, and 230-400 mesh size silica gel were used for extraction, chromatographic separation, and TLC analysis purposes. Iodine vapor was used and served as a TLC-detecting agent. Ferric chloride, potassium ferricyanide, 0.2 M potassium phosphate buffer, and trichloroacetic acid were consumed for the study of potassium ferric ion reduction antioxidant power (PFRAP), and DPPH free radical was used for the DPPH antioxidant assay. Mueller Hinton agar (MHA) and Potato Dextrose agar (PDA) media were used for antibacterial and antifungal activity evaluation, respectively, using DMSO as a positive control. Chloramphenicol, ketoconazole, and

ascorbic acid were used and served as reference drugs for the antibacterial, antifungal, and antioxidant activity assays, respectively.

Apparatuses and Instruments: An electrical laboratory blender (Torrington, CT., USA), orbital shaker (Hy-5A, Movel Scientific Instrument CO. Ltd., China), a suction filtration apparatus, and a rotary evaporator (rotary vacuum, Jainsons, India) were used for plant sample grinding, extraction, filtration, and solvent evaporation, respectively. Silica gel 60 F_{254} pre-coated aluminum TLC sheet (Merck), TLC chamber, and capillary tube were used for TLC analysis. A UV-lamp cabinet (254 and 365 nm, UVP Chromato-Vue C-70G, Analytik Jena, USA) was used and served as a non-destructive TLC-detecting tool. The PTLC and glass column were used for fractionation of the extract and isolation of the compound. A Petri dish, an incubator (Binder B28, Germany), and an autoclave (Tuttnauer 3150EL, Israel) were used for the evaluation of antimicrobial activity. A UV-Vis spectrophotometer (Cecil CE4001 UV/VIS, Cambridge, England) was employed for absorbance measurement during the antioxidant activity assay. 1D-NMR (^{1}H, ^{13}C, and DEPT-135) (BRUKER ACQ 400 AVANCE) spectroscopic instrument was employed for the structural identification of the isolated compound.

2.4. Extraction and Chromatographic Fractionation

The commonly applied successive organic solvent extraction protocol (11) was used in this study. That is, P powdered leaves (500 g) were macerated in *n*-hexane (3x, 2.5 L) by shaking overnight and then followed by filtration and concentration, resulting in a dark yellow dry extract (18 g). The remaining residue, after *n*-hexane extraction, was re-extracted successively with chloroform, chloroform: methanol (1:1), ethanol, and methanol using the same technique. The extract solutions were filtered and concentrated to yield corresponding dry extracts of 6 g, 53 g, 27 g, and 43 g. Then, the chloroform: methanol (1:1) extract, after TLC examination, was submitted to silica gel chromatographic fractionation. That is, green crude extract (30 g) was reconstituted in chloroform: methanol (1:1, 150 mL), adsorbed on normal silica gel (40 g), and concentrated. The adsorbed powder was then applied over a glass column packed with silica gel (200 g) and eluted with *n*-hexane/$CHCl_3$/EtOAc/MeOH of different polarity ratios. A total of three hundred seventy-six fractions (20-50 mL) were collected, and those with similar TLC profiles were combined. Among the combined fractions, Fr. 85-92 (dark gel, 100 mg) was repacked over the same normal silica gel-packed column (30 g) to give sixty sub-fractions after being eluted with $CHCl_3$/EtOAc in different polarity ratios. Finally, the sub-fr.11-25 (30 mg) was reapplied on a PTLC plate and run using an *n*-hexane/EtOAc/AcOH (3:1:0.1) solvent system, resulting in the isolation of calotropin (12 mg).

The chemical structure of the isolated calotropin (Figure 1) was identified using a 1D- NMR (1H, 13C, and DEPT-135) spectroscopic instrument in comparison with the reported data. The 1D-NMR (^{1}H, ^{13}C, and DEPT-135) analysis was performed on a BRUKER ACQ 400 AVANCE spectrometer with 400 MHz for ^{1}H and 100 MHz for ^{13}C, and DEPT-135. The instrument was equipped with a 5 mm proton probe and operated at a temperature of 298 K with topspin software (version 2.1). Tetramethyl silane (TMS) was used as an internal standard to measure the chemical shifts (δ_{ppm}) of the acquired spectra. Finally, the generated spectra were further processed using the software MestReNova (Mestrelab Research S.L., version 12).

2.5. In Vitro Antimicrobial Activity Evaluation

2.5.1. Antibacterial activity assay

The antibacterial activity of both crude extracts and the isolated compound was evaluated against three standard human pathogens, namely, *Staphylococcus aureus* (*S. aureus,* ATCC 25923), *Escherichia coli* (*E. coli,* ATCC 25922), and *Pseudomonas aeruginosa* (*P. aeruginosa,* ATCC 27853) bacterial strains. Experimental activity was conducted at the Microbiology Laboratory of the School of Medical Laboratory, Haramaya University. The agar medium disc-diffusion technique was followed to evaluate the antibacterial effectiveness of the extracts and isolated compound using the standard protocols of the Clinical and Laboratory Standards Institute (CLSI) (12, 13). A stock solution of each extract (200 mg in 2 mL) and isolated compound (5 mg in 5 mL) was prepared in 4% DMSO. Then, three concentrations (50,000, 25,000, and 12,500 µg/mL) of each extract and four various dilutions (500, 300, 100, and 50 µg/mL) of the isolated compound were prepared from corresponding stock solutions using the two-fold serial dilution method (14,15). Chloramphenicol-impregnated standard disc (30 µg) and DMSO solvent were used and served as positive and negative controls, respectively. The remaining detailed experimental procedures applied in this study were similar to those stated in (15, 16). Each experiment was done in duplicate, and results were presented as mean ± standard deviation after statistical analysis with SPSS software (version 20).

2.5.2. Antifungal activity assay

The chloroform: methanol (1:1) extract of *G. purpurascens* leaves was assessed for its antifungal activity against *Candida albicans* ATCC 10231 using the disc diffusion method. A PDA medium was prepared as per the manufacturer's instructions. The PDA-containing plate was then inoculated with Candida albicans suspension by streaking it with a sterilized swab very well. Similar to the antibacterial activity experiment, four concentrations (12,500, 25,000, 50,000, and 100,000 µg/mL) of the chloroform:methanol extract was prepared in DMSO, and 100 µL amount of each concentration was loaded onto a sterile Whatman filter paper disc (6 mm). The impregnated discs were placed on the surface of the inoculated agar plates using sterile forceps. Commercial ketoconazole/Tilt disc (10 µg/disc) was used as a reference drug. Then, the PDA plates were sealed with Parafilm and incubated at 27 °C for 3-5 days for fungal growth. After incubation, the diameters of the zones of inhibition around each disc were measured using a caliper (in mm). Experiments

were conducted in duplicate, and the results were expressed in as the mean and standard deviation.

2.6. In Vitro Antioxidant Activity Examination

The *in vitro* antioxidative effect of extracts and isolated compounds was evaluated against DPPH free radicals using six different concentrations (500, 250, 150, 100, 50, and 25 µg/mL) prepared from corresponding stock solutions (1 mg/mL in MeOH). Ascorbic acid (AA) was used as a standard antioxidant agent at similar concentrations. The assaying experiment was done following the procedures described previously (15-17). The antioxidant potential of each extract and isolated calotropin was evaluated in terms of percentage scavenging activity calculated using the following formula (1):

$$\text{DPPH scavenging activity (\%)} = (1 - \frac{A}{A_0}) \text{ x } 100 \quad (1)$$

where A and A_0 are the absorbances of samples with DPPH and the negative control (DPPH solution, 0.004% w/v in MeOH), respectively, the DPPH free radical trapping power of the extracts and the compound was also expressed in terms of IC_{50} (the concentration needed to scavenge the total DPPH radicals by 50%). This IC_{50} value was obtained from the regression equation derived from the percentage scavenging activity versus the concentration graph of each tested sample. The experimental activity was done in triplicate, and the results were expressed as the mean ± standard deviation.

2.7. In Silico Molecular Modeling Study

The molecular binding capacity of the isolated compound, calotropin, was studied by docking against the *P. aeruginosa* PqsA (5OE3) enzyme model. The molecular docking analysis was performed using the AutoDock Vina tools (version 4.2). All the necessary protocols were adjusted during the computational analysis, as stated previously (16). The conformation with the best-scored pose between the protein model and the isolated compound with the lowest binding energy was considered for the binding capacity. The molecular docking results were analyzed based on the binding energy (kcal/mol) and number of binding interactions between potential amino acid residues and the studied compound. The drug-likeness, ADME, and toxicity properties predictions of the isolated compound were also computed by SwissADME, PreADMET, and OSIRIS/Pro Tox-II property explorer software.

3. RESULTS AND DISCUSSION

In this study, the phytochemical investigation of the leaves of *G. purpurascens* resulted in the isolation of a cardenolide compound, calotropin (Figure 1). The *in silico* and *in vitro* antibacterial activity of this isolated compound was also evaluated herein. Besides, extracts of the leaves of *G. purpurascens* were assessed for their potential antibacterial, antifungal, and antioxidant effects against microbial strains and oxidants.

3.1. Structural Identification of Isolated Compound

Isolated compound (Calotropin, Figure 2): Black gel (12 mg); R_f 0.4 (*n*-hexane/EtOAc/AcOH, 3:1:0.1); See Table 1 for ^{1}H, ^{13}C and DEPT-135 NMR spectral data.

In the ^{1}H NMR spectrum (Figure S1) of the isolated compound, a deshielded singlet signal at δ_H 10.05 (1H, H-19) is due to the aldehydic proton. In contrast, a doublet signal at δ_H 5.93 (1H, H-22, d, J = 7.35) is due to an olefinic methine proton. Moreover, a broad singlet signal was observed at δ_H 4.72 (1H, H-1'), suggestive of oxygenated methine proton (-O-CH-O-). In addition, a broad doublet signal (integrated for two protons) was observed at δ_H 4.53 (2H, H-21, br d, J = 24.52) which underlined the occurrence of methylenoxy methylene protons (-CH_2-O-). The ^{1}H spectrum also confirmed the presence of three additional oxygenated methine protons, which appeared as a broad doublet at δ_H 4.0 (1H, H-2, br d, J = 6.06) and multiplets at δ_H 3.89 (1H, H-3) and 3.45 (1H, H-5'). A hydroxylated methine proton (-CH-OH) was also found as a multiplet signal at δ_H 3.64 (1H, H-3'). In the aliphatic region, a doublet of doublet signal appeared at δH 2.85 (1H, H-17, dd, J = 10.2, 6.4), implying the presence of a methine proton attached to a tertiary carbon adjacent to a quaternary olefinic carbon. Moreover, a doublet at δ_H 0.94 (3H, H-6', d, J = 7.1) and singlet at δ_H 0.83 (3H, H-18) signals were observed to confirm the presence of two methyl groups positioned at oxygenated tertiary and quaternary carbon, respectively. In the aliphatic region, additional twelve multiplet signals were shown at δ_H 2.47 which ascribed to nine different methylene (18H, H-1, H-4, H-6, H-7, H-11, H-12, H-15, H-16 and H-4') and three methine (3H, H-5, H-8 and H-9) groups.

The corresponding ^{13}C and DEPT-135 spectra (Figures S2 and S3) presented twenty-nine unsymmetrical recognizable signals, which attributed to seven quaternary, one hydroxylated methine (-CH-OH), four oxygenated methine (-O-CH-O-), one oxygenated methylene (-CH_2-O-), one olefinic methine (=CH-), four aliphatic methine (-CH-), nine aliphatic methylene (-CH_2-) and two methyl (-CH_3) groups. Two of the seven quaternary signals appeared at δ_C 208.0 (C-19) and 175.9 (C-23 overlapped), corresponding to the aldehyde and furan carbonyl carbons, respectively. The remaining five belonged to two aliphatic carbons at δ_C 52.6 (C-10) and 49.0 (C-13), two hydroxylated carbons at δ_C 90.4 (C-2') and 84.4 (C-14), and one olefinic carbon at δ_C 175.9 (C-20 overlapped). The signal at δC 116.6 (C-22) was ascribed to the olefinic methine carbon, and the one at δ_C 71.9 (C-3') belonging to the hydroxylated methine carbon. The signal resonated at δ_C 73.9 (C-21), representing the oxygenated methylene carbon. The overall spectral data (Table 1) is in agreement with the reported data in the literature (10) for calotropin (Figure 2).

Table 1: ^{1}H, ^{13}C, and DEPT-135 NMR (CD_3OD) data of calotropin.

	Isolated compound			Calotropin (10) in $CDCl_3$:CD_3OD (4:1)	
Attribution	**δ_H**	**δ_C**	**DEPT-135**	**δ_H**	**δ_C**
1	2.47 (m)	30.2	$-CH_2-$	*	34.2
2	4.0 (br d, *J* = 6.06)	70.5	-CH-O-	4.00 (dd, *J* = 12.5, 4.0	68.1
3	3.89 (m)	77.9	-CH-O-	3.85 (m)	71.2
4	1.68 (m)	33.0	$-CH_2-$	*	32.6
5	1.68 (m)	43.0	-CH-	*	44.1
6	1.41 (m)	28.7	$-CH_2-$	*	28.9
7	1.41 (m)	27.4	$-CH_2-$	*	27.2
8	1.26 (m)	38.8	-CH-	*	42.9
9	1.68 (m)	49.3	-CH-	*	49.9
10	-	52.6	Q	-	52.4
11	1.41 (m)	24.2	$-CH_2-$	*	21.4
12	1.26 (m)	33.4	$-CH_2-$	*	38.5
13	-	49.0	Q	-	49.2
14	-	84.4	Q	-	83.9
15	2.12 (m)	36.8	$-CH_2-$	*	38.7
16	1.68 (m)	26.5	$-CH_2-$	*	26.3
17	2.85 (dd, *J* = 10.2, 6.4)	50.4	-CH-	2.69 (dd, *J* = 9.0, 4.5)	50.1
18	0.83 (s)	14.8	$-CH_3$	0.89 (s)	15.1
19	10.05 (s)	208.0	Q (C=O)	*	207.7
20	-	175.9	Q	-	175.7
21	4.53 (br d, *J* = 24.52)	73.9	$-CH_2-O-$	4.63 (dd, *J* = 18.0, 1.0)	73.5
22	5.93 (d, *J* = 7.35)	116.6	=CH-	5.69 (br s)	116.8
23	-	175.9	Q (C=O)	-	175.5
1′	4.72 (br s)	94.1	-O-CH-O-	4.78 (s)	95.5
2′	-	90.4	Q	-	90.9
3′	3.64 (m)	71.9	-CH-OH	3.72 (m)	72.3
4′	2.37 (m)	35.4	$-CH_2-$	*	37.8
5′	3.45 (m)	66.3	-CH-O-	3.38 (m)	67.8
6′	0.94 (d, *J* = 7.1)	19.9	$-CH_3$	1.02 (d, *J* = 6.0)	20.4
OHs	2.05 (s)	-	-	*	-

***** Values not reported

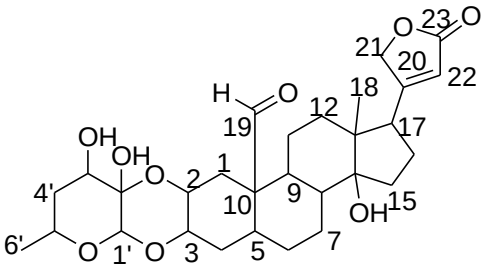


Figure 2: Structure of calotropin isolated from *G. purpurascens* leaves.

3.2. In Vitro Antimicrobial Activity Evaluation

3.2.1. Antibacterial activity assay

The antibacterial inhibition zone diameters, expressed as mean ± standard deviation, recorded by five extracts of *G. purpurascens* leaves against three standard human bacterial pathogens are reported in Table 2. As can be shown in Table 2, all the tested concentrations of all extracts indicated positive activities against all strains, except the lowest concentrations (12,500 and 25,000 µg/mL) of chloroform (against *S. aureus*), chloroform: methanol (against *P. aeruginosa*), methanol (against *E. coli*), and ethanol (against *S. aureus*) extracts, which were found inactive (< 7 mm inhibition zone). The highest inhibitory value (10.1 ± 0.1 mm) against *E. coli* at the highest concentration (100,000 µg/mL) was scored by *n*-hexane extract and then followed by 1:1 of chloroform: methanol (9.4 ± 0.0 mm) and chloroform (9.1 ± 0.3 mm) extracts. The lowest concentration (12,500 µg/mL) of the four extracts also inhibited the growth of *E. coli* (7.1 ± 0.1 to 8.1 ± 0.0 mm). The *S. aureus* bacterium was found to be sensitive to all extracts at the maximum concentration (100,000 µg/mL), with the larger diameter of the zone of inhibition value (9.1 ± 0.2 mm) recorded by the methanol extract. The *P. aeruginosa* was also found to be more susceptible to *n*-hexane extract (10.8 ± 0.1 mm) at the highest dose (100,000 µg/mL). Thus far, one similar study was reported by (6), aiming to evaluate the antibacterial activity of ethanol and methanol leaf extracts of *G. purpurascens* against the same

standard bacteria mentioned above. However, the tested concentrations (150, 300, and 600 mg/mL) in the reported study were far higher than the ones tested in the present work. According to the report, the ethanol and methanol leaf extracts scored respective inhibition zone values of 6.09 ± 0.18 to 6.90 ± 0.40 mm and 9.19 ± 0.07 to 13.20 ± 0.1 mm against *E. coli*; 9.51 ± 0.1 to 12.7 ± 0.15 mm and 7.1 ± 0.01 to 8.2 ± 0.01 mm against *S. aureus*; and 9.01 ± 0.01 to 9.77 ± 0.15 mm and 9.8 ± 0.01 to 13.8 ± 0.10 mm against *P. aeruginosa*. In essence, our result was comparable to this report, regardless of the huge concentration difference.

Table 2: Antibacterial inhibition zone diameter (mean ± sd) of leaf extracts of *G. purpurascens* against the *E. coli*, *S. aureus,* and *P. aeruginosa* standard bacterial strains.

Bacterial strains	Concentration (µg/mL)	Diameter of zone of inhibition (mean ± sd, mm) of extracts					
		***n*-Hexane**	**$CHCl_3$**	**$CHCl_3$:MeOH (1:1)**	**MeOH**	**EtOH**	**Chloramphenicol (30 µg)**
E. coli	12,500	8.1±0.0	7.8±0.2	7.9±0.3	6.8±0.0	7.1±0.1	24.3±0.8
	25,000	9.2±0.2	8.4±0.4	9.0±0.1	6.9±0.1	7.9±0.4	
	50,000	9.8±0.0	8.7±0.0	9.4±0.0	7.1±0.1	8.4±0.1	
	100,000	10.1±0.1	9.1±0.3	9.4±0.0	7.2±0.0	8.7±0.1	
S. aureus	12,500	7.0±0.0	0.0	7.0±0.8	7.1±0.4	6.3±0.0	19.4±1.8
	25,000	7.6±1.2	6.9±0.0	7.2±0.7	8.3±0.0	6.6±0.0	
	50,000	8.1±0.0	7.5±0.0	7.4±0.7	8.8±0.5	7.0±0.0	
	100,000	8.8±0.5	8.0±0.1	8.1±0.2	9.1±0.1	7.2±0.0	
P. aeruginosa	12,500	9.1±0.1	7.1±0.0	6.5±0.3	7.8±0.0	7.5±0.1	7.1±0.2
	25,000	9.2±0.0	7.9±0.0	7.7±0.2	8.2±0.2	8.1±0.4	
	50,000	9.7±0.2	8.1±0.2	8.0±0.1	8.6±0.0	8.5±0.0	
	100,000	10.8±0.1	8.8±0.0	8.6±0.0	9.2±0.0	9.0±0.1	

The isolated compound, calotropin, showed an inhibitory effect against *P. aeruginosa* at all concentrations, with a higher diameter of the zone of inhibition value of 8.8 ± 0.1 mm recorded at 1000 µg/mL concentration (Table 3). This compound also attempted to inhibit the growth of E. coli up to 300 µg/mL dose (7.7 ± 0.7 mm), whereas it was found to have no activity against the methicillin-resistant *S. aureus* (MRSA) bacterium at all concentrations.

Table 3: Antibacterial activity of the isolated compound (calotrpoin) against the *E. coli*, *S. aureus* and *P. aeruginosa* bacterial strains.

Concentrations (µg/mL)	Diameter of zone of inhibition (mean ± sd, mm) of calotropin		
	E. coli	***S. aureus***	***P. aeruginosa***
50	6.3 ± 0.0	0.0	7.4 ± 0.2
100	6.8 ± 0.1	0.0	7.8 ± 0.1
300	7.7 ± 0.7	0.0	7.9 ± 0.1
500	8.1 ± 0.3	0.0	8.6 ± 0.1
1000	8.8 ± 0.0	0.0	8.8 ± 0.1
Chloramphenicol (30 µg/disc)	25.3 ± 0.8	19.2 ± 1.6	7.3 ± 0.5

3.2.2. Antifungal activity assay

The chloroform:methanol (1:1) extract of *Gomphocarpus purpurascens* leaves was assessed for its potential antifungal activity against C. albicans ATCC 10231. The obtained result indicated that the extract displayed a slight activity only at the maximum concentration of 100,000 µg/mL with a diameter of the zone of inhibition value of 11.00 ± 1.00 mm.

However, this extract was found to be totally inactive up to 12,500 µg/mL. It was generally found to be weak compared to the standard antifungal drug ketoconazole 10 µg/disc (17.67 ± 2.52 mm).

3.3. *In Vitro* Antioxidant Potential Examination

As presented in Table 4 and depicted in Figure 3, an auspicious anti-DPPH free radical inhibitory effect was observed in all extracts with higher scavenging percentage values (95.7 ± 0.00 and 93.5 ± 0.00, respectively) recorded by the ethanol and methanol extracts at 500 µg/mL, with each having an IC_{50} value of 1.1 µg/mL. The chloroform: methanol (1:1) and chloroform extracts also displayed good DPPH radical scavenging percentage values of 91.2 ± 0.00 (IC_{50} value of 1.7 µg/mL) and 85.2 ± 0.00 (IC_{50} value of 2.7 µg/mL). The isolated compound, calotropin, exhibited far less DPPH scavenging percentage (65.09 ± 0.04) at the higher concentration (500 µg/mL) with a higher IC_{50} value of 134.0 µg/mL.

Table 4: DPPH scavenging activity percentage of *G. purpurascens* leaf extracts and the isolated calotropin.

Concentration (µg/mL)	% Scavenging activity (mean ± sd) against DPPH free radical					
	$CHCl_3$	$CHCl_3$: MeOH	MeOH	EtOH	Calotropin	Ascorbic acid
25	64.30 ± 0.00	70.1 ± 0.00	72.53 ± 0.15	73.7 ± 0.00	36.3 ± 0.00	75.45 ± 0.07
50	70.6 ± 0.00	74.6 ± 0.00	76.83 ± 0.15	78.87 ± 0.06	39.64 ± 0.03	80.99 ± 0.10
100	75.60 ± 0.00	78.07 ± 0.00	79.97 ± 0.06	82.2 ± 0.00	43.7 ± 0.03	85.29 ± 0.07
150	78.30 ± 0.20	82.3 ± 0.00	83.3 ± 0.00	85.43 ± 0.06	48.92 ± 0.07	89.01 ± 0.00
250	80.83 ± 0.06	87.7 ± 0.00	88.4 ± 0.00	90.6 ± 0.00	56.64 ± 0.04	95.79 ± 0.03
500	85.2 ± 0.00	91.2 ± 0.00	93.5 ± 0.00	95.7 ± 0.00	65.09 ± 0.04	98.89 ± 0.00
IC_{50} (µg/mL)	2.7	1.7	1.1	1.1	134.0	1.0

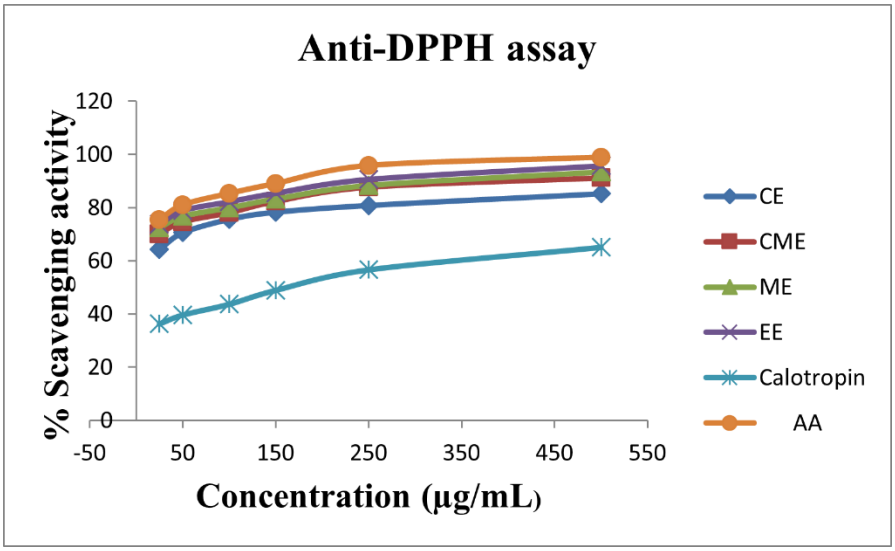


Figure 3: DPPH radical scavenging activity percentage versus concentration (µg/mL) of *G. purpurascens* leaf extracts and the isolated calotropin.

3.4. In Silico Molecular Modeling Study

The docking analysis output revealed that the isolated compound, calotropin, formed some interactions with the amino acid residues of the *P. aeruginosa* PqsA (5OE3) enzyme (Figure 4). This compound recorded a docking score of -10.3 kcal/mol, which was found to be higher than that of chloramphenicol (-7.0 kcal/mol) (Figure 5). It established six hydrogen bonds with Gly-169, Lys-172, Arg-397, Ala-170, Thr-380, and Thr-164 amino acid residues of the protein model 5OE3. This compound also formed a π-cation interaction with Arg-333 and six Van der Walls interactions with Thr-164, Thr-304, Gly-302, Ala-303, Glu-305, and Gln-369 amino residues (Figure 4).

The drug-likeness property prediction report indicated that this calotropin was found to violate one of Lipinski's rules of five (with a molecular weight > 500 g/mol). The ADME property prediction report showed that the compound scored a higher skin permeation value (logP) of -8.89 cm/s. It also exhibited high gastro-intestinal absorption and was found to be non-blood brain barrier (BBB) permeable. Besides, the compound showed an inhibitory interaction with the P-glycoprotein (P-gp) enzyme, whereas it was observed as a non-inhibitor of all the cytochrome-P (CYP) enzymes. The toxicity property prediction report revealed that this calotropin compound was found within the toxicity class of two with an LD_{50} value of 8 mg/kg. It displayed immunotoxicity and cytotoxicity properties. However, it was found to be a non-hepatotoxic, non-carcinogenic, and non-mutagenic isolate.

4. CONCLUSION

The present study reported, for the first time, the combined antimicrobial and antioxidant activities of various extracts of the leaves of *G. purpurascens*. Besides, a cardenolide compound, known as calotropin, was isolated from the leaves of the plant and reported herein, along with its binding capacity against the *P. aeruginosa* PqsA (5OE3) enzyme. This compound showed a strong binding capacity with a docking score of -10.3 kcal/mol against the target enzyme, which supported the obtained *in vitro* antibacterial activity. The various extracts of the leaves of *G. purpurascens* displayed good antibacterial activity against all tested bacterial strains, which supported the claimed traditional medicinal uses of the plant. Besides, all the leaf extracts exhibited promising DPPH radical scavenging activity comparable to that of ascorbic acid (1.0 µg/mL) at the same concentrations, and this may give a clue for the anticancer potential of the plant and the isolation of potential antioxidant compounds. Since this is a preliminary report, we believe that further biochemical investigations would be needed on this *G. purpurascens* plant species to increase the chance of obtaining additional phytochemicals with modified biological activity.

Further, it was higher than that of chloramphenicol (-7.0 kcal/mol). On the leaves of *G. purpurascens*,

they led to the isolation of a cardenolide compound called calotropin. All the extracts and isolated compound, calotropin, displayed better antibacterial activity against *P. aeruginosa* strain than chloramphenicol (7.1 ± 0.2 to 7.3 ± 0.5 mm) at all tested concentrations. However, the tested chloroform: methanol (1:1) extract was found to have negligible antifungal activity against *C. albicans* compared to the standard ketoconazole 10 µg/disc (17.67 ± 2.52 mm). All the leaf extracts exhibited promising DPPH radical scavenging activity with IC_{50} values ranging from 1.1-2.7 µg/mL equivalence to ascorbic acid (1.0 µg/mL) at the same tested concentrations. The molecular docking result revealed that the isolated compound, calotropin, showed a good binding capacity against *P. aeruginosa* PqsA (5OE3) with a docking score of -10.3 kcal/mol, which was higher than that of the chloramphenicol (-7.0 kcal/mol). The drug-likeness candidacy and the pharmacokinetic property result showed that the isolated calotropin violated one of Lipinski's rules of five (with molecular weight > 500 g/mol) and was found to be an immunotoxic and cytotoxic isolate. Since this is a preliminary report, further biochemical investigations would be needed on this *G. purpurascens* plant species to increase the chance of obtaining additional phytochemicals with modified biological activity.

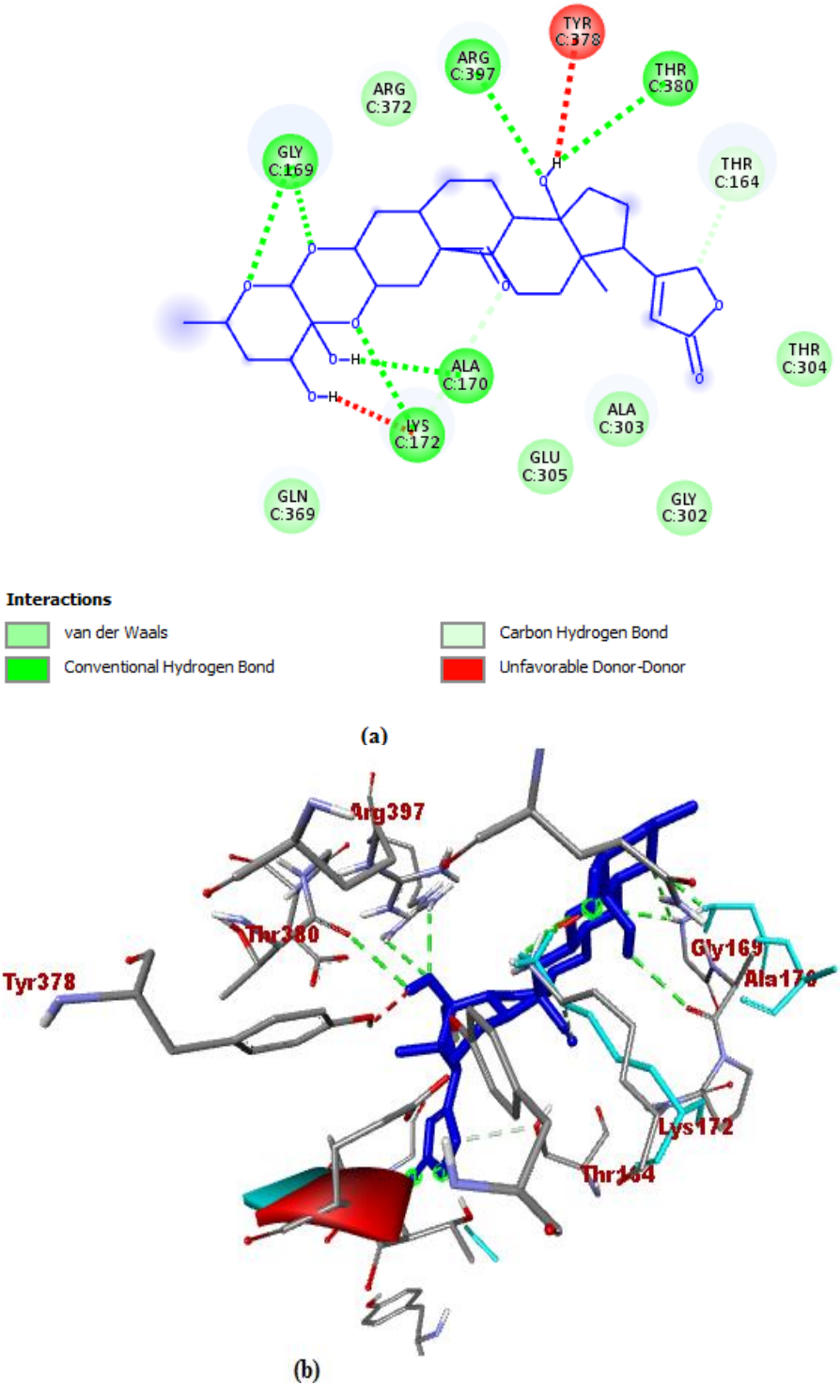
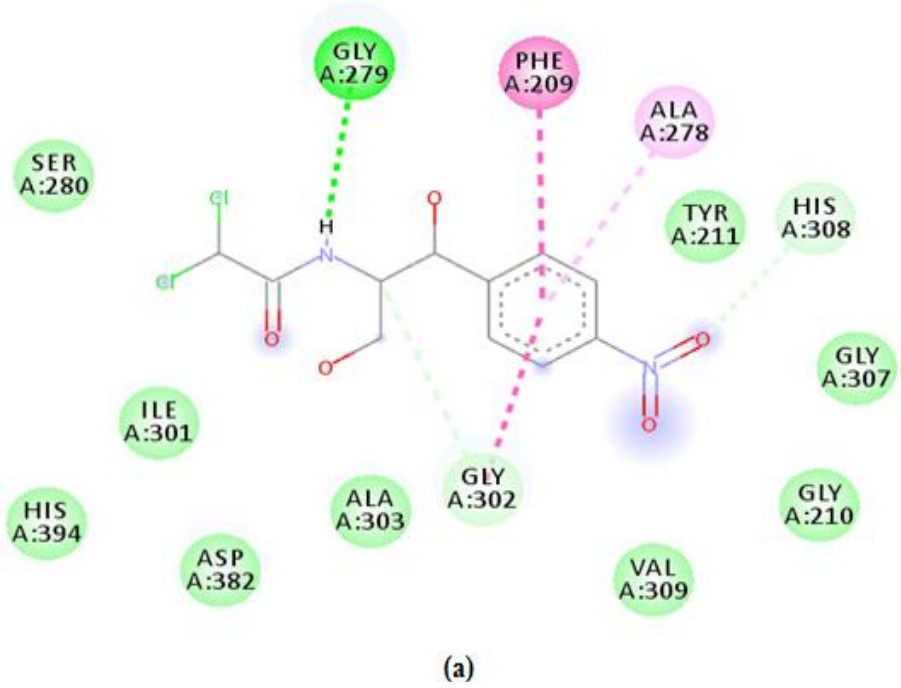


Figure 4: Ligand-protein interaction **(a)** and 3D representation **(b)** between calotropin and *P. aeruginosa* PqsA (5OE3).



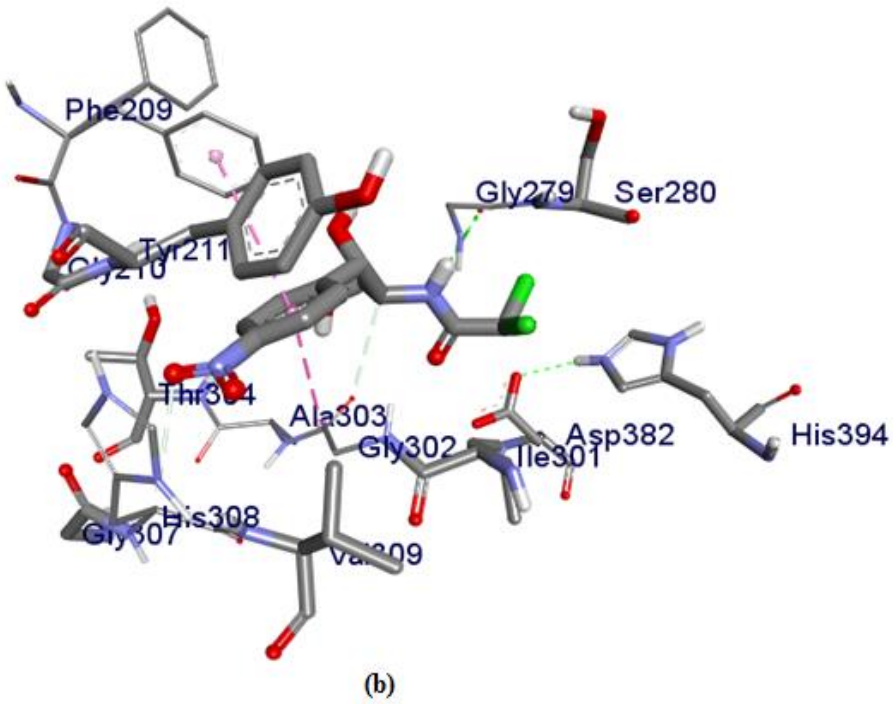


Figure 5: Ligand-protein interaction **(a)** and 3D representation **(b)** between chloramphenicol and *P. aeruginosa* PqsA (5OE3).

5. CONFLICT OF INTEREST

The authors declared no conflict of interest.

6. ACKNOWLEDGMENTS

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7. SUPPLEMENTARY MATERIALS

^{1}H, ^{13}C, and DEPT-135 NMR spectra of isolated compounds (calotropin) are available online.

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