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Determination of the antimicrobial and antioxidant activities of the leaf extracts of *Griffonia simplicifolia*

Clement Osei Akoto, Akwasi Acheampong, Prosper Dodzi Tagbor and Kingsley Bortey

Abstract
Griffonia simplicifolia has ethnomedicinal use in the treatment of depression, fibromyalgia, bladder and kidney problems, insomnia, malaria, obesity, migraine, as an aphrodisiac and a remedy for cough. The aim of this present study is to investigate the biological activities (antimicrobial and antioxidant) and isolate some of the components in the methanol and petroleum ether leaf extracts of *Griffonia simplicifolia*. Phytochemical screening, antimicrobial (agar and broth dilution method) and antioxidant [total antioxidant capacity (TAC), DPPH and H_2O_2 scavenging] assays were carried out on extracts. Thin layer and column chromatography were employed to isolate a single component which was analyzed using FTIR analysis. The petroleum ether and methanol extracts showed antimicrobial activity against test organisms with MICs ranging from 12.5 – 62.5 mg/mL. The IC_{50} values for methanol and petroleum ether extracts in the DPPH and H_2O_2 assays were 61.85 ± 0.41 and 94.26 ± 0.82 µg/mL; and 524.61 ± 0.68 and 976.75 ± 4.17 µg/mL, respectively. The TACs (gAAE/100 g) for methanol and petroleum ether extracts were 36.42 ± 0.38 and 18.47 ± 0.56 g, respectively. The phytochemical investigation revealed the presence of secondary metabolites such as alkaloids, triterpenoids, flavonoids, steroids, saponins, glycosides, phenols, tannins and coumarins. The findings of this study suggest that *Griffonia simplicifolia* leaves could be exploited as potential therapeutic candidate for the treatment of bacterial infections and diseases associated with oxidative-stress.

Keywords: Antimicrobial, antioxidant, phytochemical, therapeutics, pharmacological

1. Introduction
Medicinal plants are known to have antioxidant, anti-inflammatory, antimicrobial, anthelminthic and antimalarial activities amongst others [1-2]. Plants have always been an essential source of diverse therapeutics for various diseases and ailments worldwide. A great number of plants have been screened and found to possess various chemical compounds with varying therapeutic abilities [3]. About 80% of the world's population depend on traditional medicines for primary healthcare according to the World Health Organization [4]. Medicinal plants, therefore, are composed of important resources that can be put to use for both health and economic liberality. In Ghana, the use of medicinal plants in the form of traditional medicine is very common. Due to economic factors, coupled with some cultural preferences; traditional medicine has gained appreciable popularity among Ghanaians [5]. This popularity can also be attributed to the fact that traditional medicine is almost always readily available in many communities [6]. The exploitation of herbal medicines and medicinal plants in Ghana has been recognized and documented by many authors to curb the threat of vital information being lost since knowledge about the use of plants as medicines mostly belong to the older generation and are passed down orally from generation to generation [5, 6]. However, there are still many ethnic cultures and communities in Ghana whose traditional knowledge about herbal medicines is yet to be documented.
In this study, the leaves of *Griffonia simplicifolia* were screened for antimicrobial and antioxidant activity. *Griffonia simplicifolia* is an evergreen hard-wooded climbing shrub that grows typically in the western and central parts of Africa. It is mostly found in countries like Ghana, Liberia, Nigeria, Gabon, Congo, Ivory Coast and Togo [7]. It is commonly and locally called "Kagya" in Ghana by the Akans. Traditionally, various parts of the plant are used to treat wounds, kidney ailments, skin burns, diarrhoea, vomiting and stomach ache [8]. In Ghana, the stem and root are used as chewing sticks since it is believed to produce an aphrodisiac effect. The stem of *G. simplicifolia* is very strong and hard so it is used as a walking stick in most Ghanaian communities. The leaves are used in the treatment of kidney and bladder ailment and skin diseases [9].

The leaves are also reported for the treatment of malaria, relieving constipation and a remedy for cough [10-11]. A decoction of the stems and leaves is administered internally to stop vomiting and to treat congestion of the pelvis and externally used as a disinfectant. The seeds of *G. simplicifolia* are used as an herbal supplement for their 5-hydroxytryptophan content (5-HTP) which is a direct precursor for serotonin [7, 12]. 5-HTP is known to increase serotonin levels in the central nervous system and has widely been used in the treatment of depression, fibromyalgia, migraine, obesity and insomnia [7, 12-13]. With its abundance in the seeds of *G. simplicifolia*, it could represent a new therapeutic strategy for the treatment of serotonin-related disorders.

Most research studies conducted on the pharmacological potential of *G. simplicifolia* are mainly focused on crude extracts of the leaves [9], seeds [12], stem-bark and ethnobotanical review [13]. With the scientific evidence of the plant pharmacological properties lacking, it is important to identify the bioactive compounds responsible for each of the ascribed bioactivities. At the time of carrying out this research, a preliminary toxicity assessment report had been carried out on the plant [14] not concerning its antimicrobial activity.

The aim of this study was to examine the efficacy of *G. simplicifolia* methanol and petroleum ether extracts as an antimicrobial and antioxidant using *in vitro* assays. Additionally, to identify and confirm the presence of the phytochemicals in the leaves extract eliciting pharmacological activities using FTIR analysis.

2. Materials and Methods

2.1 Sample collection and identification

The fresh matured leaves of *G. simplicifolia* were collected in the month of September, 2018 from a local farm at Kentinkrono, in Kumasi in the Ashanti region of Ghana with the help of a local herbalist. They were taxonomically identified and authenticated at the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi by Mr. Clifford Osafo Asare, with the voucher specimen number (KNUST/HMI/2019/L005) deposited in the herbarium for reference purposes.

2.2 Chemicals and reagents

All chemicals were purchased from Sigma Aldrich Co. Ltd, Irvine, U.K., except the standard drugs. The organic solvents were of analytical grade and procured from BDH Laboratory Supplies (England).

2.3 Extraction of plant material

The leaves of *G. simplicifolia* were thoroughly washed, first under running water to remove any form of debris and subsequently rinsed in distilled water to exclude dissolve heavy metals in tap water and then distilled water. The leaves were air dried under shade for two weeks, pulverized into coarse powder, and stored in a desiccator until analysis.

2.3.1 Preparation of extracts

A mass of 250 g of the powdered sample of *G. simplicifolia* was soaked separately in 1 dm^3 of petroleum ether and methanol and extracted using the soxhlet apparatus [1]. The extracts were condensed and evaporated to dryness using the rotary evaporator at 50 ºC (BUCHI Rota vapor R -114). The extracts were dried and the percentage yield of extracts with respect to powdered plant material determined. The extracts were then stored at 4 ºC in a refrigerator.

2.4 Phytochemical screening of extracts

The pulverized sample and the crude extracts obtained were screened to assess the presence of phytoconstituents using the methods described by Trease and Evans [15].

2.5 *In vitro* Antioxidant Assays

Three main assays were employed for the antioxidant activity determination. They were the 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radicals scavenging, Hydrogen Peroxide scavenging (H_2O_2) and the Total Antioxidant Capacity (TAC) assays.

2.5.1 DPPH radical Scavenging Assay

The DPPH free radical scavenging activity of the two extracts were examined according to a modification of the standard methods previously described [1, 16]. Ascorbic acid was used as reference standard. The absorbance was measured at 517 nm. The experiment was independently repeated to obtain three independent sets of data for the analysis. DPPH radical scavenging (%) was calculated using the formula

$$\% \text{ Scavenging} = \frac{A_0 - A}{A_0} \text{ x } 100\%$$

Where

A_0 = absorbance of control; A = absorbance of test solution

2.5.2 Hydrogen Peroxide Scavenging Assay

Determination of hydrogen peroxide scavenging potential of the extracts were carried out according to a modification of the standard methods previously described [1, 17]. Gallic acid was used as reference standard. Absorbance was taken at 510 nm using a UV-vis spectrophotometer. The experiment was independently repeated to obtain three independent sets of data for the analysis. The percentage scavenging activity was calculated using the formula below

$$\% \, Scavenging = \frac{A_{test}}{A_{control}} \times 100$$

Where

A_{test} is absorbance of the test samples and $A_{control}$ is the absorbance of the negative control. The results were further reported in IC_{50}.

2.5.3 Total antioxidant capacity (TAC) assay

A modification of the methodology as previously described was used to study the total antioxidant capacity of the extracts of *G. simplicifolia* [1, 18]. Ascorbic acid was used as the reference standard. The absorbance of the solutions was measured in triplicates using a UV-visible spectrophotometer at 695 nm. The absorbance was measured and distilled water was used as the blank. The experiment was independently repeated to obtain three independent sets of data for the analysis. From the linear equation of the ascorbic acid concentration-absorbance plot, the corresponding independent variables as ascorbic acid equivalents (AAE) were determined, and the results expressed as gAAE/100g ascorbic acid.

2.6. Antimicrobial activity

Agar well diffusion and Broth micro-dilution (minimum inhibitory concentration) assays were employed to assess the antimicrobial activities of the extracts of *G. simplicifolia*.

2.6.1. Sources of microorganisms

Four bacteria and one fungus were used as test organisms. These were two Gram positive bacteria which included *Staphylococcus aureus* and *Enterococcus faecalis* and two Gram negative bacteria which included *Escherichia coli*, *Pseudomonas aeruginosa*. The fungus was *Candida albicans*. The microbial strains were provided by the Pharmaceutical Microbiology Section of the Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Science, KNUST, Kumasi. The microbial strains were sub-cultured on nutrient agar slants and incubated at 37 °C for 24 hours.

2.6.2. Inoculum preparation: Bacterial isolates were streaked onto nutrient agar (Oxoid, United Kingdom) plates and incubated for 18-24 hours at 37 °C. Using the direct colony suspension method, suspensions of the organisms were made in nutrient broth and incubated overnight at 37 °C. For the tests, colony suspensions in sterile saline was adjusted to 0.5 McFarland standard and further diluted in sterile double strength nutrient broth (~2×10^5 CFU/mL) [19].

2.6.3. Agar well diffusion

The antimicrobial activities of the different extracts were determined using a modification of the agar well diffusion standard method previously described [1, 20]. Ciprofloxacin (0.05 mg/mL) and clotrimazole (0.05 mg/mL) were used as the standard reference antimicrobial drug. The extracts and antibiotics were tested in triplicates and mean zones of inhibition were calculated for each extract and the standard antibiotic.

2.6.4 Broth micro-dilution

In the determination of the minimum inhibitory concentration (MIC), the method used was a modification of micro-well dilution standard method previously described [1, 20]. Ciprofloxacin and clotrimazole were used as positive control. The experiment was carried out triplicate.

2.7 Thin layer chromatography (TLC)

The number of components present in the extracts were determined by the analytical TLC method. The pre-coated silica gel plates (0.25 mm) with a fluorescent indicator (F254) were spotted with the extracts about 1 cm from the bottom edge of plates, with the aid of capillary tubes and allowed to dry [21]. Various solvent systems of petroleum ether/ethyl acetate, hexane/ethyl acetate, hexane/ethyl acetate/chloroform in different ratios were used. After trying out different combinations of solvents (hexane/ethyl acetate/chloroform in different ratios), the ratio of 5:3:2 (hexane/ethyl acetate/chloroform) gave the best separation of components for most of the extracts. The plates were dried and visualized by a 254 nm UV lamp. The separated spots were then marked and their sample and solvent fronts were measured.

The retardation factor (R_f) of the eluted spots was calculated as follows

$$R_f = \frac{\text{Distance travelled by spot}}{\text{Distance travelled by solvent front}}$$

2.8 Column Chromatographic Separation

Flash chromatography was performed using 40–63 µm silica gel (200 x 400 mesh) to separate the number of components present in the extracts [22]. Dry powdered petroleum ether extract was chromatographed on a column packed with silica gel and eluted with a gradient of solvent hexane to provide a fraction, A. The fraction was monitored by means of TLC (eluent Hexane/EtOAc 9:1). The fraction was evaporated to dryness using the rotary evaporator, then dried and stored at 4 °C in a refrigerator until the use.

2.9 Fourier transform infrared spectrometer (FTIR) analysis

The dried fraction (A) was subjected to (FTIR) analysis (UATR Two, PerkinElmer) to determine the functional groups present. The regions between 4000 cm^{-1} and 400 cm^{-1} were scanned, then followed by baseline correction.

2.10 Data analysis

Microsoft Excel 2016 and GraphPad Prism 6.0 for Windows (Graph Pad Sofware, San Diego, CA, USA) were used for all data analyses and graphs.

3. Results and Discussion

3.1 Extraction of plant material

The yields of the extract in relation to the powdered plant material were calculated as percentages. The yields were 14.52 and 6.72% for methanol and petroleum ether extracts, respectively.

3.2 Phytochemical screening

The therapeutic activities of plants are as a result of the presence of complex chemical constituents in different parts [23]. The phytochemical screening of *G. simplicifolia* revealed the presence of all nine secondary metabolites tested for in the pulverized sample and the methanol extract. Alkaloids, tannins, flavonoids, phenols and glycosides were absent in the petroleum ether extracts (Table 1).

Table 1: Phytochemical constituents of the pulverized sample and the extracts of *G. simplicifolia*

Phytochemical	Pulverized sample	Methanol extract	Petroleum ether extract
Saponins	+	+	+
Alkaloids	+	+	-
Tannins	+	+	-
Flavonoids	+	+	-
Phenols	+	+	-
Glycosides	+	+	-
Steroids	+	+	+
Coumarins	+	+	+
Triterpenoids	+	+	+

Key: (+) = presence of secondary metabolite; (-) = absence of secondary metabolite

The petroleum ether and methanol extracts had four phytochemicals in common, that is saponins, steroids, coumarins and triterpenoids. The absence of alkaloids, tannins, flavonoids, phenols and glycosides (which are polar due to their hydrophilic moieties) in the petroleum ether extracts might be due to the fact that the solvent is more non polar hence could not extract these phytochemicals from the powdered plant material. Methanol extract, however, showed the presence of alkaloids, tannins, flavonoids, phenols and glycosides due to its polarity. The presence of alkaloids and steroids in the leaves of *G. simplicifolia* confirms the work of Offoumou *et al.*, [11] who investigated antiplasmodial activity of ethanolic leaf extracts of *G. simplicifolia*, though they did not identify tannins, flavonoids, phenols and glycosides.

Secondary metabolites of plants which include terpenoids, flavonoids, tannins, phenols, alkaloids and steroids have been shown to exhibit various pharmacological activities such as wound healing, anti-inflammation, anticancer, antioxidant, immunomodulation, antidiarrhoeal, antimicrobial, antidepressant and antiplasmodial [24, 25]. Flavonoids, phenols and tannins have been reported to have antioxidant effects on human nutrition and health through scavenging, chelating and termination of free radicals [26, 27]. The presence of these phytochemicals in the extracts of *G. simplicifolia* leaves indicate that they will play a key role in the prevention of various bacterial infections and diseases associated with oxidative-stress.

3.3 *In Vitro* antioxidant activity

The total antioxidant potential of a plant extract depends largely on both the constituent of the extract and the test system [1]. Techniques employed in the assessment of antioxidant activity differ from one another in terms of assay principles and experimental conditions [28]. Different factors can influence the activity of the extract, and therefore more than one assay needs to be performed to make up for the various modes of action of antioxidants since a single method is not sufficient to estimate the antioxidant capacity [29]. Considering the various mechanisms of antioxidant actions, the antioxidant properties of the extracts were evaluated by (DPPH) free radicals scavenging, Hydrogen Peroxide scavenging and the Total Antioxidant Capacity assays.

3.3.1. DPPH radical scavenging capacity

The DPPH radical scavenging activity of the extracts was used to determine and study the ability of the extracts of *G. simplicifolia* to mop up free radicals that may be found in animals and humans. Methanol and petroleum ether extracts of *G. simplicifolia* and ascorbic acid (reference standard) of concentrations ranging between 6.25 µg/mL to 100 µg/mL, scavenged DPPH radical between 28.23 ± 0.001 to 70.94 ± 0.046, 26.95 ± 0.017 to 56.29 ± 0.025 and 75.74 ± 0.024 to 85.61 ± 0.046%, respectively. Methanol and petroleum ether extracts of *G. simplicifolia* and ascorbic acid (reference standard) scavenged DPPH radical in a dose dependent manner (Figure 1).

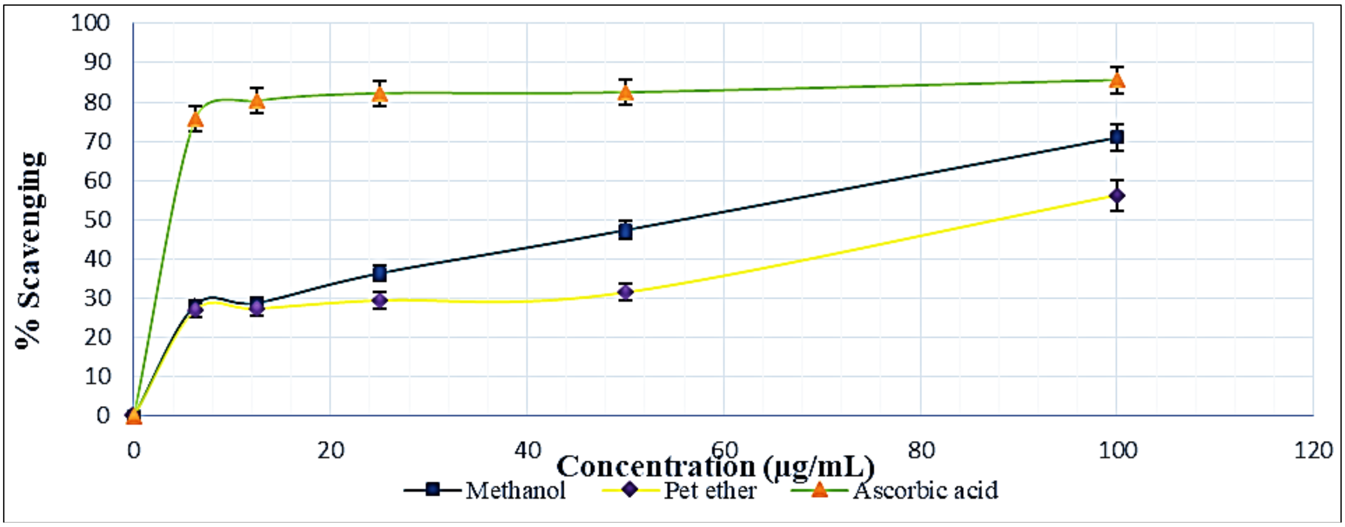

Fig 1: Comparative DPPH radical scavenging activity of the extracts of *G. simplicifolia* and ascorbic acid.

The reference antioxidant (ascorbic acid), petroleum ether and methanol extracts of *G. simplicifolia* showed antioxidant activity in the DPPH free radical scavenging assay with IC_{50} of ascorbic acid, petroleum ether and methanol ranged from 2.67 ± 0.42 to 94.26 ± 0.82 µg/mL as shown in Table 2.

Table 2: IC_{50} of DPPH Radical Scavenging Activity for Pet ether and Methanol extracts and Ascorbic Acid

Sample	IC_{50} (µg/mL)
Standard (Ascorbic acid)	2.67 ± 0.42
Methanol	61.85 ± 0.41
Petroleum ether	94.26 ± 0.82

The results implied that the activity of the test samples of extracts as antioxidants decreased in the order: methanol > petroleum ether (Figure 1). Methanol extract showed better antioxidant activity compared to the petroleum ether probably due to the presence of the flavonoids, phenols and tannins which have been reported to scavenge, chelate and terminate of free radicals [26]. Though petroleum ether and methanol extracts which comprise of a mixture of compounds were not as active as the ascorbic acid, *G. simplicifolia* leaf extracts may be useful therapeutic candidate for the treatment of diseases associated with oxidative-stress. This is due to the presence of the phytochemicals such as phenols, flavonoids, and tannins in *G. simplicifolia* leaf extracts, having antioxidant activity due to their redox properties and chemical structures.

3.3.2 Hydrogen peroxide scavenging assay

Non-radical oxidizing agents scavenging potential of the petroleum ether and methanol extracts of *G. simplicifolia* were evaluated by the use of hydrogen peroxide (H_2O_2) scavenging method.

Results showed that, the extracts demonstrated a significant antioxidant activity in concentration-dose dependent manner (Figure 2).

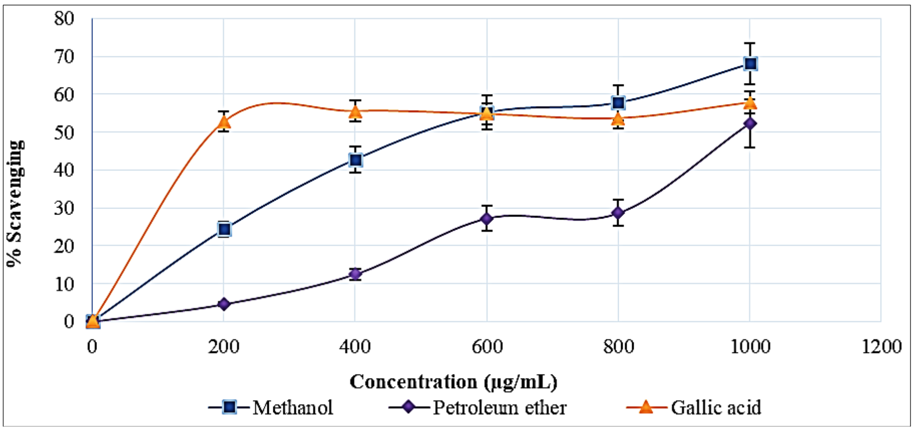

Fig 2: Comparative H_2O_2 Scavenging activity of *G. simplicifolia* extracts and Gallic acid

The IC_{50} values of gallic acid (reference antioxidant), petroleum ether, and methanol extracts ranged from 204.0 ± 0.01 to 976.75 ± 4.17 µg/mL as shown in Table 3.

Table 3: IC_{50} of H_2O_2 radical scavenging activity of extracts of *G. simplicifolia*

Sample	IC_{50} (µg/mL)
Standard (Gallic Acid)	204.0 ± 0.01
Methanol	524.61 ± 0.68
Petroleum ether	976.75 ± 4.17

From the results methanol extract was a more effective antioxidant than petroleum ether but comparable to gallic acid (reference antioxidant) although, they are all good antioxidants. Bioactive isolates from these extracts responsible for antioxidant activity could be attributed to the triterpenoids, flavonoids, tannins and phenols in *G. simplicifolia* and could be exploited for the treatment of diseases associated with oxidative-stress [25, 30].

3.3.3 Total antioxidant capacity (TAC)

Ascorbic acid also known as Vitamin C is a naturally occurring organic compound with antioxidant properties, found in both animals and plants. It is an electron donor antioxidant and this property is responsible for all its known functions. It functions as a redox buffer which can reduce, and thereby neutralize, reactive oxygen species [31].

Concentrations of ascorbic acid ranging between 6.25 to 100 µg/mL showed antioxidant activity and mean absorbances between 0.109 ± 0.03 to 0.932 ± 0.02 at wavelength of 695 nm (Figure 3).

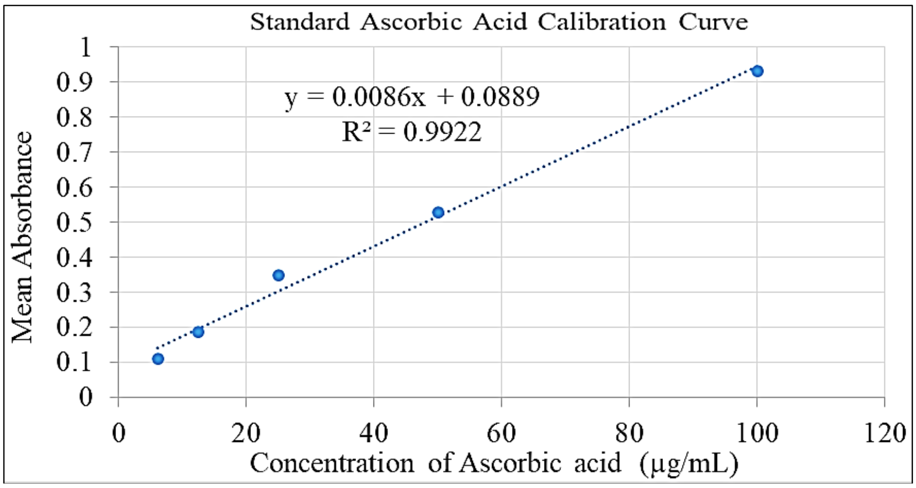

Fig 3: Absorbance of $PMo^{V}_{4}Mo^{VI}_{8}O_{40}^{7-}$ (formed in ascorbic acid solution) against concentration of ascorbic acid solution.

The TAC was found to be proportional to the concentration of extract. TAC of the extracts were examined by Phosphomolybdenum method and the results were expressed as gram ascorbic acid equivalent per 100 grams (gAAE/100g). The methanol and petroleum ether extracts had 36.42 ± 0.38 and 18.47 ± 0.56 gAAE/100g, respectively, (Table 4).

Table 4: Total Antioxidant Capacity of Petroleum ether and Methanol extracts expressed as gAAE/100g

Sample	TAC (gAAE/100g)
Methanol	36.42 ± 0.38
Petroleum ether	18.47 ± 0.56

TAC - Total Antioxidant Capacity; AAE - ascorbic acid equivalent

Methanol and petroleum ether extracts in *G. simplicifolia* demonstrated appreciable antioxidant activities due to the presence of the various phytochemicals such as flavonoids, phenols, tannins, triterpenoids among others which were associated with curing of various diseases and disorders including cancer, diabetes, gout, urolithiasis, obesity, and other diseases associated with ageing [32].

3.4. Antimicrobial assay

3.4.1 Agar well diffusion

The antimicrobial activities of the extracts were determined at four concentrations levels of 12.5, 25.0, 50.0 and 100.0 mg/mL for the agar well diffusion assay as shown in Table 5.

Table 5: Mean zones of inhibition (ZI) for methanol and petroleum ether extracts of *G. simplicifolia* and standard drugs ciprofloxacin and clotrimazole in agar well diffusion assay

Sample/Drug	Conc. (mg/mL)	Mean zone of inhibition (mm)				
		S. aureus	*P. aeruginosa*	*E. coli*	*C.albicans*	*E. faecalis*
Ciprofloxacin	50.0×10^{-3}	38.0 ± 0.5	25.0 ± 0.7	36.0 ± 0.2	NA	27.0 ± 0.75
Clotrimazole	50.0×10^{-3}	NA	NA	NA	26.0 ± 0.4	NA
Methanol	100.0	20.0 ± 0.5	21.0 ± 0.15	22.0 ± 0.46	21.0 ± 0.15	21.0 ± 0.24
	50.0	18.0 ± 0.2	19.0 ± 0.24	19.0 ± 0.26	20.0 ± 0.5	20.0 ± 0.15
	25.0	17.5 ± 0.6	18.0 ± 0.71	18.5 ± 0.12	18.0 ± 0.3	17.0 ± 0.32
	12.5	16.0 ± 0.22	16.0 ± 0.63	18.0 ± 0.26	-	-
Petroleum ether	100.0	16.0 ± 0.8	-	-	16.0 ± 0.21	15.0 ± 0.35
	50.0	-	-	-	-	-
	25.0	-	-	-	-	-
	12.5	-	-	-	-	-

NA = Not Applicable, Diameter of cork borer = 10 mm

The agar well diffusion technique simply classifies microbes as susceptible, intermediate or resistant and it is broadly used to evaluate the antimicrobial activity of plant extracts [33]. The zone of inhibition estimates the minimum antimicrobial agent (plant extract) concentration adequate to inhibit microbial growth. The higher the zone of growth inhibition, the more susceptible the organisms are to the extract or standard drug and the more potent the antimicrobial activity of the extract/standard drug [1]. The sizes of the zone of inhibition are compared to standards to determine if the microorganism is sensitive or resistant to the plant extract. The methanol and petroleum ether extracts exhibited antimicrobial activity against both gram-positive and gram-negative, and fungi organisms within the concentrations range of 12.5 to 100 mg/mL of extract with zones of growth inhibition ranging from 15.0 ± 0.35 to 22.0 ± 0.46 mm.

From the results obtained, the lowest concentration at which the methanol and petroleum ether extracts recorded a zone of inhibition was 12.5 and 100 mg/mL, respectively. No zone of inhibition was recorded for petroleum ether at concentrations of 12.5, 25.0 and 50.0 mg/mL. Even though all the test microorganisms were susceptible to the methanol extracts at a concentration range of 25.0 to 100.0 mg/mL, *E. coli* was the most susceptible with *S. aureus* and *P. aeruginosa* being the least susceptible. The petroleum ether extract showed inhibition against *E. faecalis, S. aureus* and *C. albicans* at a concentration of 100 mg/mL but no inhibition against *P. aeruginosa* and *E. coli*. The methanol extract showed inhibition against, *S. aureus, P. aeruginosa* and *E. coli* at a concentration of 12.5 mg/mL but no inhibition against *E. faecalis* and *C. albicans*. The methanol extract showed the highest activity, whereas the petroleum ether extract exhibited the least activity. All the four tested bacteria were susceptible to the ciprofloxacin (standard drug) with the gram-positive bacteria *S. aureus* showing the highest susceptibility. Both extracts and clotrimazole (standard drug) showed activity against the fungus *C. albicans*.

3.4.2 Broth microdilution

The extracts showed broad spectrum antimicrobial activity against the test organisms. Both extracts showed similar activity against the test organisms but the methanol extract showed a slightly better inhibition compared to the petroleum ether extract with antimicrobial activity ranging (at MIC of 12.5 mg/mL to 62.5 mg/mL). The results are shown in Table 6.

Table 6: Minimum inhibitory concentrations (MIC) of extracts and reference drugs against test organisms

Test organisms	Minimum Inhibitory Concentration (mg/mL)			
	Methanol (mg/mL)	Petroleum ether (mg/mL)	Ciprofloxacin (mg/mL)	Clotrimazole (mg/mL)
S. aureus	12.5	25.0	0.625×10^{-3}	NA
P. aeruginosa	50.0	50.0	2.5×10^{-3}	NA
E. coli	12.5	12.5	5.0×10^{-3}	NA
C.albicans	12.5	25.0	NA	1.25×10^{-3}
E. faecalis	62.5	25.0	0.625×10^{-3}	NA

NA=Not Applicable

The results from the antimicrobial assay performed showed that the two extracts of *G. simplicifolia* leaf exhibited varying inhibitory effects against the five selected microorganisms (two gram-positive, two gram-negative and one fungus). The minimum inhibitory concentrations (MICs) were between the range of 12.5 mg/mL to 62.5 mg/mL. The highest activity observed with the use of methanol extract was against *E. coli, S. aureus* and *C. albicans* with MIC of 12.5 mg/mL and the petroleum ether extract against *E. coli* with MIC of 12.5 mg/mL. The lowest activity was observed with the use of methanol extract against gram-positive microorganism *E. faecalis* with MIC of 62.5 mg/mL. The antimicrobial activity shown by the extracts could be attributed to the presence of secondary metabolites like triterpenoids in petroleum ether extract and alkaloids, tannins, and flavonoids all mostly in the methanol extract of *G. simplicifolia* which have been reported to exhibit antimicrobial activity [24]. Therefore, the antimicrobial activity of the methanol extracts against these test organisms may support the ethnomedicinal use of *G. simplicifolia* to treat wound infections, diarrhoea, and boils [8]. The susceptibility of *C. albicans* to the leaf extract of *G. simplicifolia* may therefore lend credence to the usage of preparations of the plant in treating such fungal infections like skin diseases [9].

3.5 Thin layer chromatography (TLC)

The analytical TLC method was used to determine the number of components present in the extracts. The chromatographic spots were observed and their R_f values determined (Table 7).

Table 7: TLC results of extract showing various components and their retardation factor using hexane/ethyl acetate/chloroform (5:3:2) as mobile phase.

Components	Retardation factor, R_f	
	Methanol	Petroleum Ether
A	0.88	0.93
B	0.82	0.88
C	0.75	0.82
D	0.60	0.75
E	0.54	0.64
F	0.41	0.54
G	0.17	-
H	0.07	-

The petroleum ether extract showed six spots and methanol eight spots with R_f values between 0.54 to 0.93 and 0.07 to 0.88, respectively. The number of spots indicating the separated components in the two extracts were less for methanol and more for petroleum ether when compared to the phytoconstituents identified to be present in each leaf extract. This means that some of the components could exist as isomers, similar functional groups or co-eluted in mixtures and it may be necessary to employ two dimensional TLC, HPLC or column chromatography to achieve complete separation of the components [1].

3.6 Column chromatographic separation
Column chromatography was employed to isolate the least polar component of the petroleum ether extract of *G. simplicifolia*. Fraction A was separated after elution with hexane.

Table 8: Chromatographic separation and fraction selected using hexane/ethyl acetate (9:1) as mobile phase.

Component	Retardation factor, R_f
A	0.935

Fraction A, the least polar compound having R_f value of 0.935 using hexane/ethyl acetate (9:1) as mobile phase was further analyzed using FTIR.

3.7 FTIR analysis
FTIR analysis was performed on the purified component A obtained from petroleum ether extract using column chromatographic separation. The results are presented in Figure 4 and Table 9 below.

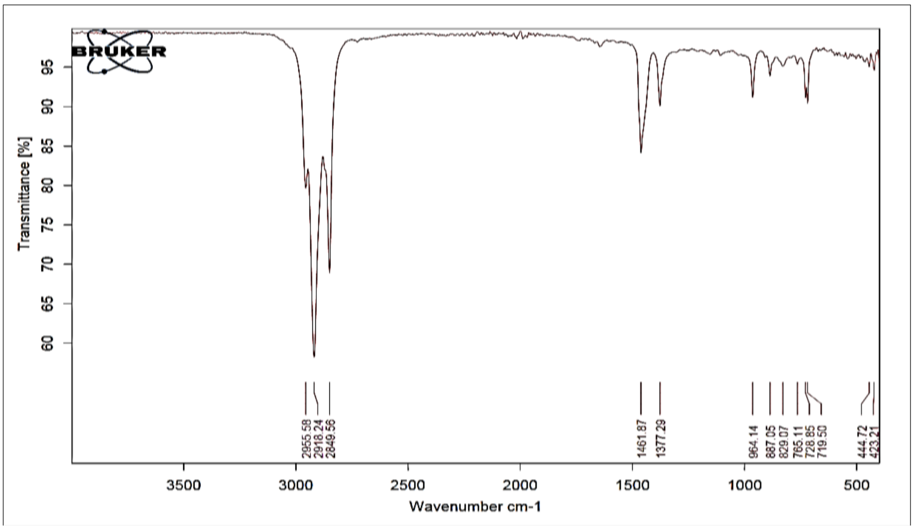

Fig 4: FTIR spectrum of Fraction A from the petroleum ether extract of *G. simplicifolia*

Table 9: FTIR Interpretation of Purified Fraction A from the petroleum ether Extract of *G. simplicifolia*

Fraction A		
Peak value (cm^{-1})	Functional group	Inference
2955 to 2849	C-H stretch	The functional groups show highly saturated compound.
1461, 1377	C-H, C-C, and/or C-O bend/stretch	

The wave numbers around 2955 to 2849 cm^{-1}, 1461 cm^{-1} and 1377 cm^{-1} positions of the spectrum are characteristic of aliphatic (C-H) and C-H, C-C, and/or C-O stretching, bending functional groups [34]. These functional groups show the presence of highly saturated compound of the identified secondary metabolites such as steroids and / or triterpenes from the screening test. Further structural characterization, identification and biological studies is ongoing in our research laboratory.

4. Conclusions
The methanol and petroleum ether extracts of *G. simplicifolia* showed the presence of varying secondary metabolites including alkaloids, flavonoids, glycosides, phenols, steroids, tannins, triterpenoids, saponins and coumarins. The extracts exhibited significant degrees of antioxidant activity in the DPPH scavenging activity, the H_2O_2 scavenging and the TAC assays. Column Chromatographic separation revealed a purified fraction A. Further studies are ongoing in our laboratory towards isolation, characterization, identification and determination of biological activities of the isolates from leaves of *G. simplicifolia*. From the results, it can be concluded that both methanol and petroleum ether extracts of *G. simplicifolia* contain promising bioactive phytocomponents against infectious and diseases associated with oxidative-stress, and could become sources of potential therapeutic agents for their treatment.

5. Disclosure
Part of this work was presented as a poster at the "8th Ghana Science Association, Research Seminar and Poster Presentations" and "8th College of Health Sciences & 12th Convention of Biomedical Research Ghana joint Scientific Conference held at the Kwame Nkrumah University of

Science and Technology, Kumasi, Ghana, in May and July 2019.

6. Conflicts of Interest
The authors declare no competing financial, professional, or personal interests that might have influenced the performance or presentation of the work described in this manuscript. The authors declare that there is no conflict of interests regarding the publication of this paper.

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8. References
1. Osei Akoto C, Acheampong A, Boakye YA, Akwata D, Okine M. *In vitro* anthelminthic, antimicrobial and antioxidant activities and FTIR analysis of extracts of *Alchornea cordifolia* leaves. Journal of Pharmacognosy and Phytochemistry. 2019; 8(4):2432-2442.
2. Acheampong A, Okyem S, Osei Akoto C, Baah AK. Antioxidant, antimicrobial and FTIR analysis of methanol root extract of *Cnestis ferruginea* and ethanol root extract of *Citrus limon*. J. Pharmacogn. Phytochem. 2018; **7**:2938-2946.
3. Govindappa M, Poojashri MN. Antimicrobial, antioxidant and *in vitro* anti-inflammatory activity of ethanol extract and active phytochemical screening of *Wedelia trilobata* (L.) Hitchc. Journal of Pharmacognosy and Phytotherapy. 2011; 3(3):43-51.
4. Tuo K, Beourou S, Toure AO, Ouattara K, Meite S, Ako AAB *et al.* Antioxidant activities and estimation of the phenols and flavonoids content in the extracts of medicinal plants used to treat malaria in Ivory Coast. Int J Curr Microbial App Sci. 2015; 4(1):862-874.
5. Addo-Fordjour P, Anning AK, Akanwariwiak WG, Belford EJD, Frimpong CK. Medicinal Plants of Ghana. Genetic Resources, Chromosome Engineering and Crop Improvement. Medicinal Plants. 2012; 6:221-246.
6. Boadu A, Asase A. Documentation of herbal medicines used for the treatment and management of human diseases by some communities in southern Ghana. Evidence-based Complement. Altern. Med, 2017, 1-12.
7. Kumar PS, Praveen T, Jain NP, Jitendra B. A review on *Griffonia simplicifollia* - an ideal herbal anti-depressant. International Journal of Pharmacy & Life Sciences. 2010; 1(3):174-181.
8. Ayensu ES. Medicinal Plants of West Africa. Reference Publication Inc. Michigan, USA, 1978
9. Eloff JN, Gurib-Fakim A, Phillips LD. African Herbal Pharmacopoeia T, Brendler (Ed.). Association for African Medicinal Plants Standards, 2010, 121-126.
10. Esposito M, Ruberto M, Pascotto A, Carotenuto M. Nutraceutical preparations in childhood migraine prophylaxis: effects on headache outcomes including disability and behaviour. Neurological Sciences. 2012; 33(6):1365-1368. Doi:10.1007/s10072-012-1019-8
11. Offoumou MR, Kipre GR, Kigbafori DS, Camara D, Djaman AJ, Zirihi GN. *In Vitro/ Ex Vivo* Antiplasmodial Activity And Phytochemical Screening Of Crude Extracts *Entandrophragma Angolense* (Welw.) C. DC., *Griffonia simplicifolia* (Vahl ex DC.) Baill. et *Uapaca guineensis* Mull. Arg. Three Plants of Ivorian Pharmacopeia In Treatment Of Malaria. Int J Curr Microbial Appl Sci. 2018; 7(03):2088-2095.
12. Carnevale C, Di Viesti V, Zavatti M, Zanoli P. Anxiolyticlike effect of *Griffonia simplicifolia* Baill. seed extract in rats. Phytomedicine. 2011; 18:848-851.
13. Pathak SK, Tahilani P, Prakash JN, Jitendra B. A review on Griffonia simplicifollia-an ideal herbal anti-depressant. International Journal of Pharmacy & Life Sciences. 2010; 1(3):174-181.
14. Nyarko RA, Larbie C, Anning AK, Baidoo PK. Phytochemical Constituents, Antioxidant Activity and Toxicity Assessment of Hydroethanolic Leaf Extract of *Griffonia Simplicifolia*. International Journal of Phytopharmacology. 2019; 10(1):6-18.
15. Trease GE, Evans WC. Pharmacognosy 16th edition. W.B. Sanders Co. Ltd., New York, 2009, 1-604.
16. Sánchez-Moreno C, Larrauri JA, Saura-Calixto FA procedure to measure the antiradical efficiency of polyphenols. J Sci. Food Agric. 1998; 76:270-276.
17. Mukhopadhyay D, Dasgupta P, Roy SD, Palchoudhuri S, Chatterjee I, Ali S *et al.* A Sensitive *In vitro* Spectrophotometric Hydrogen Peroxide Scavenging Assay using 1, 10-Phenanthroline. Free Radicals Antioxidants. 2016; 6:124-132.
18. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. Anal. Biochem. 1999; 269:337-341.
19. Wiegand I, Hilpert K, Hancock REW. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat. Protoc. 2008; 3:163-75.
20. Konning GH, Agyare C, Ennison B. Antimicrobial activity of some medicinal plants from Ghana. Fitoterapia. 2004; 75:65-67.
21. Zhao Y, Slepkov DA, Osei Akoto C, McDonald R, Hegmann FA, Tykwinski RR *et al.* Synthesis, Structure, and Nonlinear Optical Properties of Cross-Conjugated Perphenylated iso-Polydiacetylenes. Chem. Eur. J. 2005; 11:321-329.
22. Osei Akoto C, Rainier JD. Harnessing glycal-epoxide rearrangements: The generation of the AB, EF, and IJ rings of adriatoxin. Angew. Chemie - Int. Ed. 2008; 47:8055-8058.
23. Banu KS, Cathrine L. General Techniques Involved in Phytochemical Analysis. International Journal of Advanced Research in Chemical Science (IJARCS). 2015; 2(4):25-32.
24. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and Extraction: A Review. Internationale Pharmaceutica Sciencia. 2011; 1(1):98-106.
25. Boniface PK, Ferreira SB, Kaiser CR. Recent trends in phytochemistry, ethnobotany and pharmacological significance of *Alchornea cordifolia* (Schumach. & Thonn.) Muell. Arg. J. Ethnopharmacol. 2016; 191:216-244.
26. Kessler M, Ubeaud G, Jung L. Anti- and prooxidant activity of rutin and quercetin derivatives. J Pharm. Pharmacol. 2003; 55:131-142.

27. Ferguson L. Role of plant polyphenols in genomic stability. Mutation Research. 2001; 475:89-111.
28. Cao GH, Prior RL. *In vivo* total antioxidant capacity: Comparison of different analytical methods. Free Radical Biology and Medicine. 1999; 27(11-12):1173-1181.
29. Huang D, Ou B, Prior RL. The Chemistry Behind Antioxidant Capacity Assays. Journal of Agricultural and Food Chemistry. 2005; 53(6):1841-1856.
30. Kang HS, Kim JP. Butenolide derivatives from the fungus *Aspergillus terreus* and their radical scavenging activity and protective activity against glutamate-induced excitotoxicity. Appl Biol Chem. 2019; 62:43. https://doi.org/10.1186/s13765-019-0451-3.
31. Pehlivan FE. Vitamin C: An Antioxidant Agent. IntechOpen, 2017. doi:10.5772/intechopen.69660.
32. Shoaib M, Mehreen Ghias M, Shah SWA, Ali N, Umar MN, Rahman M *et al*. Synthetic flavonols and flavones: A future perspective as anticancer agents. Pak. J Pharm. Sci. 2019; 32(3):1081-1089.
33. Mbata TI, Debiao LU, Saikia A. Antibacterial Activity of the Crude Extract of Chinese Green Tea (*Camellia Sinensis*) on Listeria Monocytogenes. African Journal of Biotechnology. 2008; 7(10):1571-1573.
34. Osei Akoto C, Rainier JD. Concise Seven-Membered Oxepene/Oxepane Synthesis-Structural Motifs in Natural and Synthetic Products. Synthesis (Stuttg). 2019: 51:3529-3535. DOI: 10.1055/s-0037-1611838.