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Phenolic Derivatives Isolated from *Chamaecrista hildebrandtii* (Vatke) Lock and Their Biological Activities

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Abstract

Chamaecrista hildebrandtii is used to prepare herbal medicines as laxative and purgative agents to treat diseases such as diarrhea, stomach ulcers, healing wounds and skin infections. Thus, this plant has shown great interest to researchers, but the chemical constituent of this plant has not been determined. The objective of the study was the extraction and purification of phytochemicals from *C. hildebrandtii* crude leaf extracts and to determine their antioxidant and antimicrobial ability. Extraction of the *C. hildebrandtii* leaves was done by macerating shade-dried and milled leaves in methanol that was further partitioned using water, hexane and ethyl acetate. Ethyl acetate fraction underwent further TLC analysis and repeated column chromatography with various solvent combinations to give a total of twelve fractions. Spectroscopic analysis was done using ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY, 2D-HSQC and ^{13}C DEPT-135 and carefully compared with the literature available. Two phenolic compounds emodin (1,3,8-trihydroxy-6-methylanthraquinone) and chrysophanol (1,2-dihydroxy-6-methylanthrquinone) were isolated and identified. The antimicrobial activity was tested by disc diffusion method using Mueller Hinton agar growth medium. Emodin exhibited comparatively higher inhibitory activity against microbes in comparison with chrysophanol. Emodin showed a 55.1% DPPH inhibition while chrysophanol recorded a 58.2% inhibition. Emodin showed a desirable IC_{50} of 7.3 mg/mL compared to chrysophanol with an IC_{50} value of 12.3 mg/mL. The IC_{50} values of the two isolates were slightly higher than that of ascorbic acid (IC_{50} = 3.24 mg/mL). The outcome revealed that *C. hildebrandtii* leaf extracts may represent a promising natural source of polyphenols, which can be used as antimicrobial and antioxidant agents.

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Introduction

Plant-derived polyphenols encompass a vast group of plant-based secondary metabolites which have in common a cyclic structure with double bonds possessing one or more hydroxyl groups as substituents [1]. With more than 8000 structures, phenolic compounds are the most abundant and mostly distributed among the higher plants. Most of those are water-soluble compounds that naturally occur in combination with sugar as glycosides. These compounds show various physiological activities, including the neutralizing effects of oxidants together with the elimination of radicals in the living system. Previous *in vitro* studies have shown that phenolic compounds reduce and prevent denaturing of poly-unsaturated fatty acids (PUFA) [2]. Antioxidant and antimicrobial actions shown by polyphenols are augmented when there is a larger number of OH groups in the aromatic structure. Phenolic compounds' antioxidant activity is also brought about by high redox potentials, which make them electron donors, hydrogen ion sources and singlet reactive oxygen inhibitors [3]. Most of the latest research has shown that medicinal plants are reservoirs of many compounds that can eliminate reactive species. Tannins, saponins, carotenoids, rotenoids, terpenoids and flavonoids represent the active compounds mostly associated with polyphenols [3].

Anthraquinones comprise a class of phenolic compounds which are unique members of the quinones often extracted from medicinal plants, especially herbs. These classes of polyphenols are known to possess antioxidant activity thus inhibiting the onset of medical conditions such as cancer, aging, inflammation, liver diseases and Alzheimer's disease [4]. They are one of the largest classes of polyphenols which are abundant in many plants, including *Rheum rhabarbarum L*. from the Polygonaceae family, *Polygonum cuspidatum Sieb* (Polygonaceae), *Cassia sieberiana* (Caesalpiniaceae) and *Salvia mitiorrhiza* (Labiatae) [5]. The general structure of anthraquinones consists of three merged ringed structures with two carbonyl groups. Anthraquinone ring forms the basic structure with substituents on carbon 1 to carbon 8 in the structure [6]. Some species of higher plants have been known to contain anthraquinones, though it is less commonly found in edible plants and vegetables. *Rheum rumex, Rhamnus, Aloe* and *Cassia* plant species are plants containing anthraquinones [6, 7]. Medicinal properties of anthraquinones include laxative, anti-inflammatory, antifungal, antibacterial, anticancer and antiviral activities [6]. The main anthraquinones with medicinal properties include emodin, diacerein, physcion, cascarin and chrysophanol [8].

Fabaceae or Leguminosae, known locally as the legume or bean family, is the third largest family among the angiosperms after orchidaceae and asteraceae consisting of over 700 genera and 20000 species of trees, shrubs, vines and herbs worldwide and has been extensively researched for their bioactive compounds [9]. The twigs of *Erisema robustum*, Fabaceae showed higher bioactivity as research attributes this to flavones in this plant. *Bolusanthus specious*, (Fabaceae) showed activity against microbes *in vitro* due to irigenin, glycitein, and 8-hydroxyglycitein compounds. The bark methanolic extract of *Cassia fistula* had a IC_{50} of 213 µg/ml and showed higher free radical scavenging ability. *Cassia nigricans* (Vahl) Greene showed beneficial effects against ulcers, gastrointestinal and edema, diarrhea and skin infections [10]. *Chamaecrista hildebrandtii* is an herbaceous plant with a dense system of roots with horizontal or upright stalks from the fabaceae family. It has glabrous leaves which are compactly hairy. The central vein is noticeably odd though its axillary nerves are craspedodromous. The lateral leaves are flexible, in pairs of four and at most fourteen twosomes [10]. The sepals are acute with inflorescences of 1-4 flowers attached to 0.4 to 4 cm pedicels. It bears linear pods, which when mature, have brown to black, ovate-rhombic seeds of about 4 mm by 2.5 mm [10]. The plant has been traditionally used as an antioxidant and anti-inflammatory agent owing to its higher concentrations of phenolic compounds. The plant also holds an important role in traditional African rituals [10]. As a way of exploring the potentiality of natural products in drug development, the present study aimed at isolating and characterizing secondary metabolites from the leaf extracts of *C. hildebrandtii* to determine their corresponding antimicrobial and antioxidant capacities.

Materials and Methods

Plant collections and identification

The *C. hildebrandtii* leaves were obtained from Bondo subcounty (0.0802° S, 34.2562° E) in Kenya. The selected leaves were plucked from the plant and transported to the chemistry laboratory at the University of Kabianga. The plant was identified by a botanist at the Biological Sciences Laboratory and a voucher specimen was deposited in the herbarium.

Chemicals and apparatus

Chemicals used include methanol, hexane, dichloromethane, ethyl acetate, iodine and distilled water all of which were procured from different suppliers as analytical grade. The apparatus used included an electric weighing machine, electric mill, rotavapour, beakers, 1.5 L bottles, Whatman filter paper, development tank, columns,TLC plates, spirit lamp and UV lamp.

Preparation of plant samples

The sample was thoroughly washed to remove foreign materials and dried under a shade for about three weeks. The dried leaves were ground into fine homogenous powder to increase the surface area. The powdered leaves were macerated in 3 L of methanol for forty-eight (48) hours. The extract was filtered using Whatman filter paper No. 1 and then through cotton wool. Repeated soaking, filtering and concentration of the extract were performed to ensure higher results. The extract was concentrated using a rotary evaporator water bath at 78.5^{o}C until a semi-solid gummy black residue was obtained.

Partitioning and isolation

The black methanolic extract obtained was partitioned in hexane, ethyl acetate and water using a separatory funnel. The process was repeated three times after which three fractions were obtained: hexane fraction, water-methanol fraction and ethyl acetate fraction. The hexane fraction appeared gummy and the yield was very small. Water-methanol fraction was discarded while the ethyl acetate fraction (150 g) was further purified.

Thin layer and column chromatography

The dried ethyl acetate fraction was subjected to column chromatography packed with 100 g silica gel (100 -120 mesh) slurry in hexane. The column was eluted with ethyl acetate: hexane mixture in different combinations with increasing polarity in the ratio 1% to 100% gradient. A total of 116 fractions each with 100 ml were collected in conical flasks and after concentration and TLC analysis, the fractions with similar Rf values on the TLC plate were pooled together to give a total of 16 fractions. The combined fractions labeled CF1 –CF2 were pooled and named CFA, CF3 was renamed CFB while CF4 and CF5 had almost similar Rf values in the TLC therefore they were merged and named CFC. CF6 to CF10 were also pooled to give CFD. Pooling of CF11 and CF 12 gave CFE and finally, CFF was the product of mixing combined fractions 13 to 18.

Nuclear magnetic resonance (NMR)

Pure fractions CFA, CFB, CFC, CFD, CFE and CFF were sent for NMR analyses at the University of Surrey. NMR spectra were recorded at room temperature on a 500 MHz varian UNITY–INOVA spectrometer. ^{1}H NMR spectra were referenced against the $CHCl_3$ signal at δH 7.24 and ^{13}C NMR spectra against the corresponding signal at δC 77.0 The coupling constants are given in Hz. The NMR analyses was done on all samples, only two compounds were identified CFA (emodin) and CFE (chrysophanol), the other fractions were replicates of the identified compounds.

DPPH free radical scavenging activity

The antioxidative ability of *C. hildebrandtii* compounds was determined for the identified compounds emodin and chrysophanol using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical analytical method. Preparation of the stock solution was done by dissolving a total quantity of 0.01 mg of DPPH in 125 mL of 99% methanol to make a final concentration of 101.45 µm. The resulting mixture was then filtered using Whatman filter paper No. 1 to give a usable filtrate. The DPPH methanolic solution was diluted with methanol to obtain an absorbance of approximately 0.98$\pm$0.02 at 517 nm. From the resulting solution, 3 mL was taken and mixed with 100 µl sample at different concentrations. Normally, a 3 mL solution which contains 100 µl of 99% methanol was used as the standard. The test was performed in triplicate and the reference standard used was vitamin C [11]. Higher scavenging activity of the plant compounds was indicated by low absorbance. Evaluation of effects of scavenging was estimated based on the percentage DPPH radical scavenging ability as follows:

$$\% \text{ inhibition} = \frac{A0-A1}{A0} \times 100\ \%$$

Where A_0 is the standard experiment and A_1 is the absorbance in the presence of test compounds [12].

Antimicrobial assay

The antimicrobial activity of the identified compounds emodin and chrysophanol was evaluated by the Kirby-Bauer Disk Diffusion method [13] against the following bacterial strains: Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*Klebsiella pneumoniae* and *Pseudomonas*

aeruginosa, while the fungal strain used was *Candida albicans*. The bacteria samples were then looped on the agar plates with parallel overlapping strokes then labeled appropriately. The loop was then flamed and allowed to cool. The streak was overlapped once or twice then the plates were incubated overnight at 30°C. After 17- 20 hours of incubation, bacterial growth was checked where isolated colonies should be observed in the last streak. Selected bacterial representative colonies were marked from each plate and subcultured and incubated overnight. A pure, well-isolated colony was finally stabbed into two tubes of Mueller Hinton agar labeled and incubated. Discs of erythromycin (30 g) and Nystatin (25 g) were used as standards for antibacterial and antifungal standards. The solvent used in extraction was used as a negative control. The extracts with activity were serially diluted and then re-tested to determine the minimum inhibitory concentration (MIC). The minimum Inhibitory Concentration (MIC) of *C. hildebrandtii* leaf-extracted compounds, which had activity against test microorganisms was investigated. Varying concentrations of *C. hildebrandtii* extracts, 12.5, 25, 50, 75 and 100 mg/ml, were used to determine their inhibitory effects against selected microorganisms. It was done by the disc diffusion method. The *C. hildebrandtii* methanolic extract was put in blank discs and left to lose moisture. Small amounts were placed in bacteria using a cotton application stick and transferred into Mueller Hinton broth then it was vortexed and agitated. The bottle was then put in the densitometer. 10 µm of the bacterial suspension was pipetted and transferred into the center of Mueller Hinton agar, spread homogeneously on the surface with a sterile swab. The names of the microorganisms used were labeled on Mueller-Hinton agar and placed at regular intervals. Discs soaked in pure methanol were used as a negative control for all samples in different test organisms while tetracycline was used as a positive control. Petri plates were kept at room temperature for 25-30 minutes. Finally, the plates were incubated for 18-24 hours at 37°C. Clear zones around the discs were investigated and their zone diameter was measured [13].

Results and Discussion

The methanolic fractions were purified using column chromatography over silica gel and eluted with solvents with increasing polarity starting with hexane, the purity of fractions was confirmed using TLC. This resulted in the isolation and identification of two anthraquinones CFA and CFE renamed as compound 1 and compound 2.

Characterization of compound 1 (emodin)

Compound 1 was identified using both 1D and 2D NMR spectra and also comparison with reported literature values [14]. Compound 1 was identified as emodin (1,6,8-trihydroxy-3-methylanthraquinone). TLC fraction CFA yellow colored crystals ^{1}H NMR spectra of compound 1 (Fig. 1) presented the existence of four sets of aromatic peaks δ_H 7.52 (d, C-4), δ_H 7.28 (d, C-5), δ_H 7.07 (m, C-2) and δ_H 6.66 (d, C-7), two sets of phenolic signals δ_H (11.95(s, OH),11.87 (s, OH) and a set of methyl group δ_H 2.4 (s, CH_3) protons attached to the aromatic ring (Table 1). ^{13}C NMR band (Fig. 2) showed the existence of 12 aromatic carbon peaks δ_C166.07 (C-6), δ_C164.90 (C-8), δ_C161.85 (C-1), δ_C 135.37 (C-11), δ_C133.18 (C-14), δ_C 124.54 (C-2), δ_C 120.89 C-4), δ_C 113.74 C-13), δ_C 109.31 (C-5), δ_C 109.26 (C-12) and δ_C108.36 (C-7), 2 carbonyl δ_C 190.06 C-9), δ_C187.73 (C-10) and a methyl δ_C21.96 (benzylic) carbon). Homonuclear Correlation Spectroscopy (COSY) showed a correlation between protons on the benzene ring at δ_H 7.07 ppm (H-4), δ_H 7.28 ppm (H-5), δ_H 7.07 ppm (H-2), and δ_H 6.66 ppm (H-7). Likewise, hydroxyl groups OH in the region at δ_H 11.87 ppm and δ_H 11.95 ppm show a correlation between adjacent protons in the aromatic ring. The DEPT-135 (distortionless enhancement by polarization transfer) NMR showed a positive peak representing methyl carbon (CH_3) at δ_C 21.9614 ppm and another positive peaks for methine carbon (Table 2).

Table 1 ^{1}H-NMR data for emodin.

Position of C	^{13}C NMR chemical shifts (δ = ppm)	Reported [13] chemical shifts (δ = ppm)	Nature of C
1	161.85	162.2	Q
2	124.54	-	CH
3	148.67	149.1	CH
4	120.89	121.3	CH
5	109.26	108.7	Q
6	166. 07	166.5	Q
7	108.36	-	Q
8	164.90	-	Q
9	190.06	190.4	Q
10	181.13	182.1	Q
11	135.37	135.8	Q
12	109.31	109.7	Q
13	11.74	114.1	Q
14	133.18	133.6	Q
CH_3	21.96	22.3	CH_3

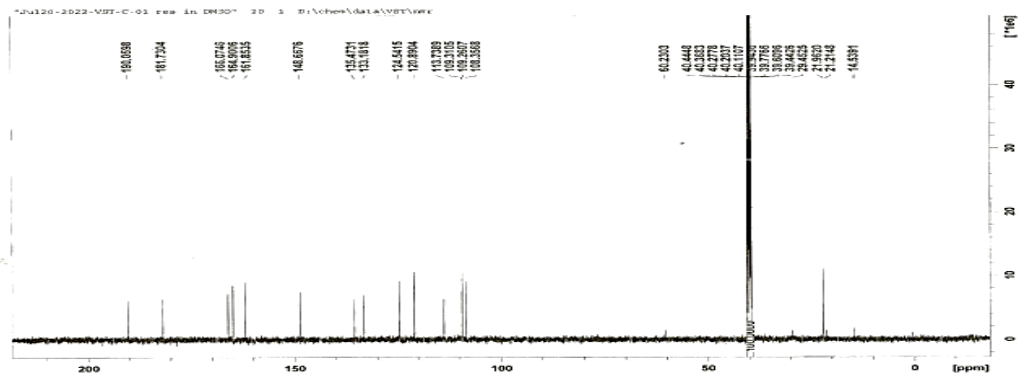

Fig. 1 ^{13}C-NMR spectrum of Emodin.

Fig. 2 ^{1}H-NMR spectrum for Emodin.

Table 2 ^{13}C-NMR for emodin at 500 MHz (DMSO solvent).

Position of C	^{13}C NMR chemical shifts (δ = ppm)	Reported [13] chemical shift values (δ = ppm)	Nature of C
1	161.85	162.2	Q
2	124.54	-	CH
3	148.67	149.1	CH
4	120.89	121.3	CH
5	109.26	108.7	Q
6	166. 07	166.5	Q
7	108.36	-	Q
8	164.90	-	Q
9	190.06	190.4	Q
10	181.13	182.1	Q
11	135.37	135.8	Q
12	109.31	109.7	Q
13	11.74	114.1	Q
14	133.18	133.6	Q
CH3	21.96	22.3	CH_3

(CH) at δ_C 124.54 ppm, δ_C 120.89 ppm, δ_C 109.26 ppm and δ_C 108.36 ppm for C-2, C-4, C-5 and C-7 one-to-one. The HSQC (Heteronuclear Single Quantum Coherence) NMR spectrum showed chemical shifts protons correlations for the following pairs: δ_H 7.07 ppm and 124 δ_C.54 ppm (C-2), δ_H 7.52 ppm and 1 δ_C 20.89 ppm (C-4), δ_H 6.66 ppm and δ_C 108.36 ppm (C-7) and δ_H 2.40 ppm and δ_C ppm 21.96 (CH_3). Compound 1, yellowish crystalline solid with

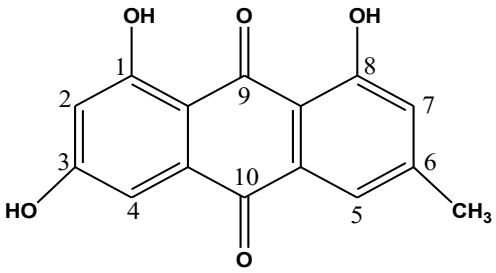

Fig. 3 Structure of emodin.

general molecular formula of $C_{15}H_{10}O_5$ was identified as emodin (Fig. 3).

Characterization of compound 2 (chrysophanol)

The CFE was identified as chrysophanol (1,6-dihydroxy-3-methylanthraquinone) from the NMR spectra and comparison with existing literature values. The compound had been reported from lichens and higher plant families belonging to *Cassia spp.* [14]. Compound 2 was an orange-red powder obtained from 12:88 ethyl acetate: hexane solvent systems. ^{1}H NMR analysis revealed two sharp singlet hydroxyl (-OH) protons involved in hydrogen bonding; a characteristic of two chelated hydroxyl groups and were assigned to OH protons at δ_H 12.03 and δ_H 12.14 at C-1 and C-8, respectively, of the anthraquinones basic structure (Fig. 4). Signals of five aromatic protons 6.9 δ_H 8 (1H, d, H-2), δ_H 7.42 (1H, d, H-4), δ_H 7.67 (1H, d, H-6), δ_H 7.30 (1H, dd, H-7), and δ_H 7.86 (1H, d, H-5) were found (Table 4). A total of fifteen carbon signals were observed in the ^{13}C NMR spectrum (Table 5), which were two carbony carbons (δ_C 190.11 and δ_C 181.87) corresponding to C-9 and C-10, five aromatic methine carbons δ_C 124.6, δ_C 120.93, δ_C 119.2, δ_C 120.93, δ_C 234.60 corresponding to C-2, C-4, C-5; C-6, and C-7 and two oxygenated aromatic quaternary carbon and a methyl carbons δ_C 161.87 and δ_C 164.91 for C-1 and C-8, five aromatic quaternary carbons δ δ_C 148.71, δ_C 133.27, δ_C 120. 83 and δ_C 113.83 corresponding to C-3, C-1a, C-4a, C-5a, and C-8a and a methyl carbon at C-3, respectively (Fig. 5).

Emodin and chrysophanol are both anthraquinone derivatives with compound 1 having an extra hydroxyl group at C-3. The study reports for the first time the isolation of emodin and Chrysophanol compounds from *C. hildebrandtii* leaves extract. However, they have been formerly isolated from the root bark of *Rummex abyssinicus*, *Cassia allata, Cassia occidentalis* and *Aloe vera* [15]. The traditional use of *C. hildebrandtii* extract as an antibacterial agent, antiviral and antifungal justifies the medicinal properties of the isolated compounds against the microbes. Emodin has been used in many

Table 3 ^{1}H NMR data for chrysophanol at 500MHz (DMSO solvent).

Position of H	Chemical shifts ppm (J in Hz)	Nature of H
2	7.07	1H, m
4	7.5	1H, d
5	7.2	1H, d
7	6.66	1H, d
CH3	2.4	3H, s
OH	11.87	1H, s
OH	11.95	1H, S

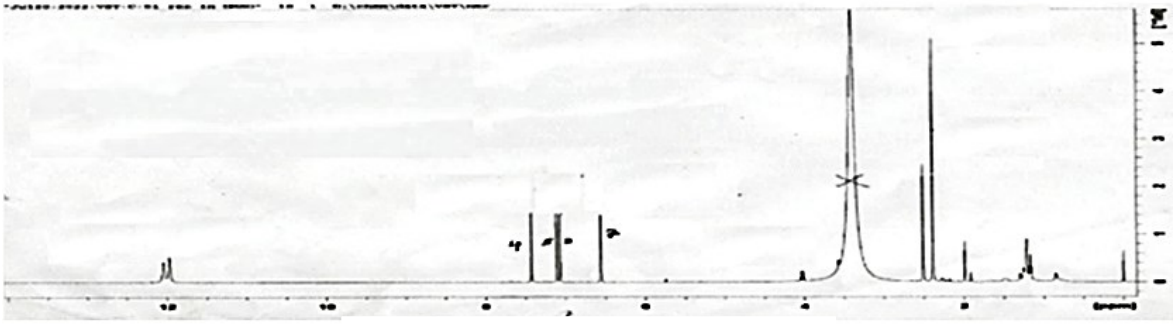

Fig. 4 ^{1}H NMR spectrum for chrysophanol.

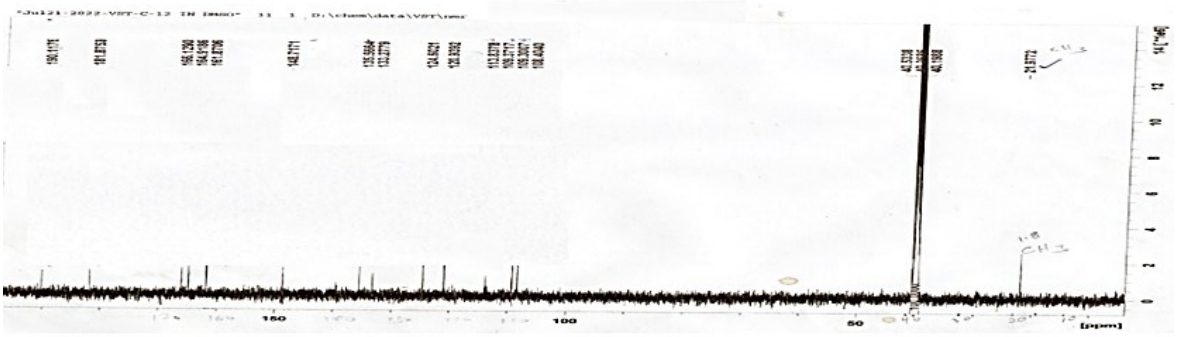

Fig. 5 ^{13}C NMR spectrum for chrysophanol.

Table 4 ^{13}C-NMR for chrysophanol at 500 MHz (DMSO solvent).

Position of Carbon atom	Experimental NMR Data		Reported values [15]	
	^{1}H (M, J in Hz)	^{13}C	^{1}H (M, J in Hz)	^{13}C
1	-	161.87	-	162.2
1a	-	113.83	-	113.6
2	6.98(1h, d)	124.60	7.22	124.9
3	2.45 (s, d)	148.71	-	149.1
4	7.42 (1H, d)	120.93	7.55	120.4
4a	-	133.27	-	109.7
5	7.86 (1H, d)	119.00	7.71	119.2
5a	-	120.00		
6	7.67(1H, d)	120.93	7.80	120.5
7	7.30 (1H, d)	124.60	7.22	123.9
8	-	164.91	-	161.4
8a	-	113.83	-	114.1
9	-	190.11	-	190.4
10	-	181.87	-	182.1
3-CH_3	2.45 (3H, s)	21.97	2.44	21.6
1-OH	12.03(1H, s)	-	11.86	-
8-OH	12.14 (1H, s)	-	11.96	-

traditional medicines from ancient times in the management of diseases like cancer, diabetes and chronic inflammatory diseases [15]. The mechanism of activity of emodin as an anti-inflammatory, anti-cancer and neuroprotection capabilities has been studied and this points to the current evidence on the therapeutic efficacy. Chrysophanol (Fig. 6) on the other hand has also been in use in traditional medicine where it was used as an anti-diabetic, anti-inflammatory and neuroprotective agent.

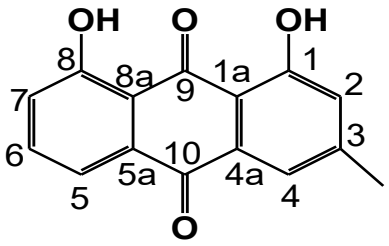

Fig. 6 Chemical structure of Chrysophanol.

DPPH free radical antioxidant assay

The results for the antioxidant assay of emodin and chrysophanol compounds isolated from the leaves of *C. hildebrandtii* and vitamin C as the standard are summarized in Table 5. When 400 µg/mL of each compound methanolic solution was tested spectrophotometrically, emodin reacted to give 55.05% inhibition while chrysophanol had 58.17% inhibition of the original concentrations of DPPH. The reduction in the absorbance of DPPH is proportional to the concentration of the antioxidant. From the spectroscopic absorbance of DPPH against *C. hildebrandtii* extract, the results indicated that emodin had a higher antioxidant activity as compared to chrysophanol. The higher activity of emodin against the free radicals could be attributed to the hydrogen-donating ability of emodin which appeared to be higher than that of chrysophanol [16]. The results indicated that hydrogen giving ability of an antioxidant depends on the position and the number of the hydroxyl substituents and in the aromatic ring structure of the polyphenol, for example, emodin has three hydroxyl substituents while chrysophanol has only two which scavenge DPPH free radicals in the solution at 517 nm.

Table 5 DPPH absorbance of the isolated compounds and control.

Compounds	Absorbance (517nm)	Antioxidant activity (%)
Control	0.961	-
Emodin	0.432	55.05
Chrysophanol	0.402	58.17

Antimicrobial activity assay

The antimicrobial activity of the isolated emodin and chrysophanol was determined against the microbes and gave the following summary of the growth inhibition zones (in mm) shown in Table 6. The results indicated that emodin and chrysophanol showed interesting antimicrobial activities, which are comparable with those of the positive controls Erythromycin and Nystatin. Higher activity was recorded with emodin against *S. aureus* with its corresponding inhibition zone of 20.1 ± 0.6 mm. The Gram-negative bacteria possess more external membranes than the Gram-positive bacteria hence

Table 5 Growth inhibition zones of isolated compounds against selected human pathogens.

Microorganisms	Zones of Inhibition (mm ± S.E.)			
	Emodin	Chrysophanol	Erythromycin*	Nystatin*
Staphylococcus aureus	20.1 ± 0.6	20.0 ± 1.0	22.0 ± 0.2	na
Pseudomonas aeruginosa	18.0 ± 1.0	19.1 ± 1.5	23.1 ± 0.3	na
Klebsiella pneumoniae	19.2 ± 2.5	16.7 ± 1.5	20.4 ± 0.7	na
Candida albicans	17.1 ± 0.2	16 .2 ± 0.3	na	20.1 ± 0.6

*na = No activity; * = Positive controls
S.E. = Standard error

poor antimicrobial activities were reported (Table 5). The cell wall structure and composition of the bacteria and the fungi explain the sensitivity to the antimicrobial agents [17]. Gram-positive bacteria and fungi have their cell walls exposed so antimicrobial drugs can interact with these cell walls and hence are prone to be more susceptible to antimicrobial penetrations [17]. The presence of hydroxyl groups in the two isolated compounds at positions 1, 3 and 8 in the aromatic ring; especially for emodin, increases the antimicrobial abilities of these compounds and it explains why an improved activity was recorded for emodin as compared to that of chrysophanol. The most susceptible organism was *K. pneumoniae* followed by S. *aureus*. All the compounds showed good activity against *C. albicans,* and reference drugs were more active on the microbes than the isolated compounds. From the data, it can be concluded that the isolated compounds from *C. hildebrandtii* ethyl acetate combined fractions had antimicrobial activity. It seemed that more compounds could be present, especially in methanolic/water fractions and hexane fractions which were not isolated and this calls for more research on the plants to isolate and characterize them**.**

Conclusion

The present study has confirmed that *C. hildebrandtii* contains phenolic derivatives of anthroquinone schofold with two compounds; emodin and chrysophanol, being isolated from *C. hildebrandtii* leaf extracts and reported for the first time. The two compounds showed moderate antibacterial and antioxidant activity, with emodin showing desirable antimicrobial activity against *S. aureus* with a corresponding zone of inhibition of 20.1 ± 0.6 mm. The reported bioactivities of the isolated compounds conform with the reported literature, where, emodin and chrysophanol had been proven to be effective against both Gram-positive and Gram-negative bacteria and fungi species *C. albicans*. The study therefore has given a scientific basis for ethnobotanical uses of the *C. hildebrandtii* medicinal plant in the management of microbial diseases.

Acknowledgment

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Conflict of Interest

The authors have no conflicts of interest.

References

[1] Ayodele OA, Ayodele SO, Adeniji OE, Asaniyan EK, Oloruntola OD, Adeyeye SA. Comparitive analysis of antioxidant, nutitional, phytochemical and enzyme inhibition properties of Justicia carnea and Alochornea cordifolia leaf meals. Sci Lett 2024; 12(2):76-83.

[2] Waheed S, Shekh M, Anwar M, Tayyab M. Comparative analysis of nutritional, antioxidant, and antibacterial properties of Moringa oleifera extract. Biomed Lett 2023; 9(1):7-15.

[3] Kumar S, Abedin M M, Singh AK, Das S. Role of phenolic compounds in plant defensive mechanisms. In: Plant Phenolics in Sustainable Agriculture. Singapore: Springer; 2020; pp. 517-532.

[4] Xie Y, Zhang L, Li YY, He D, Zheng LF. Chrysophanol localizes in mitochondria to promote cell death through upregulation of mitochondrial cyclophilin D in Hep G2 cells. Chinese Herb Med 2021; 13(2):221-227.

[5] Wang D, Wang XH, Yu X, Cao F, Cai X, Chen P, Li M, Feng Y, Li H, Wang X Pharmacokinetics of anthraquinones from medicinal plants. Front Pharmacol 2021; 12:638993.

[6] Diaz-Muñoz G, Miranda IL, Sartori SK, de Rezende DC, Diaz MAN. Anthraquinones: An Overview. Stud Nat Prod Chem 2018; 58, 313–38

[7] Chien SC, Wu YC, Chen ZW, Yang WC. Naturally occurring anthraquinones: chemistry and therapeutic potential in autoimmune diabetes. Evid Based Complement Alternat Med 2015; 2015:357357.

[8] Martorell M, Castro N, Victoriano M, Capó X, Tejada S, Vitalini S. An update of anthraquinone derivatives emodin, diacerein, and catenarin in diabetes. Evid Based Complement Alternat Med 2021; 1:3313419.

[9] Asfaw MM, Abebe FB. Traditional medicinal plant species belonging to Fabaceae family in Ethiopia: A systematic review. Int J Plant Biol 2021; 12(1):8473.

[10] Odhiambo RS, Kareru PG, Mwangi EK, Onyango DW. Antioxidant activity, total phenols, flavonoids and LCMS profile of *Chamaecrista hildebrandtii* (Vatke) lock and

Clerodendrum rotundifolium (Oliv.). Eur J Med plant 2019; 26(3):1-5

[11] Patel RM, Patel NJ. *In vitro* antioxidant activity of coumarin compounds by DPPH, Super oxide and nitric oxide free radical scavenging methods. J Adva Pharma Edu Res 2011; 1:52-68.

[12] Pisoschi AM, Negulescu GP. Methods for total antioxidant activity determination: a review. Biochem Anal Biochem 2011; 1:1.

[13] Andrews JM. Determination of inhibitory concentrations. J. Antimicrob. Chemotherapy 2001; 48: 5-16.

[14] Yi Y, Adrjan B, Wlodarz J, Li J, Jackowski K, Roszak S. NMR measurements and DFT studies of nuclear magnetic shielding in emodin and chuanxiongzine molecules. J Molecul Struct 2018; 1166:304-310.

[15] Kengne IC, Feugap LDT, Njouendou AJ, Ngnokam CDJ, Djamalladine MD, Ngnokam D, Voutquenne-Nazabadioko L, Tamokou JD. Antibacterial, antifungal and antioxidant activities of whole plant chemical constituents of Rumex abyssinicus. BMC Complement Med Therap 2021; 21(1):164.

[16] Shermatova G, Eshbakova K, Narbutaeva D, Karakulova A. Antioxidant and antihypoxic activity of emodin and chrysophanol. Aust J Techn Nat Sci 2022; 3-4:11-3.

[17] Varela MF, Stephen J, Lekshmi M, Ojha M, Wenzel N, Sanford LM. Bacterial resistance to antimicrobial agents. Antibiotics 2021; 10(5):593.