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Research Article

LC/PDA/ESI-MS/MS Polyphenols Profiling in the *In vitro* Active Leaves Extracts of *Combratum hartmannianum* against human Pathogens with Special Emphasis to *Madurella mycetomatis*

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Abstract: The present communication represent an attempt to investigate the antimicrobial and antimycetomal activity of the leaves of *Combratum hartmannianum* (Combretaceae) and to define the phytochemical profiles of the active agents. Decoctions of the leaves of *C hartmannianum* are commonly used in Sudanese traditional medicines against jaundice, external skin infections, malaria and similar febrile diseases. Air dried ground leaves of *C. hartmannianum* were extracted using 80% methanol. The methanolic extract was sequentially fractionated with petroleum ether, chloroform and ethyl acetate. The aforementioned extracts of *C. hartmannianum* were tested against two Gram positive and three Gram negative bacteria as well as two fungi. Additionally, the obtained extracts of *C. hartmannianum* were tested in vitro against *Madurella mycetomatis* the most common eumycetoma causative organisms employing a newly developed microtitre plate- based

antibacterial assay incorporating resazurin as an indicator of cell growth.Following bioactivity guided fractionation the ethyl acetate phase at both concentrations (1mg/ml, 5mg/ml) was significantly active against *Staphylococcus aureus* (20mm, 20mm) and *Escherichia coli* (20mm, 20mm). Furthermore, this fraction at a concentration of 5mg/ml possessed activity against activity against *Protues vulgaris* (17 mm), *Pseudomona. aeruginosa*,(20 mm) *Aspergillus niger* (20 mm) and *Candida albicans* (20 mm). The leaves chloroform extract (1mg, 5mg) possessed high activity against *Bacillus subtitles* (30mm, 25mm) and *S. aureus* (30mm, 25mm). the petroleum ether extracts of the leaves at two different concentrations (1mg, 5mg) showed activity against *Staphylococcus aureus* (30 mm) *Bacillus subtitles* (18mm, 23 mm) and *Escherichia coli* (25 mm). A promising inhibitory activity emerged against *Madurella mycetomatis* ranging between 78 and < 39.1 µg / ml. Most active were the ethyl acetate and chloroform fractions with MIC < 39.1 µg / ml. polyphenols were mainly accumulated in the chloroform and ethyl acetate phase which showed very similar TLC and HPLC chromatograms. Reverse phase High Pressure Liquid Chromatography coupled to Tanden Mass Spectromentry performed on the ethyl acetate fraction of the leaves of *C. hartmannianum* led to the identification of sixteen flavonoids and a phenantherene which were believed to be responsible of the activities mentioned above. These results support the different traditional uses associated with the plant studied. Pharmacological merits reported on C. *hartmannianum* were also in agreement with the results obtained.

Keywords:*Combretum. Hartmannianum,* antibacterial, antimycetoma activity, eumycetoma, polyphenols.

INTRODUCTION

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value. The genus *Combretum* belongs to the family combretaceae and consists of 350 species[1]. *C. hartmannianum* is a glabrous medium-sized tree up to 6m high with broad leaves growing mainly in semiarid or in Savannah woodland and its found mainly on rocky hills slopes throughout central Sudan mainly around Nuba mountains[2].

Combretaceae is known for its medical uses in Africa and Asia. *Combretum* spp. are widely used in folk medicine for the treatment of hepatitis, malaria, respiratory track infections, cancer, bilharzia, tuberclosis, HIV infection, bacterial and fungal infections and parasitic diseases[3,4,5,6]. In medical preparations leaves and bark of *Combretum* spp. are used predominantly[6]. Decoction of the bark of *C. hartmannianum* is used traditionally in Sudan against malaria and similar febrile diseases. The infusions of the leaves are used against jaundice and decoction of the whole plant is used for external skin infection[2]. The chemistry of *Combretum* species has been studied by a number of researchers. To date around 80 metabolites were reported from the genus *Combretum*, these include, stilbenes, phenantherenes, terpenoids, cycloarenoids, macro lactones and flavonoids[7-11].

Among the metabolites of *Combretum* stilbenes are the most important[7]. Stilbenes have been reported to interact with microtubule formation by binding to tubulin, the major structural component of microtubules, and to cause mitotic arrest, that inhibits the growth of cancer cells. Microtubules are among the most

strategic subcellular targets of anticancer chemotherapeutics. One of the most active stilbenes isolated is combretastatin A4, which is in very late stages of clinical trials.To date no secondary metabolites were reported from *C. hartmannianum.* Reported biological activities on *C. hartmannianum* include antischistosomal activity of the aqueous extract of the leaves by[12]. Reported was also antibacterial activity of the leaves and stem of *C. hartmannianum* on gram +ve and gram –ve bacteria by[13, 14] studied the activity of different parts of *C. hartmannianum* by testing their *in vitro* activity against hemoflagellates, selected bacteria, HIV-1-RT and tyrosine kinase inhibitory, and for cytotoxicity. Extracts of different parts of *C. hartmannianum* (Combretaceae) possessed significant activity against the chloroquine sensitive *P. falciparum* strain (NF54) with IC_{50} values of 0.2 µg/ml (bark), 0.4 µg/ml (stem) and 4.3 µg/ml (leaves). Most interestingly, the extracts of the leaves of *C. hartmannianum* totally inhibited the enzyme HIV-1 reverse transcriptase (HIV-1 RT) at a concentration of 66 µg/ml. A comparably strong activity against $p56^{lck}$ tyrosine kinase was also seen for this extract[14]. The present communication represents an attempt to investigate the antimicrobial and antimycetomal activity of the leaves extracts of C. hartmannianum and to define the polyphenols profiles of the active agents.

MATERIALS AND METHODS

Plant material collection, preparation and extraction: Leaves of *C. hartmannianum* (**Fig.1**) were collected from the Faculty of Agriculture, University of Khartoum, Shambat. A voucher specimen was made for each sample and was taxonomically identified at the department of Botany, Medicinal and Aromatic plants Institute, National Center for research. The herbariums was deposited at the Department of Biochemistry, Commission of Biotechnology and Genetic Engineering, National Center for Research. Collection data categorized under place, date and collector, an asterisk indicating a herbarium sample are as follows: *Combretum hartmannainum* (1*), Shambat, 20 – 08 – 2005, Omima Eiz Eldin. Leaves collected were dried separately in shade and ground coarsely. 20 g of air dried course ground leaves of *C. hartmannianum* were extracted using 80% methanol. The methanolic extract was sequentially fractionated with petroleum ether, chloroform, ethyl acetate. Extracts were obtained by removing solvents in vacuum **(Fig.2).**

Figure 1: Plant parts studied (A) *Combretum hartmannianum*, Shambat 2005 (B) *C. hartmannainum* leaves

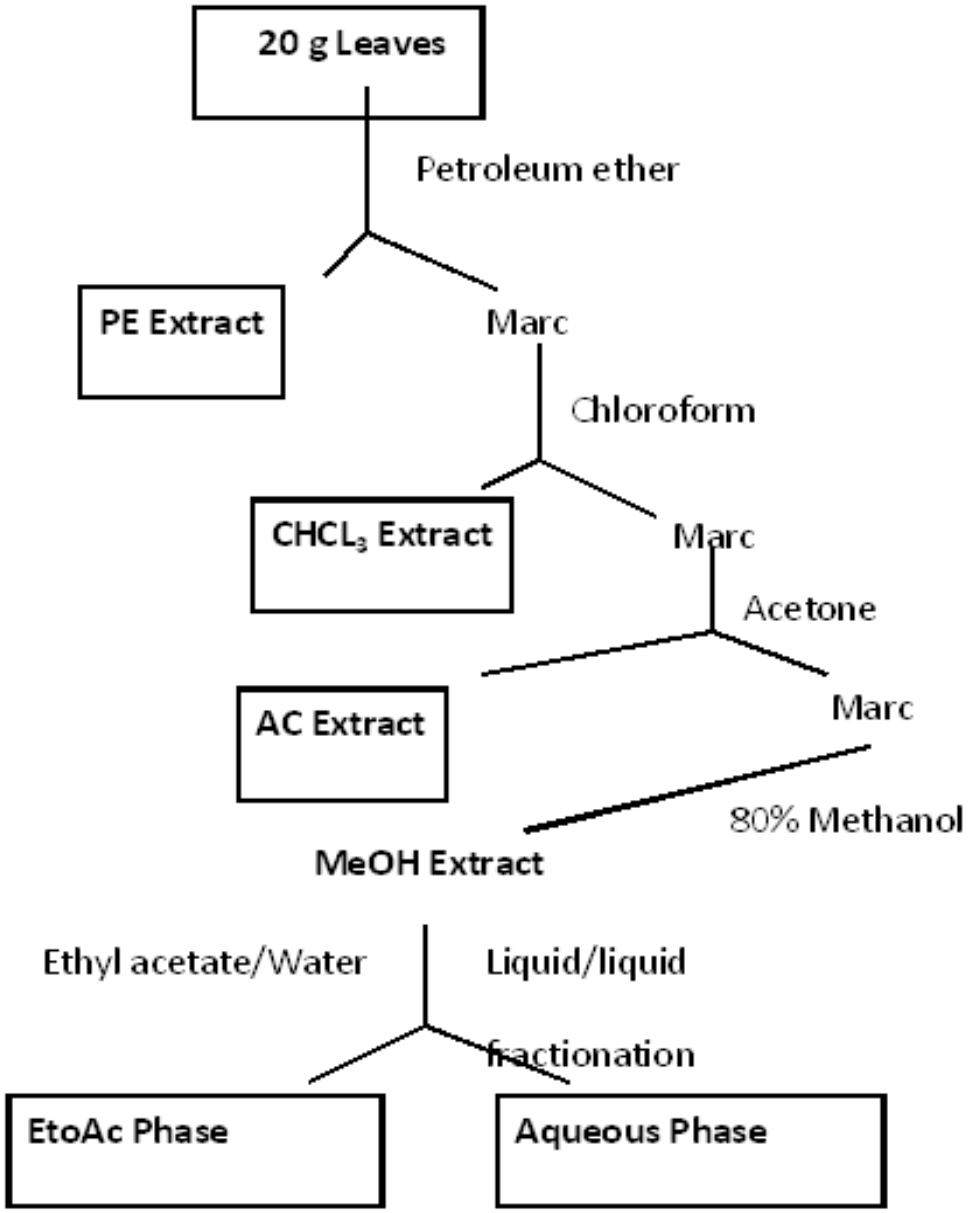


Fig.2: Extraction and fractionation of *C. hartmannianum* leaves.

Antimicrobial Activity: The extracts of *C. hartmanianum* were tested for antimicrobial activity, the method used was cup-plate agar diffusion method[15] with minor modifications. Plant extracts were tested against the following human pathogens:

Bacillus subtiIis (NCTC 8236), *Escherichia coli* (ATCC 25922, *Pseudomonas aeruginosa* (ATCC27853) ,*Staphylococcus aureus* (ATCC 25923- 8/29/2005), *Protues vulgaris* (ATCC 6380-8/29/2005), *Aspergillus*

niger (ATCC 9763-8/29/2005) and *Candida albicans* (ATCC 7596-8/29/2005. Tested stock organisms were supplied by the institute for medicinal and aromatic plants, National Center for Research.

In vitro susceptibility testing against *Madurella mycetomatis:* The obtained extracts of *C. hartmannianum* were testes in vitro against *Madureela mycetomatis* the most common eumycetoma causative organism employing a newly developed microtitre plate- based antibacterial assay incorporating resazurin as an indicator of cell growth[16].

Thin Layer Chromatography (TLC): Aluminium silica gel plates 60 F_{254} (Merck 5554) or pre-coated TLC plates SIL RP-18W / UV 254 (Macherey-Nagel) were used as stationary phase in carrying out TLC of the different plants extracts. Standard chromatograms were prepared by applying 20 µl solution (5 mg/ml) to a silica gel plate and developing it in different solvent systems depending on the type of extract. Chromatograms were detected under UV light (254 and 366) and sprayed with diagnostic reagents which include: vanillin-H_2SO_4 reagent, Dragendorff, 5% Aluminum chloride and Natural Product Reagent (NPR)[17].

High Pressure Liquid Chromatography (HPLC) analysis: *C. hartmannainum* leaves extracts were analysed using a Finnigan HPLC system composed of a model LCQ pump, LCQ Deca XP MAX ion trap mass spectrometry (San Jose CA, USA) coupled with the column oven. Plants extracts and fraction were separated on a column with phenomenex (4u, polar-RP80A, 250 x 2.00mm, 4micron; phenomenex, USA) reverse-phase column (phenomenex LC-18) at 35^0 C and a flow rate of 200 µLmin^{-1}). The column was eluted with a gradient mobile phase consisting of 0.014% TFA (trifloroacetic acid) in 5% acetonitrile (phase A) and 0.14% TFA in 50% acetonitrile using the following gradient program: 0 min (85% A, 15% B), 40 min (35% A, 65% B), 45 min (35% A, 65% B), 50% min (85% A, 15% B), UV detection was performed at 320 – 380 nm targeting flavonoids and stilbenes.

Triple quadrupole mass spectrometric analysis (LC-MS/MS): The HPLC was joined with a Finnigan LCQ pump, LCQ Deca XP MAX ion trap mass spectrometry (San Jose CA, USA) mass spectrometer with the Electrospray Ionization (ESI) interface at positive ion mode. For the condition of positive ion mode, the capillary temperature was set to 280ºC and the spray voltage was set to 5000 V. nitrogen was used as sheath gas, and the flow was set to 40 U. Helium was used as collision gas at 0.8 m Torr. Collision Induced Dissociation (CID) or IT-MS experiments were performed for fragmentation of glycosyl flavonoids Neutral loss scan were investigated with scan range from m/z 300 to 700 at collision energy of 15 and 30 eV.

RESULTS AND DISCUSSION

Antimicrobial activity of C. *hartmanianum* leaves extracts: The antimicrobial activity of the different extracts of *C. hartmannianum* leaves, against standard human pathogens **Table 1**. Plant extracts which possessed ≥ 14 mm inhibition zones were considered to be active. The petroleum ether extract of the leaves at two different concentrations (1mg/ml**,** 5mg/ml**)** showed activity against *Staphylococcus aureus* (30 mm), *Bacillus subtitles* (23 mm, 18 mm) and *Escherichia coli* (25mm). TLC revealed the presence of terpenoids with antimicrobial activity were reported in the genus of Combretum[18]. The leaves chloroform extracts possessed activity against *Staphylococcus aureus* (30 mm, 25 mm) and *Bacillus subtitles* (30 mm, 25 mm).

The same extract was only active at the heights concentration (1mg/ml, 5mg/ml) against *Proteus vulgaris, Pseudomonas aeruginosa, E coli, Aspergillus niger, Candida albicans* showing inhibition sones of 23 mm, 20 mm, 27mm, 15 mm, 15 mm respectively. Additionally the leaves extracts was the ethyl acetate phase at both concentrations (1mg/ml, 5mg/ml) against *Staphylococcus aureus* (20mm, 20mm), and *Escherichia coli*

(20mm, 20mm). the ethyl acetate extract possessed activity against *Proteus vulgaris, Pseudomonas aeruginosa, Aspergillus niger,* and *Candida albicans* at a concentration of 5mg/ml of 17mm, 20mm, 20mm, 20mm respectively. The aqueous phase was only active at a concentration of 5mg/ml against *Bacillus subtitles, Staphylococcus aureus, Proteus vulgaris, Pseudomonas aeruginosa, Aspergillus niger,* and *Candida albicans* showing inhibition zones of 20mm, 18mm, 20mm, 20mm, 20mm, 20mm respectively.

Polyphenols, mainly flavones, flavonols, flavonoids were accumulated in the chloroform and ethyl acetate phase.Since flavonoids are known to be synthesized by plants in response to microbial infections, it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. More lipophilic flavonoids may also disrupt microbial membranes[19]. Different *Combretum* spp. were previously reported to have significant antimicrobial activity[6]. Similar antibacterial activates were reported on the ethyl acetate fraction of *C. Hhartmamniaum* heart wood by[18,20].

Table 1: Antimicrobial activity of leaves and bark extracts of *C. hartmannianum*

Extract/drug concentration	**Extract/drug**	**Measurement of inhibition zones diameter (mm) Bacteria (MIZD) fungi**						
		***Bacteria**					***Fungi**	
		S.a	**B.s**	**P.v**	**P.a**	**E.c**	**A.n**	**C.a**
1mg/ml	Petroleum ether	30	23	-	-	25	-	-
	Chloroform	30	30	-	-	25	-	-
	Ethyl acetate	20	18	-	-	20	-	-
	Aqueous							
5mg/ml	Petroleum ether	-	18	20	-	-	-	-
	Chloroform	25	25	23	20	27	15-	15-
	Ethyl acetate	20	-	17	20	20	20	20
	aqueous	20	18	20	20	-	20	20
	Gentamycin							
	Clotrimazole							

B .s = Bacillus subtitles,S . a = Staphylococcus aureus, E.c = Escherichia coli, P.v = Proteus vulgaris, P. a = Pseudomonas aeruginosa, A.n =Aspergillus niger ,C. a . = Candida albicans

MIZD (mm) :> 18 mm : Sensitive

: 14 – 18 mm : Intermediate; : <14 mm : Resistant

Anti mycetoma activity: A promising inhibitory activity emerged against *M mycetomatis* with MIC ranging between 78.1 and 39.1 µg/ml. most active were the ethyl acetate and chloroform fractions with MIC 39 µg/ml **(Table 2).** The antifungal activity of the genus Combretum was documented by[18]. It has not been reported for *C. hartmanianum* through.

Table 2: Activity of the leaves extracts of *C. hartmannianum* against *Madurella mycetomatis*

***Combretum. Hartmannianum* leaves extracts**	**MIC (µg/ml)**
Methanolic (Crude)	78.1
Petroleum ether	39.1
Chloroform	39.1
Ethyl acetate	39.1
Aqueous	78.1
Ketoconazole (+ cotrol)	0.25

RP-HPLC-DAD polyphenols profiling in the leaves ethyl acetate phase of *C. hartmanianum:* A comparison RP-HPLC (320 - 380) chromatogram of the ethyl acetate and chloroform is presented in (Fig 1). This UV range enabled the detection of metabolites classes of interest (flavonoids and astilbenes). It is clear from this chromatogram that similar poly phenol exist among the active extracts of the leaves of the plants studied namely the chloroform and ethyl acetate fractions. TLC with the aidof NPR reagent revealed that targeted polyphenols were mainly accumulated there two fractions.

All flavonoids aglycones contain at least one aromatic ring and, consequently efficiently absorb UV light. The first maximum, which is found in the 240 – 285 nm range, is due to the A-ring and the second maximum, which is in the 230 – 550 nm range, to the substitution pattern and conjugation of the C- ring. Simple substitutions such as methyl, methoxy, and non-dissociated hydroxyl groups generally affect minor changes in the position of the absorption maxima[21]. LC with multiple-wavelength or diode-array detection (LC-DAD) was used for the detection and /or subgroup classification of flavonoids contained in the active extracts studied. Characteristic UV spectra of the main classes of flavonoids were reported by[21]. The utility of RP HPLC separation for more specific and selective identification of stilbenes and flavonoids derivatives was greatly enhanced by mass-spectrometric detection; in particular the use of MS-MS enabled the sate identification of co-eluting peaks in the complex biological matrix[22].

Compounds strictures assignment in the ethyl acetate fraction of the leaves of *C. harmannianum* :Assignment of strictures of the polyphenols recorded in the leaves ethyl acetate phase was done by studying the results of LC-MS/MS CID experiments fragments and comparing them to the reported data or to injected standards when available (Table 3 , Fig 2). The overall polarity and stereochemistry of the compounds are key factors governing their chromatographic behavior. It has been found that sugars with a D-configuration namely glucose, glactose, xylose and glucuronic acid are usually linked to the glycine by β bonds, whilst α linkages occur to L-arabinose and L-rhamnose[23].

RP-HPLC and MS-MS CID experiments data are presented (Table 3) and figure (2). Compound 1, (m/z 611) is the least polar compound (8.7 min). Glucose and rhamnose fragmentation ([M+H] $^{+}$ 146- 162]) gave rise to the main peak in positive mode. This was a product ion (Y^{o}) of (m/z 303). According to the fragmentation pattern of this products ion and after comparison with already established data[24]. This compound was com firmed to contain Quercetin. Additionally, the intensity of the aglycone product ion suggest both sugars to be attached at position 7[25] suggesting compound 1 to be Quercetin 7-O α rhamnoside-(1-6)-O- β-glucopyranoside.

Similarly, the MS/MS data of compound 2 (m/z 463, 9.9 min) are presented in **table (3).** Loss of a glucose molecule ([M+H] +- 162]) gave the main peak of the product ion (m/z 303). Intensity of the aglycone product ion in addition to the fragmentation of the glucose moiety (m/z >150) suggests compound 2 to be Quercetin-7-o- β-glucopyranoside[25].

Compound 3 (11. 3 min, m/z479) MS/MS data table (3) shows loss of glucose unit (m/z 162) giving rise to prominent aglycone product ion (Yo) (317 m/z). The fragmentation pattern of Yo ion is in agreement with that of isorhamentin[24]. The aglycone ion of Compound 3 was not observe in M3 spectrum typical of flavonoids with .glycosidation at. Position 7[23]. Compound 4 (12.8 min, m/z 449) CID experiment fragmentation resulted in aglycon ion (m/z287) which expected to be kaempferol. Loss of a glucose unit (162) and the Appearance of Prominent y∘ peak in M3 spectrum suggests compound 4 to be Kaempferol-3-o-β glycosides. Compound 7 is an aglycone (m/z287) with a fragmentation Pattern similar to the standard kaempferol hence assigned kaempferol. Compounds 5&8 (13.5, 20.0 min) gave the same aglycone mass (m/z 301) up on the loss of a glucose unit (162). According t the fragmentation Pattern of their products ion and after comparison with reported data[24]. They were confirmed to be derivative of Chryseriol .Differences in their retention time Suggest them to be isomers. Additionally the intensity of the aglycone product ion depends on the attachment position of the sugar moiety[23].

Accordingly. Compound 5 was assigned Chryseriol-7-o-β glycoside and Compound 8 Chryseriol-3 -o-β glycoside **(Table 3, Fig.2**). Compound 6 &12 (493, 331 m/z) were also expected to be a glycoside and it's a glycone after analyzing their fragmentation Pattern. Loss of one glucose unit from Compound 6 (15min) released the aglycone (m/z) ,331) similar in mass to compound 12 (26.9 min). Differences in these compounds retention time obeys the rule of elution of glycoside being elution before it's a glycone in reverse phase HPLC system (Rijke *et al*,. 2006). Fragmentation pattern, and intensities of the a glycone product ion after Consulting reported data suggests Compound 6 to be isorhamentin 3 methoxy-7-o-β glycoside and compound 12 isorhamentin -3- methoxyl, (**Table 3, Fig 2).**

Compound 9 was a glycoside (m/z 477, 20.8 min), which gave a prominent a glycone .ion. (m/z315) upon fragmentation of glucose (162).The a glycone mass together with the Loss of two methyl groups (m/z 15) suggests compound 9 to be kaempfero,3 ,7 dimethoxy – 4'-o- β glycoside after consulting literature[23,24]. (Table 3, Fig 2).Another a glycone of flavnoidal base was assigned to compound 10 (m/z 271).It was Suggests being flavones Apigienin after comparing it to an injected standard and reported data[24]. , (Table 3, Fig 2). Similarly compound11 (m/z 301, 24.6min) possessed a fragmentation pattern similar to the flavones chrtseriol (Table 3, Fig 2). Results of CID experiments on compound 13 (m/z 301, 29.5) Suggest it to be 4', 5, 7-trihydroxy-6-methoxy flavones after comparing them to established data[24], (Table 3, Fig 2).

Compound 16 (m/z315, 36.5 min) CID experiments results in n pattern comparable to that reported on phenantheren[8]. .Loss of aseriec of four methyl groups (15) product the assigned structure, (Table 3, Fig 2).Compounds 14, 15, and 17 possessed the same mass (m/z 345) with clear difference in their retention times (30.5, 32.7, 39.3min) and fragmentation pattern, (Table 3, Fig 2). Compound 15 was assigned Ayanin after comparingits fragmentation with standards and reported data[24]. Loss of some of methyl groups (m/z 15) and analyzing the intensities of the resulting fragments in comparison with reported data[11], suggest compound 14 to be 3, 7, dihydroxy-3, 4,5' tri methoxy flavones and compound 17 to be 3, 5, dihydroxy-3, 4,7' tri methoxy flavone.

Table (3) Peak No.(Fig.), HPLC data(Rt), UV data(nm), molecular weight(m/z), MS/MS data (m/z) and assigned Structures of C. hartmannianum ethyl acetate fraction

Compound peak	**Rt (min)**	**M+ (*m/z*)**	**UV λ_{max}(nm)**	**[b] CID M^n main fraction ions *(m/z)***	**Assigned structures**
1	8.7	611	220-255-300sh[a]-360	490-303-270	Quercetin 5α -O-rhamnoside(1-6") β-O-glucopyranoside
2	9.9	465	255- 230sh-350	407-395-365-303	Quercetin 7 -β- glucopyranoside
3	11.3	479	230-310	317-302-285-217	Isorhamnetin-7-O-β- glucopyranoside
4	12.8	449	225- 265sh -340	287--263-203-153	Kaempferol-3- O -β- glucopyranoside
5	13.5	463	225- 270sh-335	301-153-259-273-302-345-258	Chrysoeriol 7-O -β- glucopyranoside
6	15	493	225-255sh-355	331-316-301	Isorhamnetin 3-methoxy-7- O -β- glucopyranoside
7	19. 6	287	250- 290sh-340-360sh	287-153-269-227	Kaempferol
8	20.0	463	225sh -255-360	301-280	Chrysoeriol 3- O --β- glucopyranoside
9	20.8	477	225- 270sh-235	315-300	Kaempferol-3,7-di methoxy-4' glucopyranoside
10	23.9	271	230sh 265-335	271-118-171-153	Apigenin
11	24.6	301	250-340	286-258-229	Chrysoeriol
12	26.9	331	230sh –255-245	316-301-273245	Isorhamnetin 3-methoxy
13	29.5	301	225-250sh-345	301-286-166-259	4',5,7 - trihydroxy - 6 - methoxy flavone
14	30.5	345	240-275-340	330-312-284	3,7 dihydroxy-3',4',5 trimethoxy flavone
15	32.7	345	230sh-250-350-	330-287	5',5,dihydroxy 3,4',7 tri methoxy flavone
16	36.5	315	225sh-250-345	300-243-256-272	Phenantherene
17	39.3	345	230sh-255-350	330-287-259	3, 5, dihydroxy 3',4',7 tri methoxy flavone

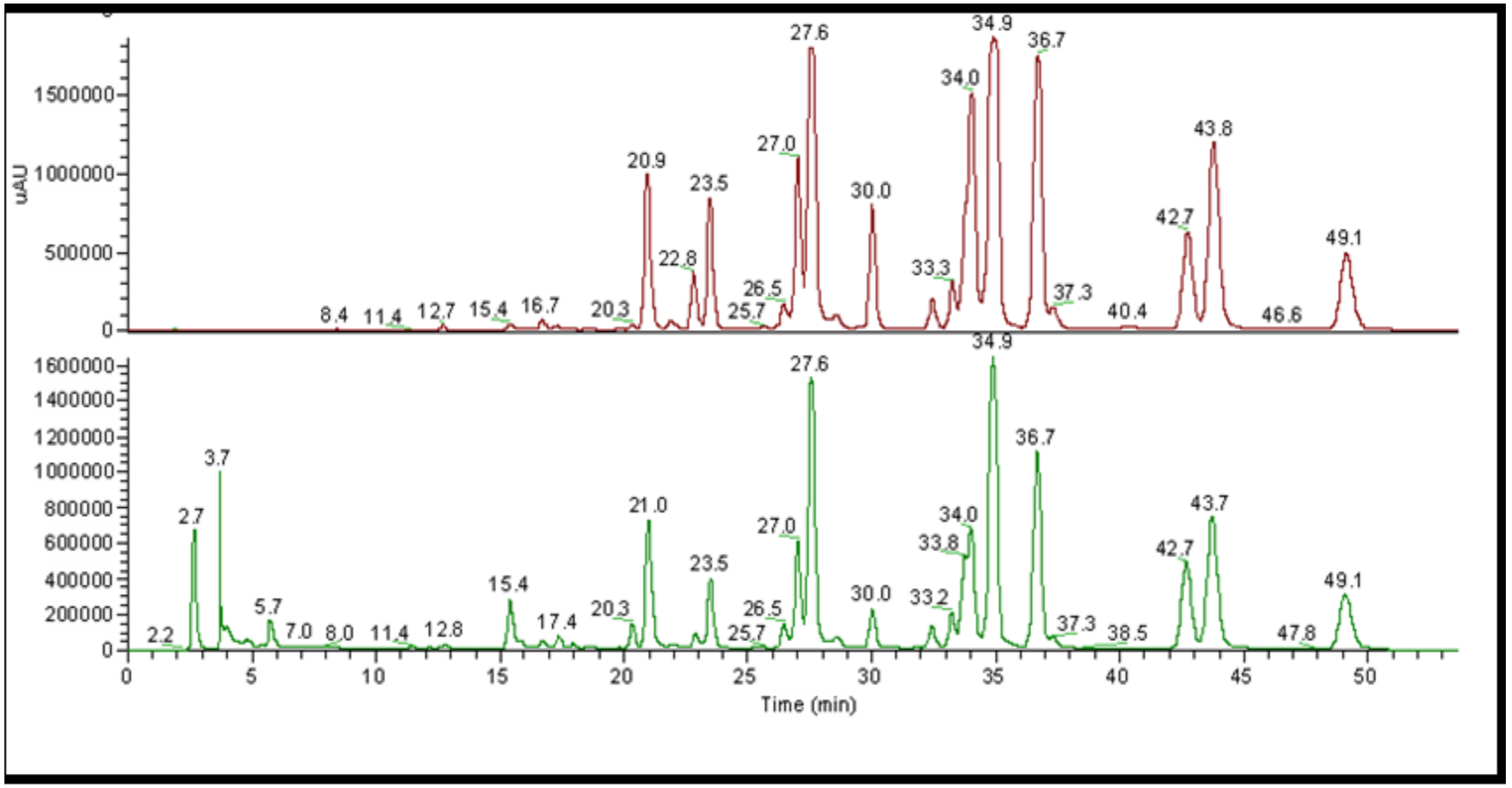


Figure 3 : RP-HPLC –DAD Chromatogram of the different extracts of *C. hartmannianum* leaves recorded at λ_{max} 320 - 380 nm. A = chloroform extract, B = ethyl acetate extract

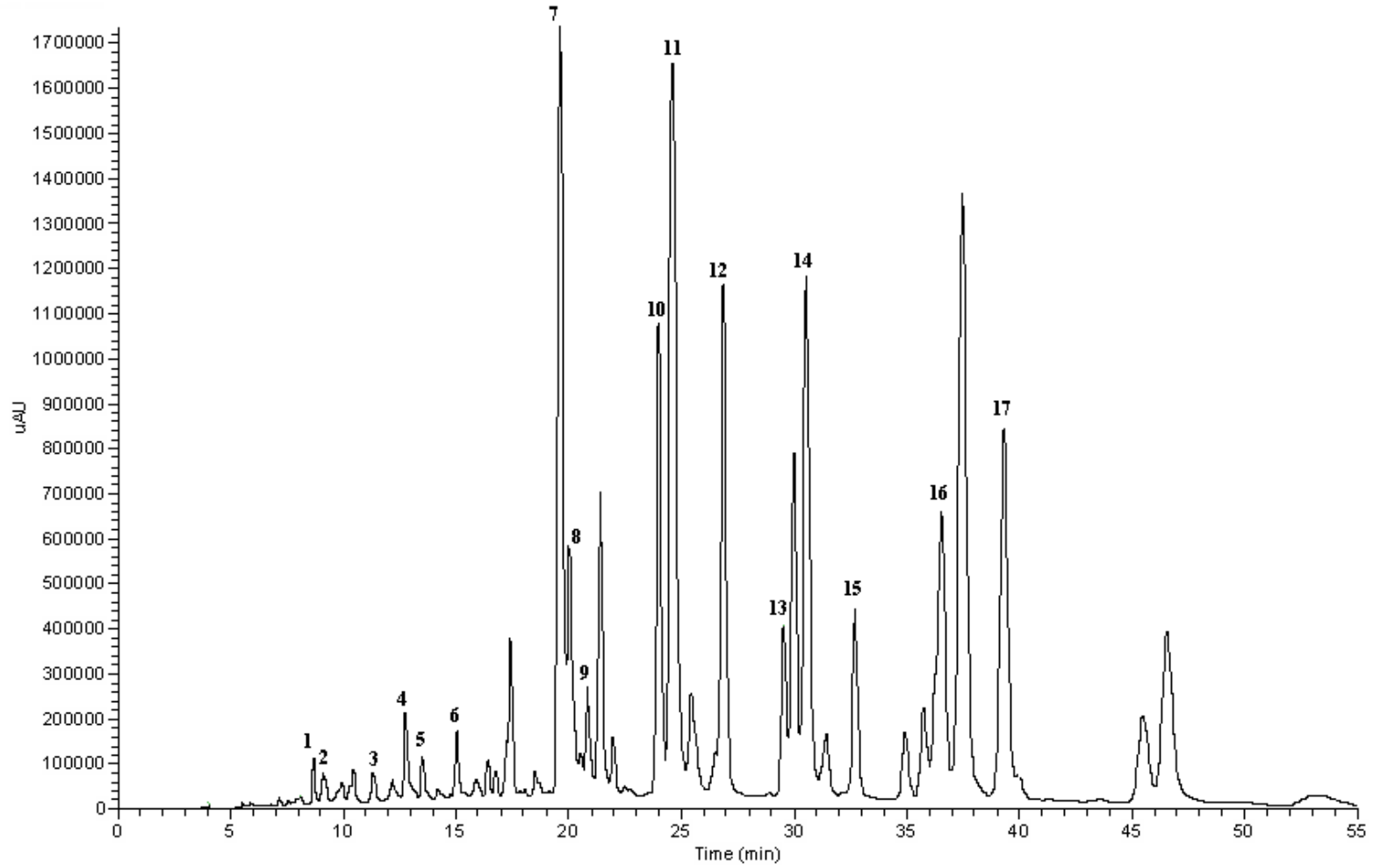
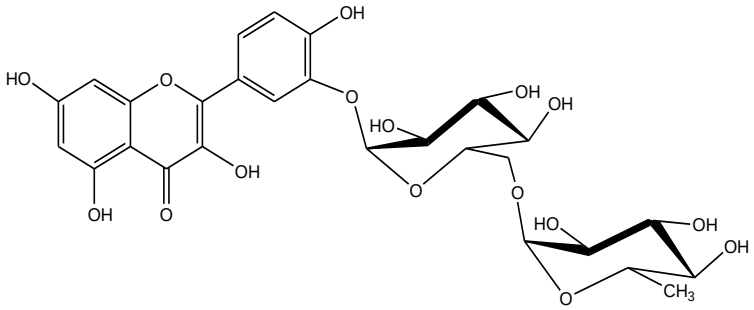
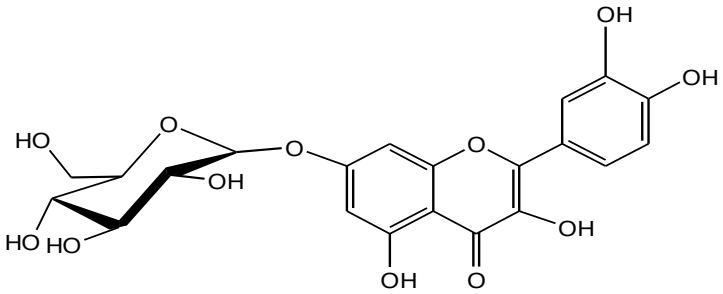


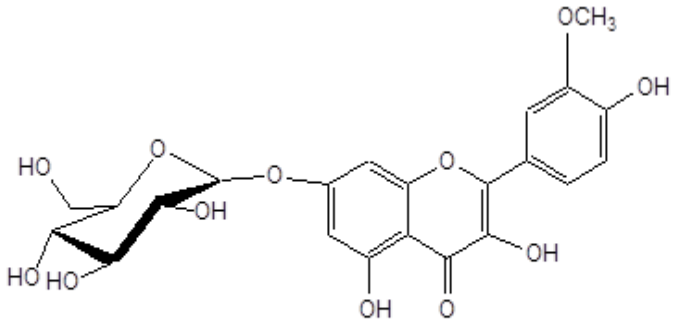
Figure 4: RP-HPLC –DAD Chromatogram of the ethyl acetate extract of *C. hartmannianum* leaves recorded at λ max 320 - 380 nm.



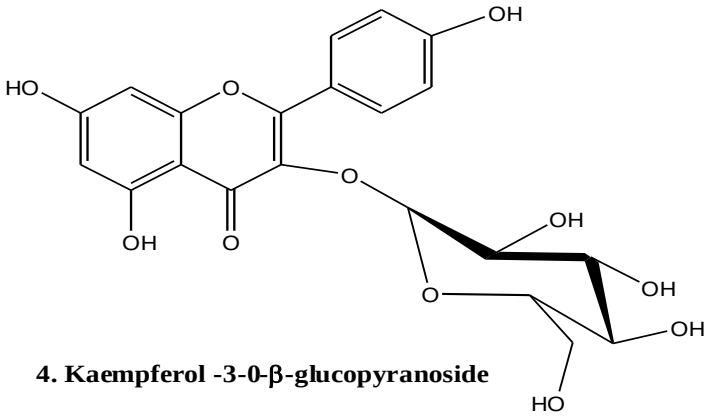
1.Quercetin-5'-α–O- rhamnoside-(1-6")-β–O-glucopyranoside



2. Quercetin 7-0-β-glucopyranoside

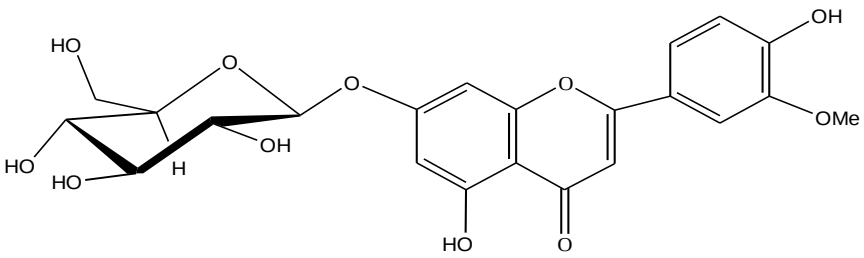


3. Isorhamnetin 7-0-β-glucopyranoside

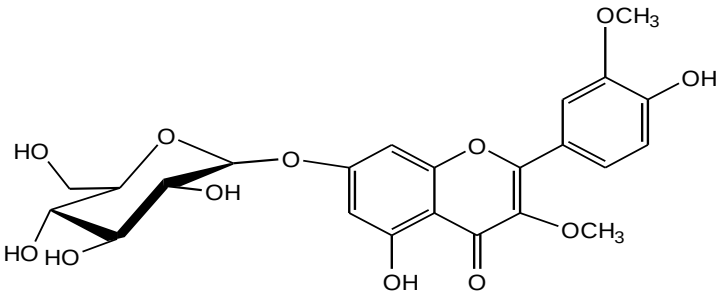


4. Kaempferol -3-0-β-glucopyranoside

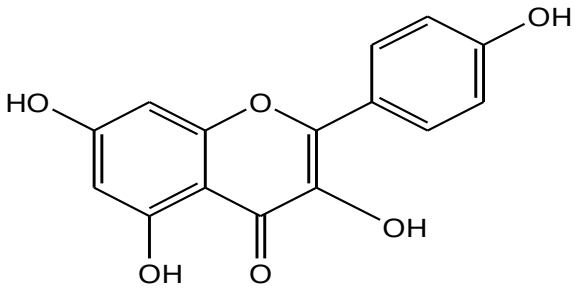
C



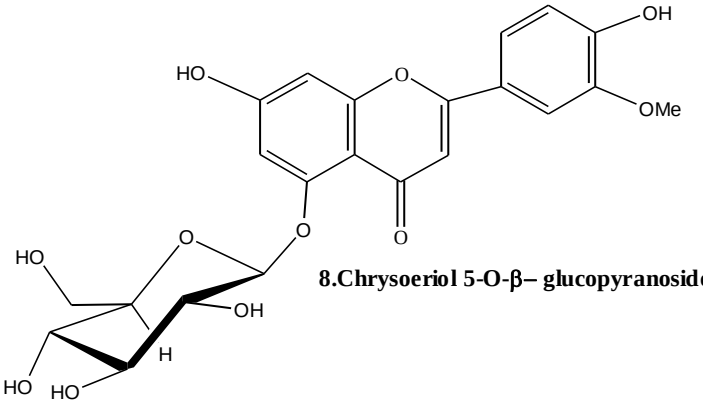
5. Chrysoeriol 7-O-β– glucopyranoside



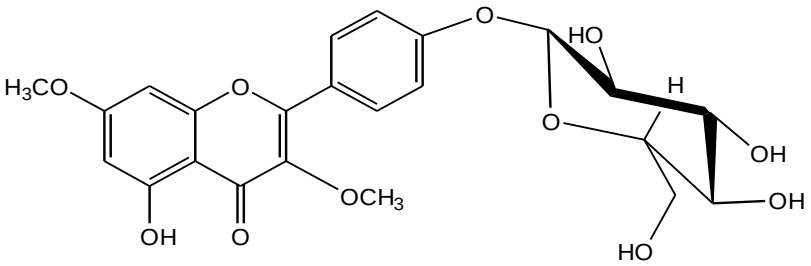
6. Isorhamnetin 3- methoxy-7-0-β-glucopyranoside



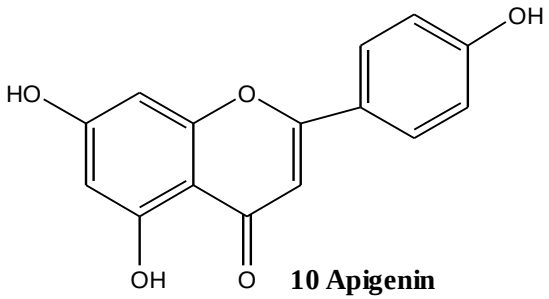
7. Kaempferol



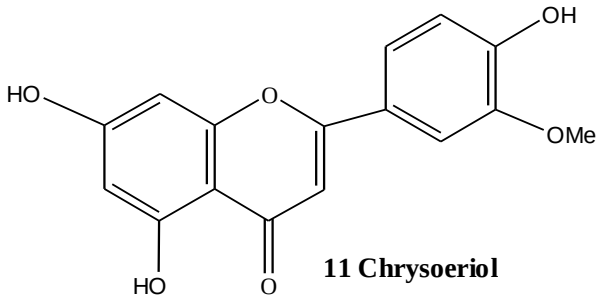
8.Chrysoeriol 5-O-β– glucopyranoside



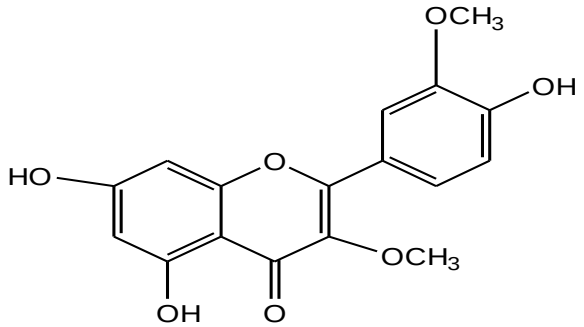
9. Kaempferol 3,7 -dimethoxy -4' glucopyranoside



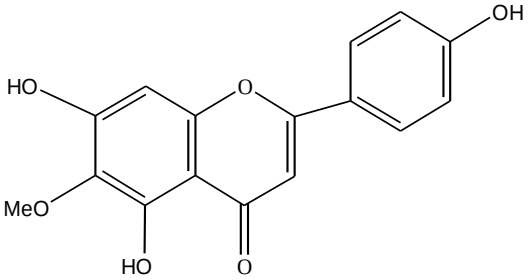
10 Apigenin



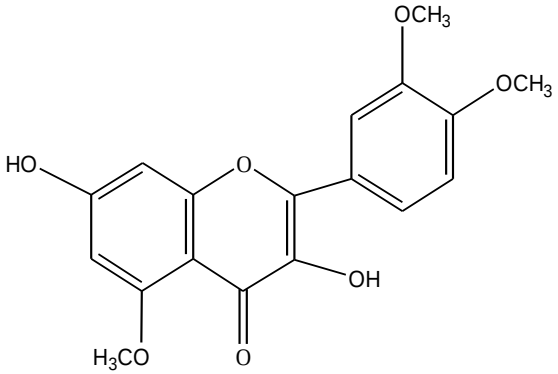
11 Chrysoeriol



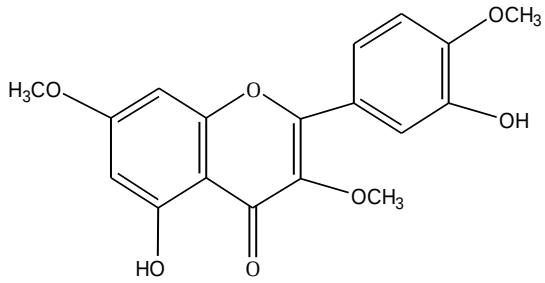
12 Isorhamnetin 3- methoxy



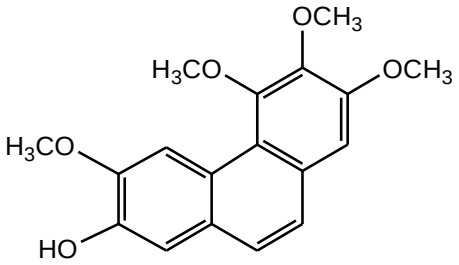
13. 4',5,7 -trihydroxy - 6 - methoxy flavone



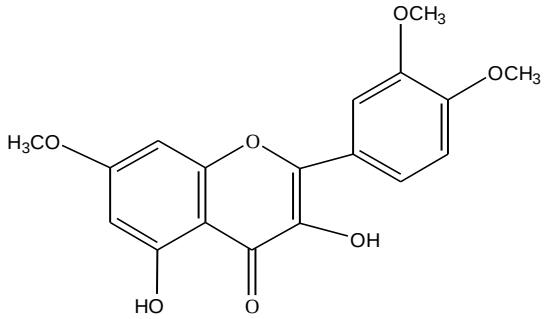
14. 3,7 dihydroxy -3',4',5 trimethoxy flavone



15. 5',5 dihydroxy -4',3,7,trimethoxy flavone (Ayanin)



16 Phenantherene



17. 3,5 dihydroxy -3',4',7 trimethoxy flavone

Fig .5: Structure of compounds isolated from Combretum hartmannianum leaves acetone extract

CONCLUSION

The extracts of the leaves of *C. hartmannianum* were found to be significantly active against tested standard human pathogenic bacteria and fungi. Additionally they possessed significant activity against *M. mycetomatis.* polyphenols possess strong antimicrobial activities[19].

Reverse phase High Liquid Chromatography coupled to Tanden Mass Spectrometry performed on the ethyl acetate fraction of the leaves of *C. hartmannianum* led to the identification of sixteen flavonoids and a phenantherene which were believed to be responsible of these activities. The anti-angiogenic and anti-proliferative effects of the plant may be due to their potential antioxidant activity, which further attributes to the collective contribution of phenolics and flavonoids present it the respective extracts.

In the previous study we proved the safeness of *C. hartmannianum* leaves metabolic extract against cell lines HT-29 (Human adenocarcinoma cells) and L-6 (Rat skeletal muscle myoblasts)[14],. This was confirmed by[26] where *C. hartmannianum* extracts proved to be non-cytotoxic against tested normal cells.

Our results prove the efficacy and safety of the leaves extracts of the *C. hartmannianum* and justifies their usage by traditional healers in Sudan against skin infections. Polyphenols are profiled for the first time in *C. hartmannianum* leaves together with their activity against mycetoma with suggests other members of the family Combretaceace to be screened. More investigations into the antimicrobial activity of compounds, already isolated from the selected fractions of this plant, against other bacteria and fungi species are justified.

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