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Chemical Constituents, Antimicrobial and Antioxidant Activity of Sudanese *Bauhinia rufescens* Bark Extract

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Abstract

The bark of *Bauhinia rufescens* was collected in January 2018 from plant garden house in Eldeain East Darfur-Sudan and it was identified at the herbarium of Medical and Aromatic Plants Research Institute, Khartoum-Sudan. The aim of the present study is to investigate the chemical constituents of the ethanolic bark extract from *Bauhinia rufescens* (L), evaluate its potential antioxidant and antibacterial activity. The plant extract was prepared by using maceration method to. The chemical constituents were identified and quantified using GC/MS. Paper disc diffusion assay was employed to evaluate the antibacterial activity and the antioxidant activitiy was evaluated using 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity. Twenty nine components have been identified, four of them are major namely; (Scopoletin(31.68%), Cholesterin methyl ester (12.42 %), Palmiticacid (6.73%) and Oleic acid(4.01%). The antibacterial activity showed moderate inhibitory effect against *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa and Staphylococcus aureus* but it was in active *against Candida albicans, Aspergillus niger*; The DPPH assay, showed (5±0.08) activity. In conclusion, the results showed that the *Bauhinia rufescens* (L) bark is a good source of natural antibacterial and antioxidant, and justify its uses in folkloric medicines.

Keywords: *Bauhinia rufescens*, Chemical Composition, GC/MS, antioxidant Antibacterial activity

1. INTRODUCTION

Natural products from different plants continue to be used in pharmaceutical preparations either as plant extracts or as pure isolates. The isolation of many physiologically active natural products like artemisinin, harmaline, morphin, atropine showed the real importance to investigate plants that can be sources of new compounds with potent clinical activities. Plants can produce a large number of compounds that can provide a wide spectrum of biological properties [1].

Bauhinia-known as kulkul in Arabic. It is a tree or shrub which grows up to 8m in height. It is recognized by it is one-direction branches, thorn-like shoots about 10 cm in length, and small leaves. This identities of the tree are: deciduous in a dry season, white and flagrant flowers; long, twisted pods about 10 cm in length, green to dark brown fruits the existence of the tree extends from Ethiopia in the East to Senegal in the west Africa. Darfur, Kordofan states are the areas where it is found in Sudan. The tree has multi uses [2,3].

Bauhinia species are utilized as folk medicines worldwide, including Africa, Asia, South America and Central America, An extract of the root is used as an astringent or antipyretic in local medicine. Leaves and fruit are applied for the treatment of diarrhea, dysentery and ophthalmic diseases. The bark of the roots and trunk is used to cure chest complaints, syphilis and other venereal diseases, leprosy and to reduce fever [4,5]. This paper is conducted to study the identification of chemical constituents, antioxidant and antimicrobial activities of *Bauhinia rufescens* bark extract.

2. MATERIALS AND METHODS

2.1. Plant material

The bark of *B. rufescens* was collected in January 2018 from plant garden house in Eldeain East Darfur- Sudan. The plant was authenticated by herbarium-National Center for Research Medicinal and Aromatic Plants Institute Khartoum-Sudan.

2.2. Chemicals

All chemicals and solvents of highest analytical grade were used as received without further purification.

2.3. Methods

2.3.1. Extraction of Plant Constituents

Powdered Air-dried bark of *B. rufescens* (100g) was macerated with700 mL of ethanol, at room temperature for three days. The extract was filtered, air dried and weighed yellow solid extract obtained yield; 5.7% was kept for further uses.

2.3.2. GC/MS analysis

The qualitative and quantitative analysis of the sample was carried out by using GM/MS technique model (GC.MS-QP2010-Ultra) from Japanese Simadzu Company, with serial number 020525101565SA and capillary column (Rtx-5ms-30m×0.25 mm×0.25µm).The sample was injected by using split mode, helium as the carrier gas passed with flow rate 1.69 ml/min, the temperature program was started from 50°C with rate 7°C/min to 180°C then the rate was changed to 10°C/min reaching 300°C as final temperature degree with 2 minutes as hold time, the injection port temperature was 300°C, the ion source temperature was 200°C and the interface temperature was 250°C. The sample was analyzed by using scan mode in the range of m/z 40-600 charges to ratio and the total run time was 28 minutes. Identification of components for the sample was achieved by comparing their retention times and mass fragmentation patents with those available in the library, the National Institute of Standards and Technology (NIST) [6].

2.3.3. Preparation of bacterial suspensions:

One mL aliquots of a 24 hours broth culture of the test organisms were aseptically distributed on to nutrient agar slopes and incubated at 37ºC for 24 hours. The bacterial growth was harvested and washed off with 100 mL sterile normal saline, to produce a suspension containing about 108-109 C.F.U/mL. The suspension was stored in the refrigerator at 4°C till used. The average number of viable organisms per mL of the stock suspension was determined by means of the surface viable counting technique. Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 mL volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The

plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37°C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 mL) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension. Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained [7].

2.3.4. Preparation of fungal suspension

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25°C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspension in 100mL of sterile normal saline and the suspension were stored in the refrigerator until used [8].

2.3.5. Testing of antibacterial susceptibility

Paper disc diffusion method was used to screen the antibacterial activity of plant extract and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines. Bacterial suspension was diluted with sterile physiological solution to 108cfu/mL (turbidity = Mc Farland standard 0.5). One hundred micro liters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whitman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20µl of a solution of each plant extracts. The inoculated plates were incubated at 37°C for 24hrs in the inverted position. The diameters (mm) of the inhibition zones were measured [8].

2.3.6. Antioxidant assay

The DPPH radical scavenging was determined according to the method of Shimada with some modification. In 96-wells plate, the test 0.5mg samples were allowed to react with 2.2Di (4-tert-octylphenyl)-1-picryl stable free radical (DPPH) for half an hour at 37°C. The concentration of DPPH was kept as (300µM).The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517 nm using multi-plate reader spectrophotometer. Percentage radical scavenging activity by sample was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate [9].

3. RESULTS AND DISCUSSION

3.1. Chemical constituents

The chemical constituents of *B. rufescens* bark extract were identified and quantified by GC/MS running time was 27.5 minutes; results revealed the presence of twenty nine components , four of them are major; (Scopoletin(31.68%), Cholesterin methyl ester (12.42 %), Palmiticacid (6.73%) and Oleic acid(4.01%). The chromatogram is presented in Figure 1. The name of plant constituents with their Retention Time (RT) are presented in the Table 1. Mass spectrum of major constituents as following;

The mass spectrum of Scopoletin (31.68 %) is shown in Fig 2. The peak m/z at 192, which appeared at R.T. 16.325 in total ion chromatogram, corresponds $M^+[C_{10}H_8O_4]^+$. The mass spectrum of Cholesterin methyl ester (12.42 %) is shown in Fig 3. The peak m/z at 400, which appeared at R.T. 21.858 in total ion chromatogram, corresponds $M^+[C_{28}H4_8O]^+$. The mass spectrum of n-Hexadecnoic acid (6.73 %) is shown in Fig 4. The peak m/z at 256, which appeared at R.T. 16.008 in total ion chromatogram, corresponds $M^+[C_{16}H_{32}O_2]^+$. The mass spectrum of Oleic acid (4.01 %) is shown

in Fig 5. The peak m/z at 282, which appeared at R.T. 17.699 in total ion chromatogram, corresponds $M^+[C_{18}H_{34}O_2]^+$.

3.2. Antimicrobial activity

Assessment of antimicrobial activities of *B. rufescens* bark extracts with different concentrations (100, 80, 60 and 40 mg/mL) were carried out against four types of bacteria, two gram positive (*B. subtilis* and *S. aureus*) and two gram negative (*E. coli* and *P. aeruginosa* and two fungi (*C. albicans* and *A. niger*). The antimicrobial activities of the plant extract against bacteria and fungi were shown in Table 2. The plant extract exhibited intermediate sensitivity against four tested bacteria with the different concentrations but it was in active against two fungi *C. albicans and A. niger.*

3.3. Antioxidant activity

The percentage of DPPH Radical Scavenging Activity (RSA) of plant extract was 5±0.08 lower than Standard as shown in Table 3. The results indicated that of *B. rufescens extract* contain anti-oxidant compounds this is in agreement with previously reported studies [10].

Table 1. Constituents of *B. rufescens* bark extract.

S. No	Name	R. Time	Area	Area%
1.	1-Propanamine, ethyl-N-methyl	3.806	463739	1.43
2.	n-Formyl-alpha-alanine	4.471	1167767	3.61
3.	2,5-dimthyl-4-hydroxy-3(2H)-furanone	5.517	561096	1.73
4.	1-pentadecene	9.526	348454	1.08
5.	3-Furanacetic acid, 4hexyl-2,5dihydro-2,5-dioxo	10.848	989737	3.05
6.	Z-5-Nonadecene	12.001	677921	2.10
7.	Quinoline,4-isobutyl-	13.553	300747	0.93
8.	1-Nondecene	14.235	557166	1.72
9.	6-(3isopropenylcyclopropan-1-enyl)-6-meth	14.766	686202	2.12
10.	Bicyclo[2,2,2]octa-2,5diene,1,2,3,6-tetramthyl-	14.998	1043858	3.23
11.	Acetic acid, (2-isopropenylcyclopentyldene)	15.408	367158	1.13
12.	2-(2diethylaminoethylamino)ethanol	15.586	762093	2.36
13.	n-Hexadecanoic acid	16.008	2176638	6.73
14.	N-[1-[(damantan-1-ylmethyl) –amino]-2-2,	16.047	743045	2.30
15.	Scopoletin	16.325	10247881	31.68
16.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.264	946660	2.93
17.	9-Octadecenoic acid (Z)-, methyl ester	17.311	265289	0.82
18.	Methyl stearate	17.532	549929	0.59
19.	Lenolelic acid, methyl ester	17.665	192289	1.70
20.	Oleic acid	17.699	1298121	4.01
21.	Octadecadienoic acid	17.888	322066	1.03
22.	17-Pentatriacontene	18.112	554462	1.71
23.	Pentadecanal	19.304	417216	1.29
24.	Hextriadecanal	20.143	749936	2.32
25.	Eicosane	20.649	457871	1.42
26.	Oxirane,hexadecyl	20.945	485167	1.50
27.	Cholest05-ene, 3-methoxy, (3.beta)	21.858	4019100	12.42
28.	Hextriadecontane	22.157	346787	1.07
29.	Squalene	23.157	644216	1.99
		23.157	32349621	100.00

Table 2. Antibacterial activity of *B. rufescens* bark extract in (mm).

Conc mg/mL	S.a	Ps.	Bs.	Ec.	C.a	A.u
100	5	7	7	11	-	-
80	3	6	8	10	-	-
60	2	5	7	7	-	-
40	1	4	10	6	-	-

E.c = *Escherichia coli*, Ps. = *Pseudomonas aeruginosa*, B.s = *Bacillus subtilis*
S.a = *Staphylococcus aureus*, C.a = *Candida albicans*, A.u = *Aspergillus niger*

Table 3. Antioxidant activity of *B. rufescens* bark extract.

S.No	Sample Code	%RSA ±SD (DPPH)
1	Plant extract	5±0.08
2	Propyl gallate (Standard)	93±0.01

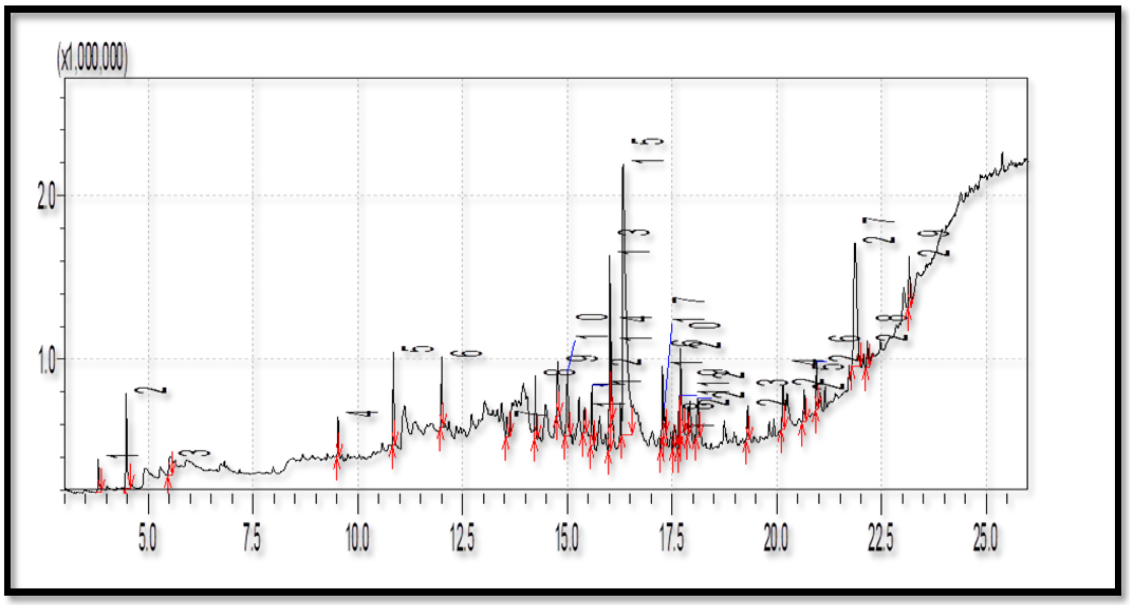

Figure 1. Chromatogram of *B. rufescens* bark extract.

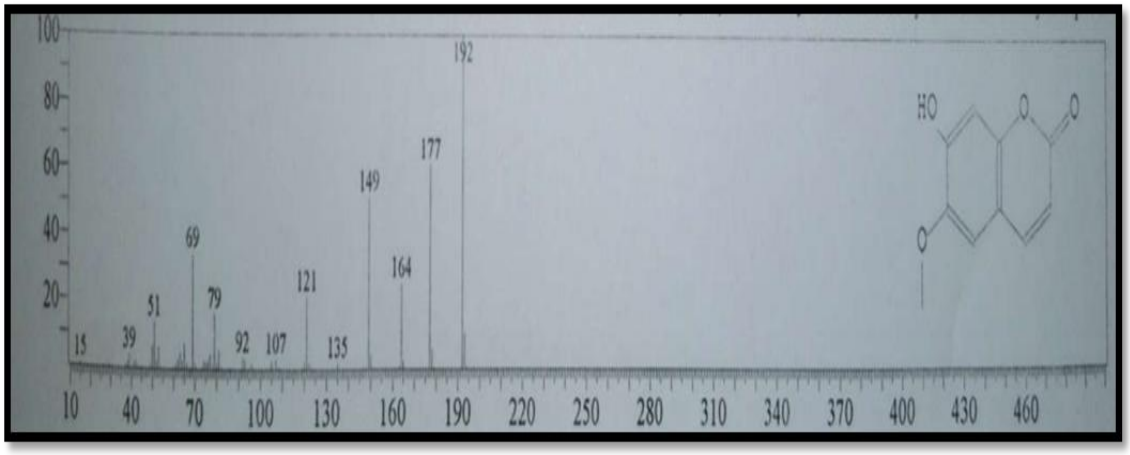

Figure 2. Mass spectrum of Scopoletin.

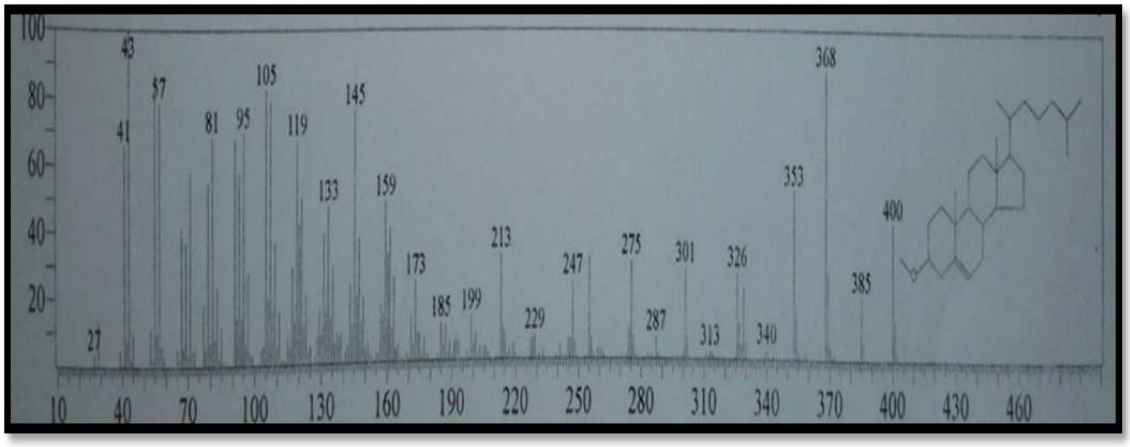

Figure 3. Mass spectrum of Cholesterin methyl ester.

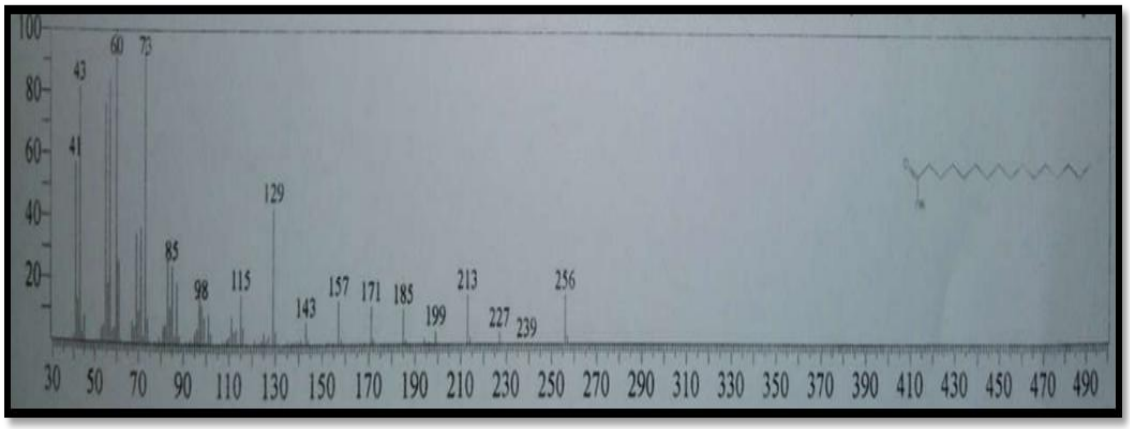

Figure 4. Mass spectrum of Palmatic acid.

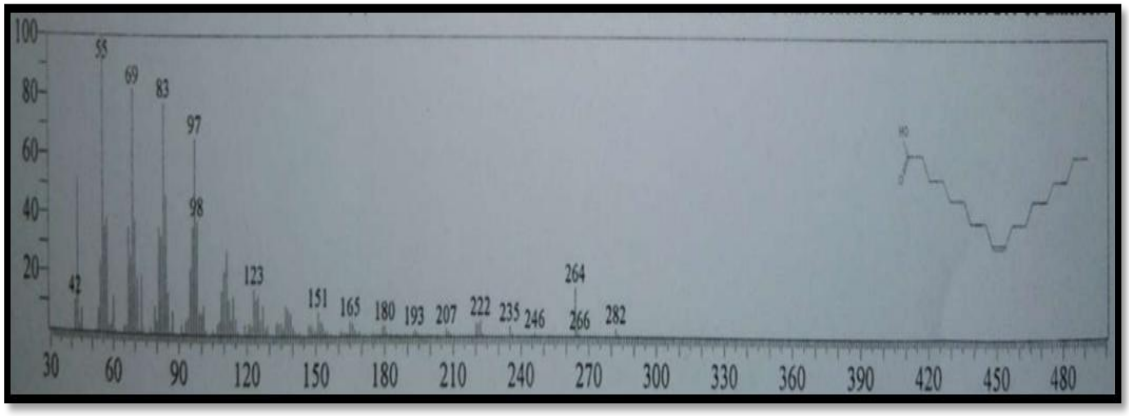

Figure 5. Mass spectrum of Oleic acid.

CONCLUSION

The results indicated that the ethanolic extract of *B. rufescens* content twenty nine compounds and has considerable antimicrobial and anti-oxidant activity. The plant extract showed activity against *B. subtilis, P. aeroginosa, S. aureus, E. coli* and no activity against *C. albicans* and *A. niger.*

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CONFLICT OF INTEREST

There is no conflict of interest.

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