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Commiphora africana Resin Phytochemical Analysis & Some Biological Aspects

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Medicinal plants are the richest bioresources of drugs. There is need to validate medicinal and aromatic plants researches results through an organized database.
Objective: To identify the terpenoids in *Commiphora africana* resin and some biological activity specially antiviral activity.
Materials and Methods: Two different methods were done to identify Terpenoids in this resin, dipping the resin in CH_2Cl_2 and isolation using column chromatography [1], ethanol and water extract were extracted for phytochemical screening and the biological aspects.
Results: Phytochemical screening identified that Flavonoids, Saponins, Alkaloids and Terpenes were the major constituents of *C. africana* resin ethanolic and water extracts. GC-MS analysis of CH_2Cl_2 extract and the isolated samples by column chromatography revealed compounds which

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can play important role in therapy, so as Betulin $C_{30}H_{50}O_2$. Urs-12-en-28-al, 3-(acetyloxy) $C_{32}H_{50}O_3$. Cholest-22-ene-21-ol, 3-5-dehydro-6-methoxy-, pivalate $C_{33}H_{54}O_3$ and Phthalic acid, bis(2-pentyl) ester $C_{18}H_{26}O_4$. LC50 values of water and ethanolic extracts were found to be 100 and 630.9 respectively. While the LC50 for the aqueous water solution of the crude resin was found to be 63.1.

High and clear antiviral activity towards Newcastle virus was observed at 400 ug/ml *C. africana* water solution, 400 ug/ml *C. africana* water extract and 100, 200, 400, 500 ug/ml *C. africana* ethanol extract. The highest antimicrobial activity recorded was obtained for *C. africana* ethanol extract against *Staphylococcus aureus, Klebsiella porwonia, Asperogillus niger and Candida Albicans*.

Conclusion: Antiviral activity shown by *Commiphora africana* resin extracts may be due to different compounds included.

Keywords: Burseraceae; terpenes; NDV; GCMS; LC50.

1. INTRODUCTION

Many peoples all over the world use herbal medicine for some aspect of primary health care [2], historical experiences with plants for therapeutic purposes have helped to introduce chemical entities in modern medicine.

Commiphora africana from the family *Burseraceae* is wide spread in African countries , Sudan (Darfur, Kordofan), followed by Ethiopia and Eritrea, and finally Somalia before curving southwards through Kenya and Uganda, rare in Tanzania, abundant again in Mozambique, Rhodesia and south-west Africa [3].

Photo 1. *C. africana* resin

Photo 2. *C. africana* tree

It is used traditionally for many purposes, treat measles, insecticides, antilipidaemic and management of cardiovascular disorders [4].

Resins usually contain complex mixture of Terpenes, gas chromatography mass spectrometry (GCMS) is useful technique for identifying volatile compounds in complex mixtures [5]. The aim of this study was to investigate the phytochemical composition, terpenoids content, and also screen the antiviral activity of *Commiphora africana* resin which could be useful for the development of new tools as antiviral agents for the control of antiviral diseases.

2. EXPERIMENTAL SECTION

2.1 Plant Material

Commiphora Africana resin was collected from the western Sudan (Jabal Mara) by Nertety forest office, and authenticated by Dr. Mohamed Elmubark head of gums unit University of Science and Technology Khartoum Sudan.

2.2 Extraction of Plant Materials

200 g *Commiphora africana* resin was extracted after grinded well with the help of mortar and pestle at room temperature by cold percolation process using 500 mL of 85% ethanol in a conical flask, wrapped with aluminium foil, shacked using a shaker for 6 hours for continuous agitation at 160 rev/min and kept at room temperature for 24 hours and then filtered by Whatman filter paper No.1, the residue was re-extracted under the same condition and the filtrates were concentrated on rotary evaporation at 40℃. 200 g of the resin were extracted with

the same steps mentioned above using 500 ml distilled water instead of ethanol and the water extract dried using freeze drier. [6], the ethanol and water extracts were preserved for phytochemical screening and biological assay.

2.3 Phytochemical Screening

Various phytochemical tests were carried out on *Commiphora africana* resin ethanol and water extracts to detect the presence of bioactive constituents of the resin using the methods described by Martinez & Valencia (1999), Sofowora (1993) Harborne (1984) and Wall et al. (1952) with few modifications [7].

(Table 1) shows the phytochemicals present in *C. africana* resin.

2.4 GC-MS Analysis

CH_2CL_2 extract and the isolated samples by column chromatography (AF1, AF2, AF3 and AF4) of *C. africana* resin were analyzed by GC/MS at the (Instrumentation lab of Central Petroleum laboratories Khartoum Sudan) and performed on Varian CP-3800 gas chromatograph coupled with Varian 4000 GC/MS/MS, fitted with VF-5MS capillary column (30 m × 0.25 mm with 0.25 µm film thickness). The initial oven temperature was 80℃ held for 1 minutes and ramp at 10℃/min until 300℃ and held for 10 minutes, injector temperature 275℃, helium was the carrier gas at a flow rate of 1 ml./min, split ratio 50. The transfer line temperature was 280℃ and the MS ion trap is set at 220℃, the acquisition mass range was 45-450 m/z. National institute of standard and technology (NIST) was the spectral Library.

2.4.1 *Commiphora africana* resin CH_2Cl_2 extract

In order to detect Terpenoids from *Commiphora africana* resin it was dipped in methelene dichloride (CH_2Cl_2) for twenty four hours, filtered and the filtrate let to dry at room temperature for 72 hours and then examined using GCMS [1].

2.4.2 Column chromatography

Column chromatography is a convenient method for isolation of different terpenoids, column chromatography for this resin was done at (Plant Physiology Lab Botany Department Faculty of Science University of Khartoum). *Commiphora africana* resin was prepared for column chromatography by dipping grinded 38.5 g of the resin in chloroform, filtered and the filtrate let to dry, dissolved in 25 ml 95% ethanol and 25ml 4% lead acetate and filtered ready for column chromatography, silica gel is used as a stationary phase and eluted with chloroform and methanol (99:1) [1] The separated layers were collected and four samples AF1, AF2, AF3, AF4 were analyzed by GCMS.

Photo 3. Column chromatography of *Commiphora africana* resin

3. BIOLOGICAL ASSAY

3.1 Antiviral Screening

Antiviral test was held at Veterinary Research institute (Virology section) towards Newcastle virus (NDV) on chickened embryos nine days old [8]. Various Concentrations 100, 200, 400 and 500 ug/ml of *C. africana* ethanol extract, water extract, and water solution (dissolve the crude resin in distilled water to form water or aqueous solution) were prepared. 0.1 ml of NDV suspension was treated with 0.1 mL of 100, 200, 400 and 500 ug/ml of each extract and the aqueous solution. The treated viruses were incubated at 4ºC for about 1 h. The treated viruses and the controls were then inoculated via the allantoic sac of 9 days old chick embryos. Hank's balanced salt solution (negative control) and the virus without treatment (positive control) were used as controls. Triplicate tests were carried out for each extract against the virus, the results were compared to the sample without treatment.

High and clear antiviral activity was observed at 400 ug/ml *commiphora africana* water solution and 400 ug/ml *commiphora africana* water extract and 100, 200 400, 500 ug/m *commiphora africana* ethanol extract.

3.2 Antimicrobial Assay

Bacterial pathogens selected were gram positive bacterium *Staphylococcus aurus (ATCC 25923)* and the gram negative bacteria *Klebsiella porwonia (ATCC 3657)*, and Fungal pathogens were *Asperogillus niger (ATCC 9763)* and *Candida albicans (ATCC 8196).*

According to (Kavanagh, 1972) sterilized nutrient agar was used as culture medium and the bacterial cultures were spread on the agar surface using sterile cotton swab on sterile Petri dish, after solidification of the agar two wells of 7 mm diameter was made in the agar medium using sterile cork borer, 100 ug/ ml aqueous of each ethanol and water extracts of *C. africana* resin were transferred into the two wells each extract in a separate Petri dish, the dishes were left at room temperature for 2 hours to allow diffusion of test sample and incubated at 37º for 48 hours, tests were performed in duplicate and means were taken, the diameter of the zones of inhibition was measured in mm with scale.

3.3 Brine Shrimp Lethality Bioassay

Brine shrimp bioassay is a simple bioassay for testing medicinal plants extracts lethality which in many cases correlates well with both cytotoxic and anti tumour properties [9], the experiment was performed according to the procedure described by Meyer [10]

Brine shrimps (*Artemia salina)* were hatched using sterile artificial seawater (prepared using sea salt 38 g in one litre distilled water) 1% yeast extract was added for feeding, and let for 24 hours. Three concentration of each extract were prepared 10, 100 and 1000 ug/ml.

Ten freshly hatched free swimming nauplii were drawn from the brighter portion through a glass capillary and placed in a small beaker containing the prepared extracts and artificial sea water were added to complete five millilitre and maintained at room temperature, for 24 h under light and surviving larvae were counted Three replicates were done for each dose level LC50 values (the concentration at which 50% of the larvae were killed), was determined based on the percent mortality and the best-fit line was obtained from the curve data by means of regression analysis [11] Caffeine LC50 306 ug/ml was used as positive control and ethanol LC50 1000 ug/ml negative control and solvent.

Table 1. Phytochemical screening of ethanolic and water extract of *C. africana* resin

Extract	Saponins	Cumarins	Triterpenes & Sterol	Flavonoids	Alkaloid	Tannin	Anthraquinone
ethanol extract	+++	+++	T+++ S -	+++	+++	-	-
Water extract	+++	++	T++ S -	++	++	-	-

(+) presence, (++) moderate presence and (+++) high presence, T Triterpenes, S sterol

Table 2. Bacteria and fungi inhibition zone in mm by *Commiphora africana* resin extracts

Extract	*Klebsiella*	*Staphylococcus*	*Asperogillus*	*Candida*
Ethanol ex.	19	17	16.5	19.5
Water ex.	14	14	14	14

Table 3. Brine shrimp bioassay results of *Commiphora africana* resin in 24 hours

Extract	Percent death 10 ug/ml	Percent death 100 ug/ml	Percent death 1000 ug/ml	LC50
Water extract	13.33	50	86.62	100
Resin, water sol	13.33	56.7	96.67	63.1
Ethanol extract	6.67	26.67	56.67	630.9

4. RESULTS

4.1 GCMS Analysis of *C. africana* CH_2Cl_2 Extract

The following six compounds were recorded from GCMS analysis of *Commiphora africana* extract prepared by CH_2Cl_2

4.2 GCMS Analysis of Column Chromatography Isolated Samples

These following compounds were recorded from GCMS analysis of samples AF1, AF2, AF3, AF4 isolated using Column Chromatography.

N	Name	Formula	Rate	M.W	Area	Area%
1	Tricyclo[5.4.0.0(2,8)]undec-9-ene, 2,6,6,9-tetramethyl-	$C_{15}H_{24}$	10.61	204	1094000	11.29

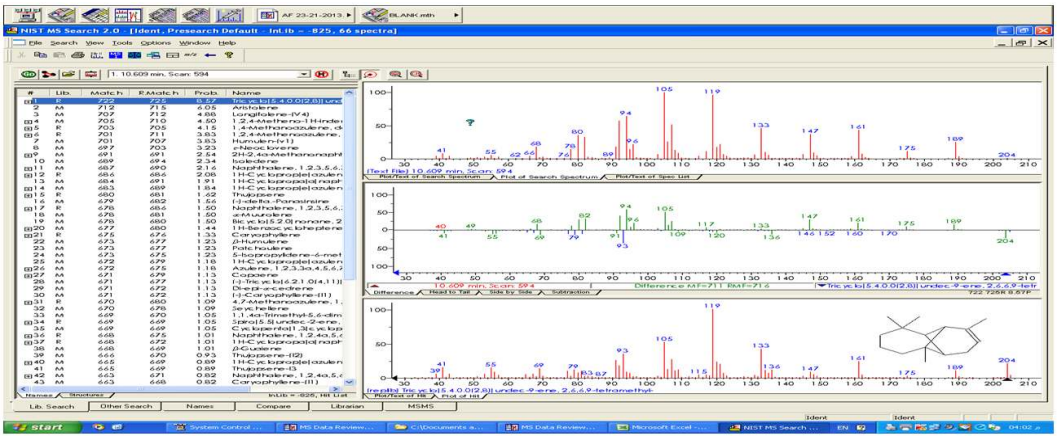

Fig. 1. Tricyclo[5.4.0.0(2,8)]undec-9-ene, 2,6,6,9-tetramethyl- $C_{15}H_{24}$

N	Name	Formula	Rate	MW	Area	Area%
2	2-Isopropenyl-2,3-dihydrofuro[3,2-g]chromen-7-one	$C_{14}H_{12}O_3$	17.681	228	762328	7.868

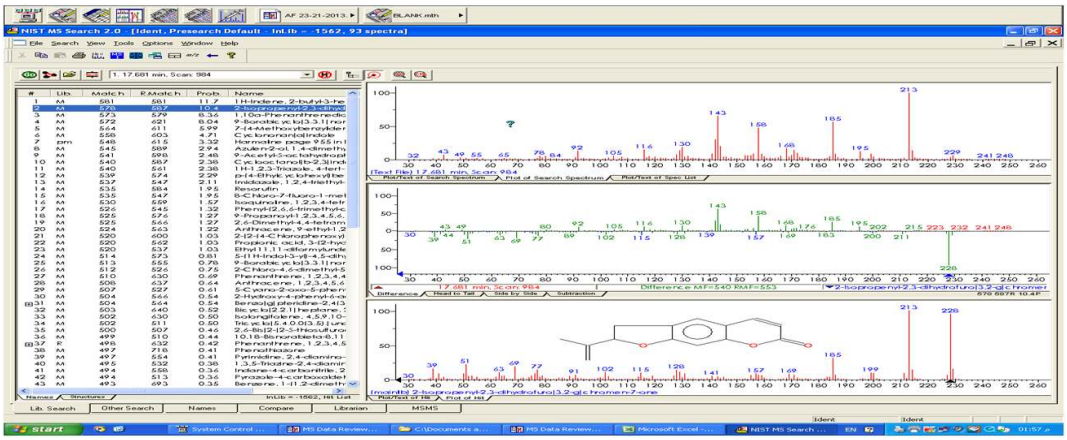

Fig. 2. 2-Isopropenyl-2,3-dihydrofuro[3,2-g]chromen-7-one $C_{14}H_{12}O_3$

N	Name	Formula	Rate	M.W	Area	Area%
3	6,7,8,9,10,11-Hexahydro-5H-cycloocta[b]quinolin-12-one	$C_{15}H_{17}NO$	18.49	227	937826	9.679

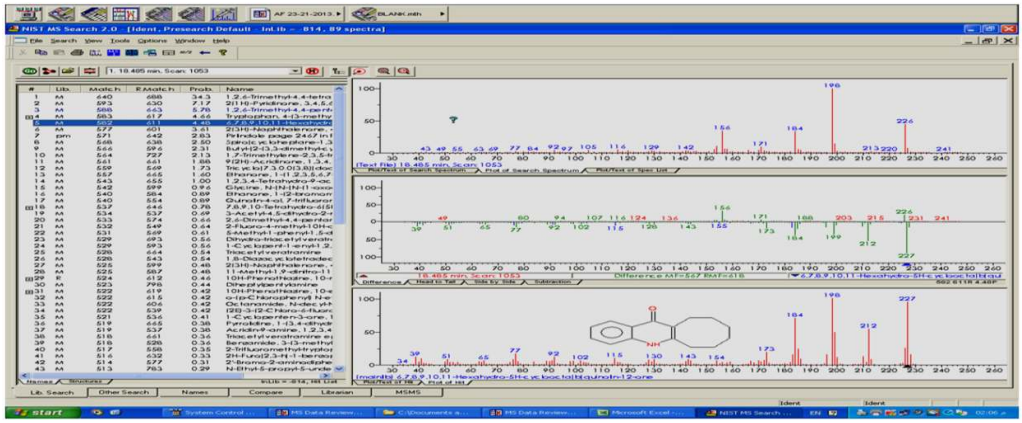

Fig. 3. 6,7,8,9,10,11-Hexahydro-5H-cycloocta[b]quinolin-12-one $C_{15}H_{17}NO$

N	Name	Formula	Rate	M.W	Area	Area%
4	Phthalic acid, bis(2-pentyl) ester	$C_{18}H_{26}O_4$	22.102	306	3716000	38.35

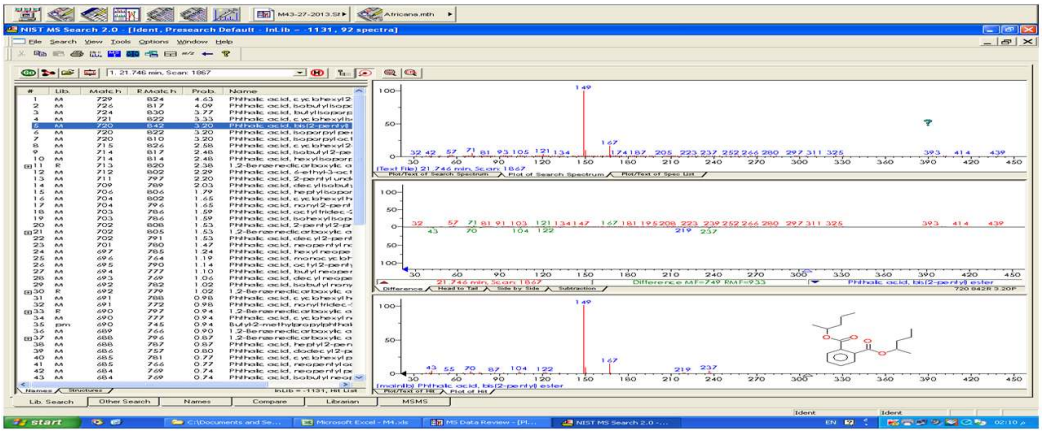

Fig. 4. Phthalic acid, bis(2-pentyl) ester $C_{18}H_{26}O$

No.	Name	Formula	Rate	M.W	Area	Area%
5	Acetic acid,3-hydroxy-7-isopropenyl,1,4adimethyl-2,3,4,4a,5,6,7,8,octahydronaphthalen-2-yl ester	$C_{17}H_{26}O_3$	27.30	278	2260000	23.33

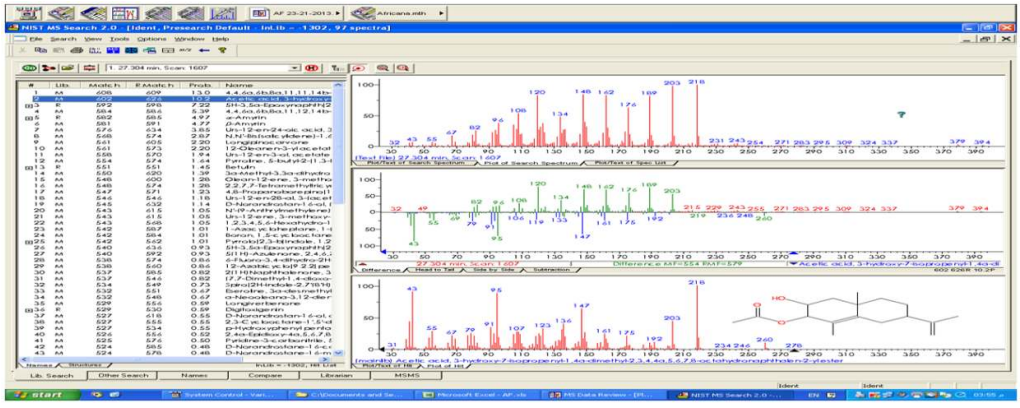

Fig. 5. Acetic acid, 3-hydroxy-7-isopropenyl-1,4a-dimethyl-2,3,4,4a,5,6,7,8-octahydronaphthalen-2-yl ester $C_{17}H_{26}O_3$

N	Name	Formula	Rate	M.W	Area	Area%
6	1-Azaspiro[5.5]undecan-8-ol, 7-(1-buten-3-ynyl)-2-(4-pentynyl)-, [6R-[6à(R*),7á(Z),8à]]-	$C_{19}H_{27}NO$	30.98	285	918650	9.482

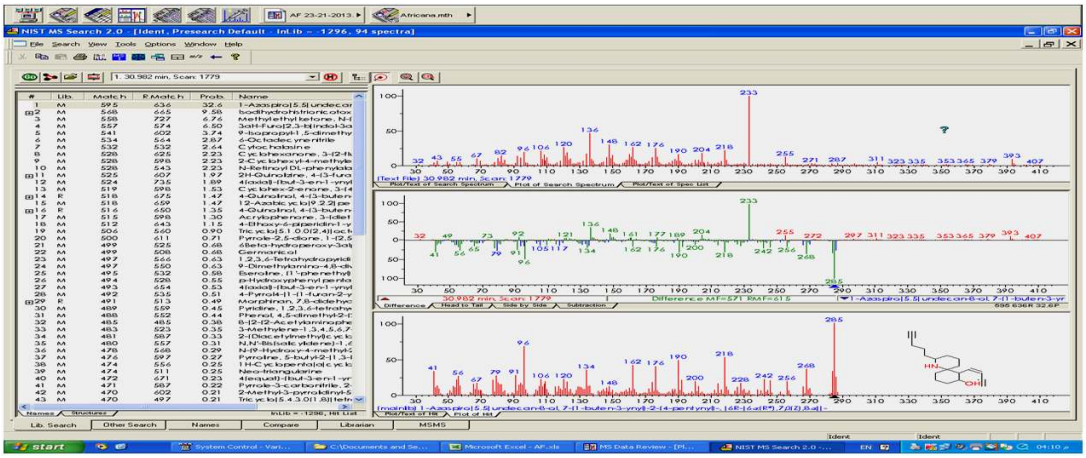

Fig. 6. 1-Azaspiro[5.5]undecan-8-ol, 7-(1-buten-3-ynyl)-2-(4-pentynyl)-, [6R-[6à(R*),7á(Z),8à]]- $C_{19}H_{27}NO$

4.2.1 Sample AF1 GCMS results

N	Name	Formula	Rate	M.W	Area	Area%
1	Cyclohexane, 1 ethenyl-1-methyl,4,bis(1,methylethenyl)-	$C_{15}H_{24}$	12.57	204	12990000	2.017

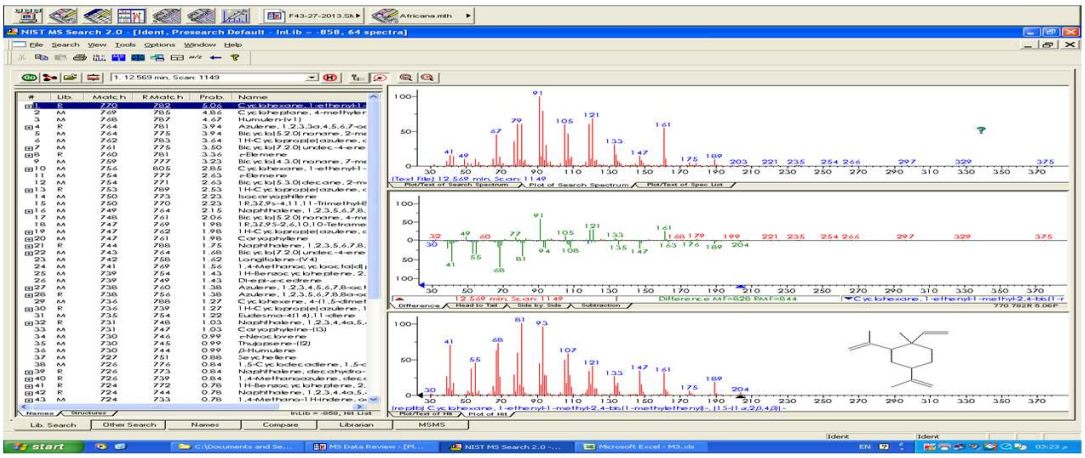

Fig. 7. Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl) $C_{15}H_{24}$

N	Name	Formula	Rate	M.W	Area	Area%
2	Cholestan-3-ol, 2-methylene-, (3á,5à)-	$C_{28}H_{48}O$	18.88	400	46450000	7.213

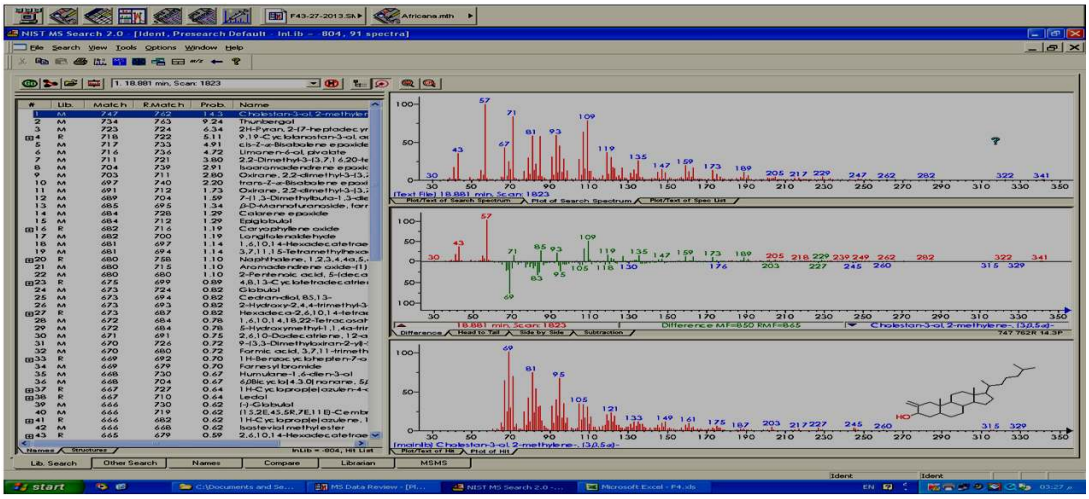

Fig. 8. Cholestan-3-ol, 2-methylene $C_{28}H_{48}O$

N	Name	Formula	Rate	Mw	Area	Area%
3	Cholest-22-ene21ol,3,5Dehydro,6methoxy-, pivalate	$C_{33}H_{54}O_3$	19.72	498	11110000	1.725

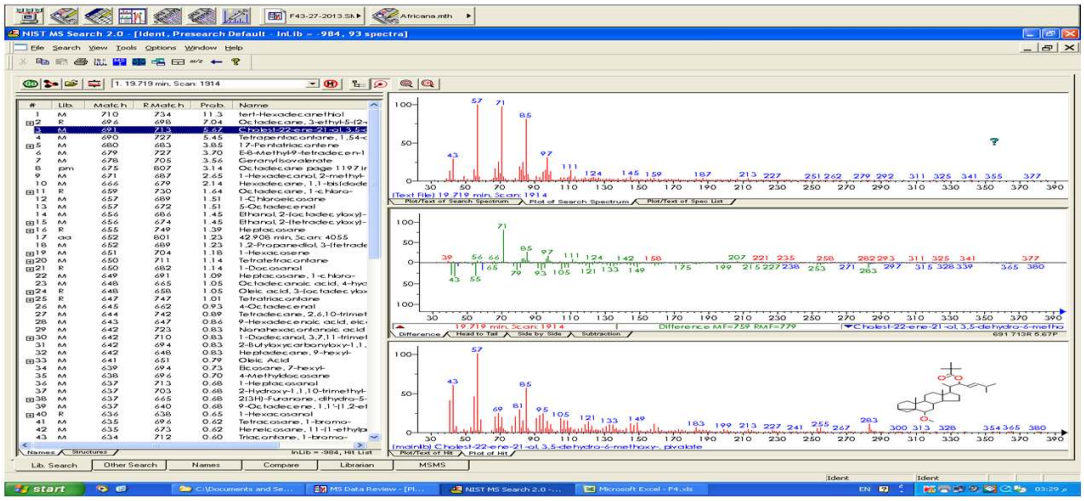

Fig. 9. Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate $C_{33}H_{54}O_3$

N	Name	Formula	Rate	M.W	Area	Area%
4	Phthalic acid,bis (2-pentyl) ester	$C_{18}H_{26}O_4$	21.74	306	421900000	65.51

Compound 4 Phthalic acid, bis(2-pentyl) ester $C_{18}H_{26}O_4$ also found in Fig. 4 in CH_2Cl_2 extract

N	Name	Formula	Rate	M.W	Area	Area%
5	3Oxatricyclo[20.8.0.0(7,16)]triaconta-1(22),7(16),9,13,23,29-hexaene	$C_{29}H_{42}O$	25.78	406	20060000	3.115

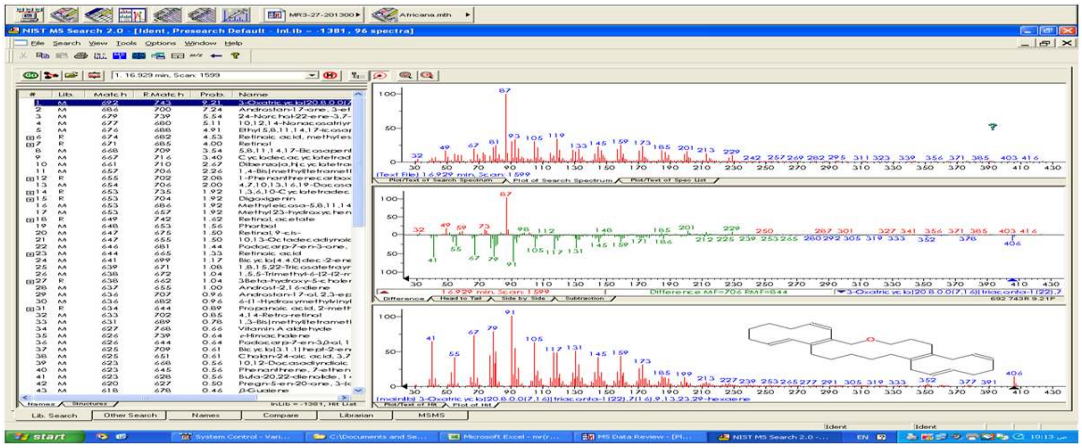

Fig. 10. 3Oxatricyclo[20.8.0.0(7,16)]triaconta1(22),7(16),9,13,23,29-hexaene $C_{29}H_{42}O$

N	Name	Formula	Rt	Mw	Area	Area%
6	Urs-12-en-28-al, 3-(acetyloxy)-, (3á)-	$C_{32}H_{50}O_3$	25.91	482	19100000	2.966

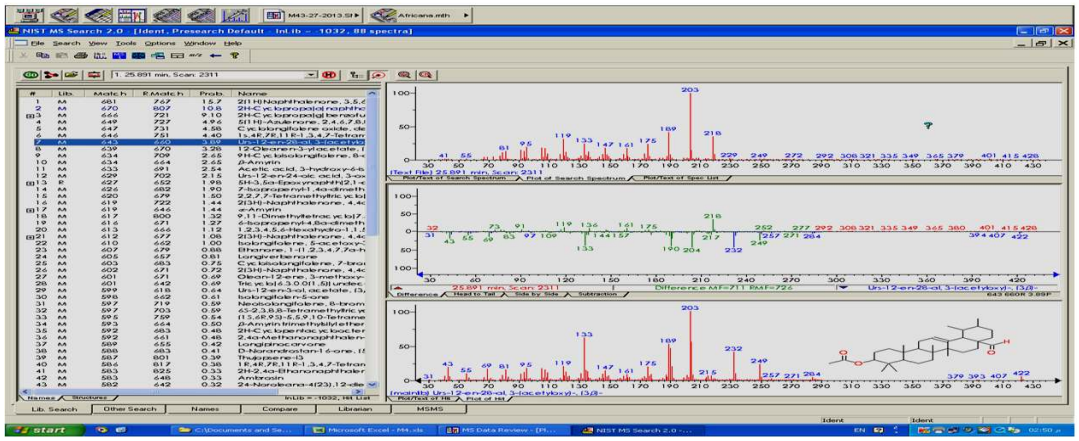

Fig. 11. Urs-12-en-28-al, 3-(acetyloxy) $C_{32}H_{50}O_3$

N	Name	Formula	Rate	Mw	Area	Area%
7	Betulin	$C_{30}H_{50}O_2$	26.41	442	57200000	8.882

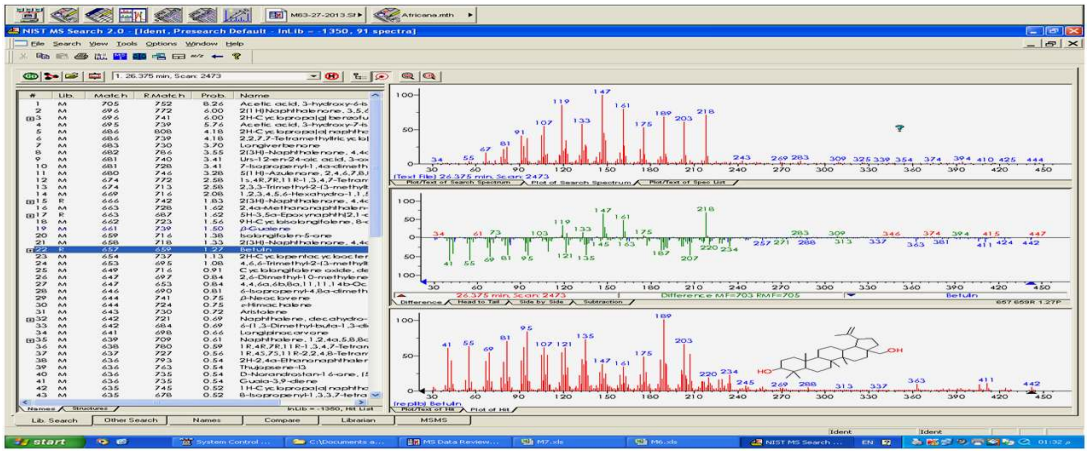

Fig. 12. Betulin $C_{30}H_{50}O_2$

N	Name	Formula	Rt	Mw	Area	Area%
8	9,19-Cyclolanost-24-en-3-ol, acetate, (3á)-	$C_{32}H_{52}O_2$	29.67	468	55170000	8.567

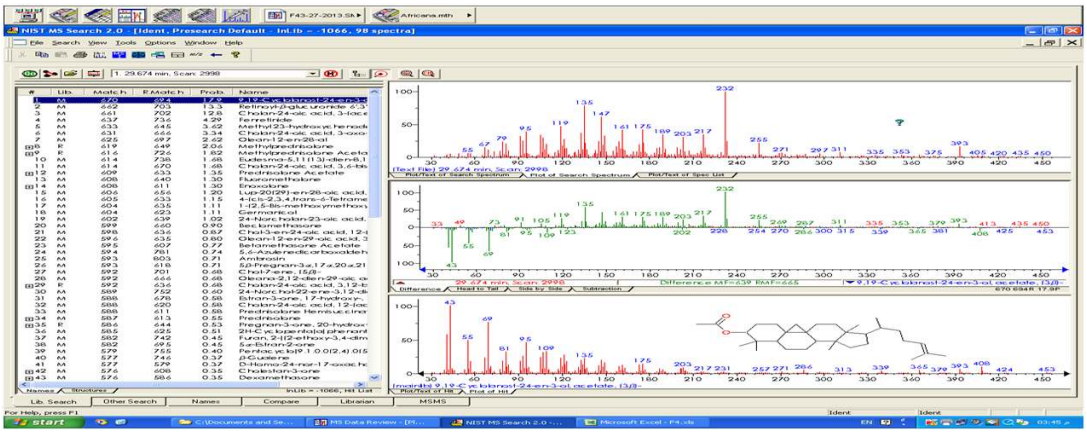

Fig. 13. 9,19-Cyclolanost-24-en-3-ol, acetate $C_{32}H_{52}O_2$

4.2.2 AF2 GCMS results

N.	Name	Formula	Rate	M.W	Area	Area%
1	Phthalic acid, heptyl isoporpyl ester	$C_{14}H_{18}O_4$	21.89	250	4054	100

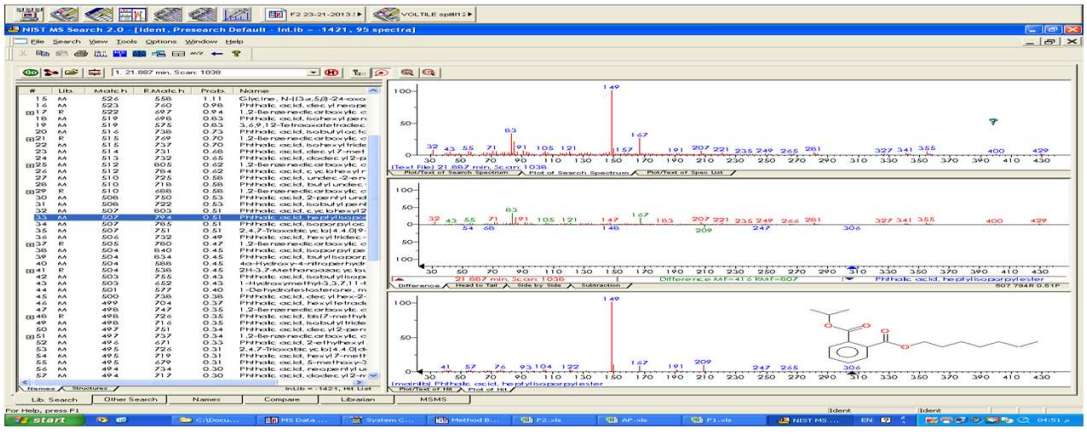

Fig. 14. Phthalic acid, heptyl isopropyl ester $C_{14}H_{18}O_4$

4.2.3 AF3 GCMS results

Phthalic acid, bis(2-pentyl ester) $C_{18}H_{26}O_4$ found in AF1 Fig. 4.

Urs-12-en-28-al, 3-acetyloxy $C_{32}H_{50}O_3$ also found in sample AF1 Fig. 11.

4.2.4 AF4 GCMS results

In this sample only Phthalic acid, bis(2-pentyl $C_{18}H_{26}O_4$ was found in AF1 Fig. 4.

$C_{19}H_{27}NO$ which found in the extract prepared by CH_2Cl_2 is found as pain reliever [12].

5. CONCLUSION

Sesquiterpene lactones were the main constituents of CH_2Cl_2 extract and triterpenoids were the main constituents of the isolated samples by column chromatography of *C.africana* resin ,generally we can suggest that *C.africana* resin possess compounds which can play important role in therapy , Betulin $C_{30}H_{50}O_2$ which found in sample AF (1) was indicated to have anti-tumour activity especially in combination with cholesterol [13], and Betulinic acid derivatives are highly selective inhibitors of HIV-1 [14], Pentazocine $C_{19}H_{27}NO$ which found in the extract prepared by CH_2Cl_2 is found as pain reliever [12].

The clear antiviral results of this resin towards NDV virus can lead to valuable work in antiviral drug discovery companies for this virus and other related viruses which threaten humans lives.

6. RECOMMENDATIONS

More declaration is needed about this resin using different methods of isolations and identifications, and also different samples of the resin from different climate areas. The procedure of medicinal plants researches must include chemical analysis and biological test to a definite plant in the way that generally used in traditional medicine beside extracting the active ingredient of the medicinal plant, this can help regulating using medicinal plants by public specially in developing countries, Medicinal plants and herbs researches need more than one researcher for more investigation to a certain plant, botanical, pharmacological chemical and biological researcher, and the research can be done in more than one country in the same time to compare between the different results This procedure can lead to useful work for different societies.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee of Veterinary Research Institute (VRI), Soba, Khartoum.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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