Materials and Methods

2.1 Materials

2.1.1 Plant material

Seeds of *Hibiscus asper*, *Merremia dissecta*, *Cucumis prophetarum*, *Citrullus lanatus* and *Cleome gynandra* were purchased from the local market – Khartoum – Sudan. The plant material were authenticated by direct comparison with a reference herbarium sample.

2.1.2 Gas chromatography-Mass spectrometry analysis

The GC-MS analysis was conducted on a Shimadzo GC-MSQP2010 Ultra instrument with a RTX-5MS column (30m,length ; 0.25mm diameter ; 0.25 μm, thickness)**.**

2.1.3 Test organisms

The studied oils were screened for antimicrobial activity using the standard microorganisms shown in Table(2.1).

Table 2.1 : Test organisms

Ser. No.	Micro organism	Type
1	Bacillus subtilis	G +ve
2	Staphylococcus aureus	G +ve
3	Pseudomonas aeruginosa	G -ve
4	Escherichia coli	G -ve
5	Candida albicans	Fungi

2.2 Methods

2.2.1 Extraction of oil

Powdered plant material (300g) was exhaustively macerated with n-hexane. The solvent was removed under reduced pressure to afford the oil.

The oil was esterified as follows: the oil (2 mL) was placed in a test tube and 7mL of alcoholic sulphuric acid. The tube was stoppered and shaken vigorously for five minutes and then left overnight. (2 mL) of supersaturated sodium chloride were added, then (2 mL) of normal hexane were added and the tube was vigorously shaken for five minutes. The hexane layer was then separated. (5 mL) of hexane extract were mixed with 5 mL diethyl ether. The solution was filtered and the filtrate (1 µL) was injected in the GC-MS vial.

2.2.2 Gas chromatography-Mass spectrometry analysis

The studied oils were analyzed by the hyphenated technique gas chromatography-mass spectrometry. A Shimadzo GC-MSQP2010 Ultra instrument with a RTX-5MS column (30m,length ; 0.25mm diameter ; 0.25 µm, thickness) was used. Helium(99% pure) was used as carrier gas. Oven temperature program and other chromatographic conditions are presented below:

- Oven temperature program Rate Temperature(^{o}C) Hold Time (min.-1)

Rate: ----------; Tempt. 150.0 ^{o}C ; Hold time (min.$^{-1}$) , 1.00

Rate: 4.00 ; Tempt. 300.0 ^{o}C ; Hold time (min.$^{-1}$) , 0.00

Column oven temperature	150.0 C^{0}
Injection temperature	300.0 C^{0}
Injection mode	Split
Flow control mode	Linear velocity
Pressure	139.3 KPa
Total flow	50.0 ml/min
Column flow	1.54 ml/sec
Linear velocity	47.2 cm/sec
Purge flow	3.0 ml/min
Split ratio	- 1.0

2.2.3 Antimicrobial assay

Mueller Hinton and Sabouraud dextrose agars were the media used as the growth media for the bacteria and fungus respectively. The media were prepared according to manufacture instructions.

Broth culture (5.0×10^{7} cfu/mL) were streaked on the surface of the solid medium contained in Petri dishes. Filter paper discs (Oxid, 6 mm) were

placed on the surface of the inoculated agar and then impregnated with 100 mg/mL of test sample. For bacteria the plates were incubated at 37^{o}C for 3 days. The assay was carried out duplicates and the diameters of inhibition zone were measured and averaged. Ampicillin, gentamycin and clotrimazole were used as positive control and DMSO as negative control.