

Quercetin and β -sitosterol from *Commiphora myrrha* Fum Extracts

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Abstract

The present study was carried out to investigate the antimicrobial activity of crude extracts of *Commiphora* gum. Methanol extract of *Commiphora myrrha* was *antifungal* to *Aspergillus niger*, whereas petroleum ether extract of *Commiphora myrrha* was strongly antibacterial against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and antifungal to *Aspergillus niger* and *Candida albicans*.

Pure quercetin and β -sitosterol were isolated from the fractions C1 and C2 of aqueous and petroleum ether extracts, respectively and screened in vitro for antimicrobial activity.

Key words: *Commiphora myrrha*, antimicrobial activity, *Quercetin*, β -sitosterol.

Introduction

Myrrha is a gummy substance exuded by shrubs growing in eastern Africa, eastern Mediterranean and Arabian Peninsula. In Sudan it is called Murra, the maceration is used locally for treatment of inflamed throat and tonsillitis. The volatile oil and mucilage in myrrha

demonstrate antimicrobial and anti-inflammatory activities. Myrrha oil is used in treatment of amenorrhea, athlete's foot, bronchitis, mouth sores and toothache (Hanrahan, 2001).

The essential oils of *Commiphora* species are rich in furanosesquiterpenoids, which have been found to possess anesthetic, antibacterial, antifungal, antihyperglycemic properties. Four furanosesquiterpenoids; 3-methoxy-4-furanogermacra-10(15)-dien-6-one, 2-methoxy-4-furanogermacra-1 (10)-en-6-one, furanogermacra-1(10)-4-dien-6-one and curzerenone (6,7-dihydro-5-isopropenyl-3, 6-dimethyl-6-vinyl benzofuran-4-one) were isolated from myrrha gum (Brieskorn and Nobel, 1980, 1981, 1983).

Chemical investigation of *Commiphora* has been done on some Kenyan species occurring also in Ethiopia include limonene, p-cymene, α -terpineol, β -bisabolene and furanoeudesma-1, 3-diene (Demissew, 1993; Blay *et al.* 1996).

Ubillas *et al.* (1999) isolated two antihyperglycemic compounds, furano -eudesma-1, 3-diene and 2-O-acetyl-8, 12-epoxygermacra-1(10), 4, 7, 11-tetraene, from the resin of *Commiphora myrrha* aqueous, ethanol and petroleum ether extracts.

Six furanosesquiterpenoids were isolated from Chinese sample of *Commiphora myrrha*, one of these compounds epoxy-furanogermacra-10(15)-en-6-one exhibited weak cytotoxic activities against a breast tumor cell in a clonogenic assay (Zhu *et al.*, 2001). Also, some aromatic sesquiterpenes were isolated from ethyl acetate extract of exudates of *Commiphora myrrha* collected from China, and identified as sesquiterpene myrrhone, epicurzerenone, furanogermacra-1E,

10(15)-dien-6-one, 2-methoxy-furanogermacra-1(10), 4-diene, T-cadinol, 3 α -hydroxy-Tcadinol, and eudesm-4(15)-ene-1 β ,6 α -diol (Zhu *et al.*, 2003).

Hili *et al.* (2003) tested fifty-one essential oils extracted from *Commiphora myrrha* for their antimicrobial activity against three bacteria, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and four yeasts, *Torulopsis utilis*, *Schizosaccharomyces pombe*, *Candida albicans* and *Saccharomyces cerevisiae* using the drop diffusion method. All showed antimicrobial activity against at least one of the micro-organisms. Also, the essential oil extract of *Commiphora myrrha* showed significant antimicrobial activity against *Escherichia coli*, *Proteus vulgaris*, *Shigella flexneri*, *Salmonella paratyphi B* and *Klebsiella pneumoniae* (Abdalla *et al.*, 2005).

Materials and Methods

Plant material

Commiphora myrrha gum was purchased from local market, Khartoum state. The samples were authenticated and a voucher was deposited at Herbarium of the Medicinal and Aromatic Plants Research Institute (MAPRI), Sudan.

Plant material Extraction and liquid fractionation

Powdered air-dried gum of *Commiphora myrrha* (500 g) was extracted by methanol and sonicated at room temperature for 1 hr, and the solution was filtered. The procedure was repeated two times and the filtrates were centrifuged. The solvent was removed under reduced

pressure. The dried extract was dissolved in 300 ml water and was partitioned with hexane (3x300 ml), chloroform (3x300 ml) and ethyl acetate (3x300 ml), respectively.

Phytochemical screening

Phytochemical tests were carried out on the methanolic extract of the gum of *Commiphora myrrha* using standard procedures of plant constituent's identification (Harborne, 1973; Sofowora *et al.*, 1982).

Determination of antimicrobial activity of the extract

The antimicrobial activity of crude extract was tested against *Bacillus subtilis* NCTC; *Staphylococcus aureus* ATCC; *Escherichia coli* ATCC; *Pseudomonas aeruginosa* ATCC; *Aspergillus niger* ATCC and *Candida albicans* ATCC using the agar diffusion method (Kavanagh, 1972; Collins *et al.*, 1995).

Column and thin layer chromatography

Aqueous extract of *Commiphora myrrha* gum was chromatographed on Diaion HP-20 column (2.5 x 40 cm) and eluted subsequently with 500 ml of H₂O, 25% MeOH, 50% MeOH, 75% MeOH, 100% MeOH and CH₃COCH₃ to give one active fraction C1 (100% MeOH). A fractionation and isolation were monitored by TLC with visualization under UV (λ_{\max} 254 and 365 nm), using the chloroform/ ethyl acetate solvent system. C2 crystallized from petroleum ether extract of *Commiphora myrrha* gum. Both C1, C2 were further tested for their antimicrobial activities. Further purification with preparative TLC using chloroform/ methanol solvent system was carried out for fractions C1 and C2 and resulted in compounds; quercetin and β -sitosterol.

NMR measurement

Each pure compound was dissolved in MeOD for ¹H NMR, in which the spectra was recorded on 500 MHz Bruker DMX 500 Spectrometer. Chemical shifts (δ) are given in ppm.

APCI Mass Spectrometry

A spectrum was recorded on Agilent LC-MS Spectrometer using probe positive-ion and phenomenex RP 18 (4.6 x 150 mm, 5 micron) column. The mass scan range was 100 – 800 m/z. The solvent system was MeOH: H₂O: Formic Acid (90:10:0.1) with flow rate of 1 ml/minute and injection volume of 10 μ L/minute. The mass Spectroscopy data was subjected to the library search using metabolmics query bank.

Results and Discussion

Commiphora myrrha gum was found to contain constituents such as coumarines, terpenes, tannins and flavonoids.

The methanol extract of the gum showed slight antibacterial and antifungal activity against *Bacillus subtilis* and *Aspergillus niger*. Out of the solvents used for liquid extraction, the water extract showed activity ranging between (15 - 20 mm) against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Aspergillus niger* and *Candida albicans*. The fraction C1 isolated from the water extract by Diaion HP-20 column has a remarkable antifungal activity against *Aspergillus niger*.

The petroleum ether extract exhibited high activity (17-20 mm) against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, and high antifungal activity (23-24 mm)

against the two fungi. The petroleum ether extract was more active to Gram negative bacteria rather than Gram positive one and has approximately similar activity against both *Aspergillus niger* and *Candida albicans*. Also, its active fraction C2 was potent against *Aspergillus niger* and *Candida albicans* (Table 1). The demonstration of the antibacterial and antifungal activity of the plant confirmed the findings of Dolara et al. (2000), Hili et al. (2003) and Abdalla (2005), who reported the highest activity of Commiphora myrrha essential oil against Escherichia coli.

Table 1. Antimicrobial activity of Commiphora myrrha gum extracts against standard organisms

Type of Extract	Standard organisms used*/ (MDIZ) mm					
	<i>B.s</i>	<i>S.a</i>	<i>E.c</i>	<i>Ps.a</i>	<i>As.n</i>	<i>C.alb</i>
Petroleum ether	17	19	20	19	23	24
Methanol	14	12	-	13	16	12
Water	18	15	-	17	20	16
Fraction C1	21	17	-	19	28	19
Fraction C2	11	12	14	11	30	21

* *B.s* = *Bacillus subtilis*, *S.a* = *Staphylococcus aureus*, *E.c* = *Escherichia coli*, *Ps.a* = *Pseudomonas aeruginosa*, *As.n* *Aspergillus niger*, *C.alb* = *Candida albicans*, MDIZ = Mean diameter of inhibition zone (mm). Conc. used 100 mg/ml at 0.1 ml/ cup.

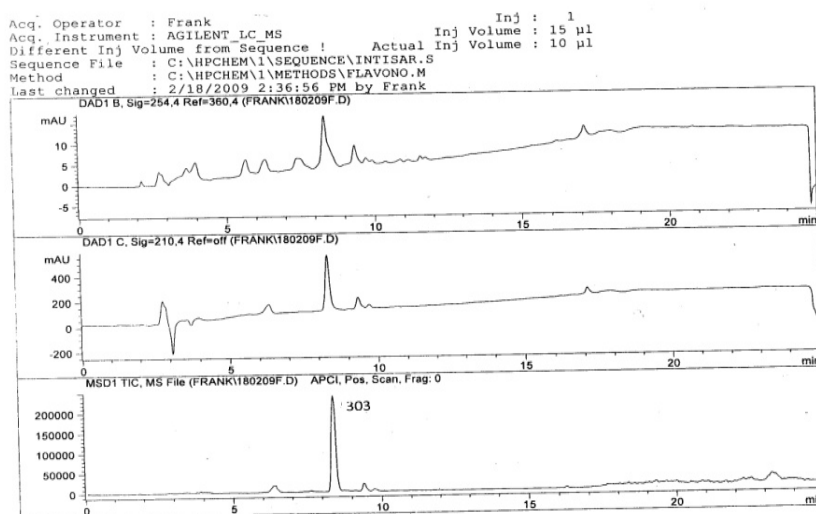
Interpretation of result: MDIZ >15 Sensitive, 14 – 15 Intermediate <14 Resistant, - No inhibition zone.

Structures elucidation

Compound C1 obtained as yellow crystals (melting point 305-307°C, lit 310-317°C) from the active fraction of water extract was supposed to contain polysaccharides and proteins. Therefore, the extract was

subjected to Diaion HP-20 resin which has been known to remove sugars and ascorbic acid from poly phenolics in aqueous plant extracts and purifying the polyphenols (Einbond *et al.*, 2004).

The APCI-MS spectra of this compound (Fig 1) provide a molecular ion peak $[M+H]^+$ at m/z 303, corresponding to quercetin molecular weight 302.24 and in agreement with it is molecular formula $C_{15}H_{10}O_7$ (Fang *et al.*, 2002; Prasain *et al.*, 2004).



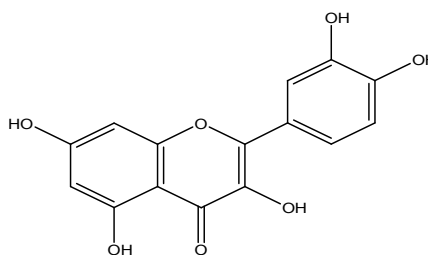
The characteristics of flavone 1H NMR spectra were applied to **C1** and it showed a similar assignment of ring A and B between δ 6.0-7.0 ppm attributed to the protons adjacent to the hydroxy in benzene ring, and the signals above δ 7.0 ppm which are attributed to the protons with meta position to hydroxy in benzene ring B (Table 2).

Therefore, it is identified as flavonol according to its spectral data which is consistent with the literature values (Eldahshan *et al.*, 2008).

The result was quite acceptable considering that quercetin and some of its derivatives quercetin-3-O- α -L-arabinoside, quercetin-3-O- β -D-galactoside, quercetin-3-O- α -L-rhamnoside and quercetin-3-O- β -D-glucuronide were the major flavonoid components of the flowers of *Commiphora mukul* (Hanuš *et al.*, 2005).

Table 2 ^1H (500 MHz) Chemical shift of quercetin in MeOD(ppm)

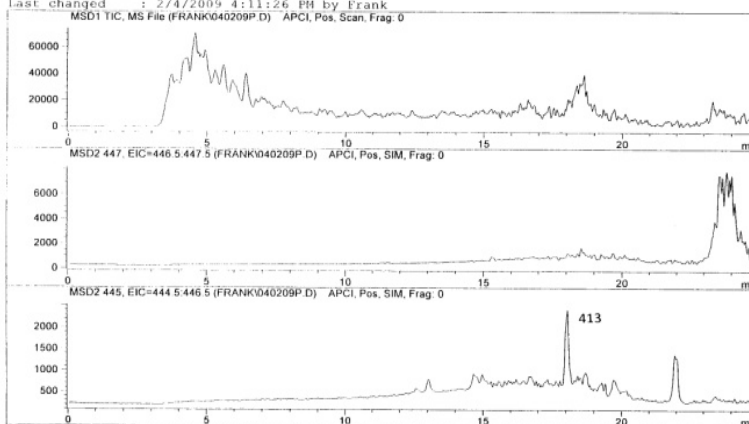
Position	Chemical shift
6	6.17(1H,d)
8	6.45(1H,d)
2'	7.89 (1H,d)
5'	6.80(1H,d)
6`	6.98(1H,s)



Quercetin

Compound C2 isolated from Petroleum ether extract as yellow needle crystals, melts at 136-137(lit 137-138) (Zhu *et al.*, 2003). APCI-MS spectra (Fig 2) showed molecular ion peak $[\text{M}+\text{H}]^+$ at m/z 413, which resemble the molecular weight of β -sitosterol and its molecular formula $\text{C}_{29}\text{H}_{49}\text{O}$ (Arora *et al.*, 1971).

Acq. Operator : Frank Inj : 1
Acq. Instrument : AGILENT LC MS Inj Volume : 15 µl
Different Inj Volume from Sequence : Actual Inj Volume : 5 µl
Acq. Method : C:\MSDCHEM\1\METHODS\FLAVONO.M
Last changed : 2/4/2009 4:13:17 PM by Frank
Analysis Method : C:\MSDCHEM\1\METHODS\HORMONES.M
Last changed : 2/4/2009 4:11:26 PM by Frank

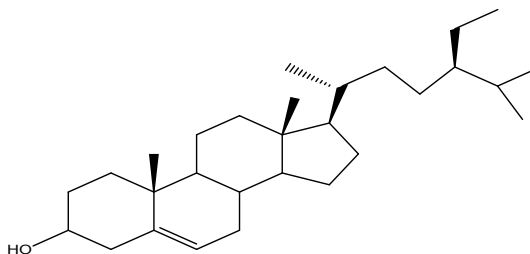


The ^1H NMR spectrum indicated the presence of six methyl groups, an olefinic proton H-6 signal at δ 5.50 (1H, brs) and β -8 proton at δ 3.31 (1H, dt) which were typical signals of an oleanene skeleton (Kriwacki *et al.*, 1989; Gallo *et al.*, 2006) and it was identified as steroid (Table 3).

This result was supported by Amjad *et al.* (1967), who isolated β -sitosterol and myricyl alcohol as crystalline compounds from the unsaponifiable portion of the ether-soluble residue of *Commiphora mukul* and also by findings of Hanuš *et al.* (2005), who isolated steroid from petroleum ether extract of *Commiphora mukul* as crystalline needles.

**Table 3 ¹H (500 MHz) Chemical shift of β -sitosterol
in MeOD(ppm)**

Position	Chemical shift
3	3.55(1H,s)
6	5.10(1H,brs)
8	3.39(1H,dt)
CH ₃	0.81-1.29(1H,m)



β -sitosterol

Conculusion

It can be concluded that methanol extract of *Commiphora myrrha* was antifungal to *Aspergillus niger*, whereas petroleum ether extract was strongly antibacterial against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and antifungal to *Aspergillus niger* and *Candida albicans*.

Quercetin and β -sitosterol were isolated from the aqueous and petroleum ether extracts, respectively.

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