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# Extraction, Isolation and Characterization of *Maerua oblongifolia* ('Sanagana')

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**ABSTRACT**

*Maerua oblongifolia* was among other medicinal plants those were extensively used medicinal plants for treatment of human and domestic animals in addition to serving as detergent for washing purpose.The present study was developed to contribute to the documentation of the pharmacological and biological activity testing through extraction, isolation, and characterization of crude extracts of leaves of most popularly used traditional medicinal plant, *Maerua oblongifolia*. Air dried leaf powder of *Maerua oblongifolia* was exhaustively extracted with methanol at room temperature. Fractionation of the column elution started with 30 ml of pure diethyl ether, followed by increasing polarity of solvent system, diethyl ether, ethylacetate, ethanol, methanol, water, 32 fractions were collected. This study has resulted in the isolation and characterization of MOM-19**.**Characterization was done by spectroscopic technique, Nuclear Magnetic Resonance (NMR).The $^{13}C$ NMR spectrum of the compound showed well resolved resonance of 38 carbon atoms. The number of carbon atom indicated that it was a triterpene. The result of this study was important as the search for new pharmacologically active compounds from plant extracts that may led to the discovery of many clinically useful drugs.

## Introduction

### Background of the study

Traditional medicine is referred to as the system of medicine with diagnostic and treatment mechanisms and roles of specialists, where in the dominant model of disease and health significantly differs from the scientific medicine (Zerihun Doda, 2005). Traditional medicine is cheaper and more accessible to most of the rural population and could become the potential source of the new drugs. It was reported that 60-85 % of the population in every country of the developing world has to rely on traditional or indigenous forms of medicine (Sofowora, 1982). About half of the word's medicinal compounds are still derived or obtained from plants (Humann, 1991, cited in Frankel et al.,

1995). Many of the most important drugs of recent times were first isolated from plants (Frankel et al., 1995; Dawit Abebe et al., 2003). The therapeutic value of any plant thus lies in the quality and quantity of the secondary metabolites or active principles (Dawit Abebe et al., 2003). Many people have practiced the use of plants universally for religious ceremonies, as specific against magic and for the treatment of various diseases for many years (Kokwaro, 1979; Abbink, 1995; Efrem et al., 2004; Mathewos Agize et al., 2013). This knowledge involves collection of raw materials, preparation of remedies, traditional diagnosis, and its prescription to the patients.

One plant may treat more than one condition. The treating action of a given plant may vary from others on the basis of the site of action, dosage of the constituent, and the different constituents it may embody (Dawit Abebe et al., 2003). Many problems can be identified in relation to the use of medicinal plants in Ethiopia. Over-dosage and lack of adequate knowledge (Kokwaro, 1979), beliefs (Kebu Balemie et al., 2004), over-harvesting, destruction and conversion of their habitats to other purposes (Frankel et al., 1995; Tizazu Gebre, 2005) and keeping the knowledge secrete and confidential (Kokwaro, 1979; Abbik, 1995; Afework Kassu, 2004; Kebu Balemie et al., 2004).

Most traditional healers practice using the same plant species for treating different diseases of human and the domestic animals. For example, according to Mathewos Agize et al. (2013), it was found that *Maerua oblongifolia* used to treat more than 12 different types of human and domestic animals in addition to serving as soap in the study area.

## Description of the plant

*Maerua oblongifolia* (Forssk.) A. Rich. [Family: Capparaceae] is a low woody bushy under-shrub, sometimes scan dent, to 2–3 m high, with thick rootstock and thick leaves, flowers strongly scented, occurring in savanna woodland from Senegal to N Nigeria and in Sudan to the Red Sea, India, Pakistan, Africa and Saudi Arabia. The plant survives annual burning by throwing up shoots from its thick rootstock.

*M. oblongifolia* is also found in Ethiopia in different dry and sub humid areas. It is a woody twining straggler having elliptic-obtuse leaves with a mucro at the apex. The flowers are greenish-yellow, in axillary and terminal corymbs and the fruit is a moniliform berry. It is distributed in the dry forests of the riverin of Gojeb and Omo rivers of Dawuro Zone and is locally known as 'Sangana'. According to Mathewos Agize et al. (2013),it is among other medicinal plants those were extensively used medicinal plants for treatment of human and domestic animals in addition to serving as detergent/ as soap for washing purpose in Loma and GenaBosa Districts of Dawuro Zone.

According to the same authors, it is woody shrub whose bark, leaf, root and whole parts were pound; chewed; dry bath and taken either with others or alone in the form of drink; smoke (except for pregnant women) through orally, nasally or anally to treat Evil eye; Anthrax, stomachache, severe abdominal cramp; hook worm, body swelling; mamp, tetanus, eye disease; liver cirrhosis; gonorrhea; For different disease; meningitis for both human and cattle. For immediate access and for the purpose, it is kept either in the house or in the pocket (Mathewos Agize et al., 2013). It was also reported (Mathewos Agize et al., 2013) that about 99.11% of traditional healers were collected it from wild that means only one informant (traditional healer) out of 112 traditional healers, was seen cultivating *Maerua oblongifolia* in the home garden. This indicated that how far the species was threatened.

## Statement of the problem

The more the multiple uses for local people, the more conservation of that plant resource through cultivation and protection in and around home gardens. However, the size of home garden, the agroecology and the type of soil it needs for its growth and individual needs determine the number

and type of each species grown in the home gardens. Plant species with many uses were observed scarcely distributed in nearby forests of the study area. For example, *Maerua oblongifolia* and *Lannea fruticosa* were more threatened because of their scarcely growing in limited places (only in lowland riverine vegetation) and being used as medicine intensively (especially their bark and roots respectively) by local people in addition to their multipurpose value (Mathewos Agize et al., 2013). It was suggested that eestablishing a field gene bank for some medicinal plants like *Maerua oblongifolia* and for other multipurpose plants (Mathewos Agize et al., 2013) because almost all of the traditional healers for medicinal purpose and community for washing purpose/to use as detergent use its root frequently. Hence, it was critically endangered.

*Maerua oblongifolia is* as yet no documented report on this plant's content and other issues that the community frequently and extensively using for treatment of various diseases, to treat more than 12 different types of diseases in the thematic area, in the study area (Mathewos Agize et al., 2013). To preserve the indigenous knowledge of this useful plant in general and the medicinal plant itself in particular, the extraction of active principles, characterization, isolation and preparing conditions for further drug manufacturing is very important/crucial. Thus, the present study is developed to contribute to the documentation of the pharmacological and biological activity testing of most popularly used traditional medicinal plant, *Maerua oblongifolia* that is considered as backbone of the health life for both human and the domestic animals. That may further contribute positive conservation effects and benefit the community.

## Objectives

### General objective

To extract, isolate, and characterize the constituents of biologically active crude extracts of leaves of *Maerua oblongifolia*.

### Specific objectives

- To extract the leaf parts of the plant
- To isolate possible active ingredients from the leaves
- To characterize the isolated compounds by spectroscopic, NMR.

## Materials and methods

### Plant material collection

Fresh roots, and the aerial parts of the plant leaves were collected from the vicinity of riverine dry forests around Gojeb and Omo Rivers. Botanical identification of the plant was carried out using flora of Ethiopia at the herbarium of Ethiopia at Addis Ababa University.

### Physico-chemical studies/ preparation of the extracts

Plant parts collected were ground in to powder by electrical grinding mill and in mortar and Pestle. Powdered plant materials were carefully sieved, collected in plastic bags, and refrigerated at (70°C) until extraction. The air dried ground plant was extracted with methanol as solvent at room temperature in Erlenmeyer flasks. After shaking well, flasks containing the solution were put on orbital shaker and left for 24 hours at speed of 120 revolutions per minute. After 24 hours a solution was filtered by using 15 cm size Whatmann filter paper. The filtrate is then dried by using rotary evaporator at temperature of about 40-45°C. The dried extract was purified by TLC and finally collected in labeled sterile small bottles and put in deep freezer unitl needed for the experiment.

### Characterization

The characterization was done by spectroscopic techniques like Nuclear Magnetic Resonance (NMR). TLC analysis was carried out on 0.2mm thickness TLC plates of Merck silica gel 60 $F_{254}$ coated on aluminium plate. Compounds on TLC were detected using UV light: while column

chromatography was carried using silicagel, 60(mesh).

## Coding system

In the coding system used for compounds, M stands for the Genus name *Maerua*, O stands for the species name *oblongifolia*, M stands for Methanol extract, the number behind MOM indicates the fraction number of fractionation in which it is obtained in the increasing polarity of the solvent system.

## Fractionation of crude extract

The column was packed with 60g silicagel. The crude methanol extract of 7 g was adsorbed on silicagel with 25 ml of petroleum ether. The adsorbed sample was then applied to the top of packed silcagel in column chromatography using spatula. The column elution started with 30 ml of pure diethyl ether, followed by increasing polarity of solvent system, diethyl ether, ethylacetate, ethanol, methanol, water, 32 fractions were collected each 30ml as follows (Table 1).

**Table 1.** Solvent systems and fractions collected from crude extract.

| Fraction | Solvent system | Ratio | Volume (ml) relation | Remark |
|---|---|---|---|---|
| 1 | $C_2H_5OC_2H_5$ | 100% | 30 | |
| 2 | $C_2H_5OC_2H_5$:EtOAC | 9:1 | 27:3 | |
| 3 | $C_2H_5OC_2H_5$:EtOAC | 8:2 | 24:6 | |
| 4 | $C_2H_5OC_2H_5$:EtOAC | 7:3 | 21:9 | |
| 5 | $C_2H_5OC_2H_5$:EtOAC | 6:4 | 18:12 | |
| 6 | $C_2H_5OC_2H_5$:EtOAC | 5:5 | 15:15 | |
| 7 | $C_2H_5OC_2H_5$:EtOAC | 4:6 | 12:18 | |
| 8 | $C_2H_5OC_2H_5$:EtOAC | 3:7 | 9:21 | |
| 9 | $C_2H_5OC_2H_5$:EtOAC | 2:8 | 6:24 | |
| 10 | $C_2H_5OC_2H_5$:EtOAC | 1:9 | 3:27 | |
| 11 | EtOAC | 100% | 30 | |
| 12 | EtOAC : $C_2H_5OH$ | 9:1 | 27:3 | |
| 13 | EtOAC : $C_2H_5OH$ | 8:2 | 24:6 | |
| 14 | EtOAC : $C_2H_5OH$ | 7:3 | 21:9 | |
| 15 | EtOAC : $C_2H_5OH$ | 6:4 | 18:12 | |
| 16 | EtOAC : $C_2H_5OH$ | 5:5 | 15:15 | |
| 17 | EtOAC : $C_2H_5OH$ | 4:6 | 12:18 | |
| 18 | EtOAC : $C_2H_5OH$ | 3:7 | 9:21 | MOM-18 |
| 19 | EtOAC : $C_2H_5OH$ | 2:8 | 6:24 | MOM-19 |
| 20 | EtOAC : $C_2H_5OH$ | 1:9 | 3:27 | |
| 21 | $C_2H_5OH$ | 100% | 30 | |
| 22 | $C_2H_5OH$: $CH_3OH$ | 9:1 | 27:3 | |
| 23 | $C_2H_5OH$:$CH_3OH$ | 8:2 | 24:6 | |
| 24 | $C_2H_5OH$: $CH_3OH$ | 7:3 | 21:9 | |
| 25 | $C_2H_5OH$: $CH_3OH$ | 6:4 | 18:12 | MOM-25 |
| 26 | $C_2H_5OH$: $CH_3OH$ | 5:5 | 15:15 | |
| 27 | $C_2H_5OH$: $CH_3OH$ | 4:6 | 12:18 | |
| 28 | $C_2H_5OH$: $CH_3OH$ | 3:7 | 9:21 | MOM-28 |
| 29 | $C_2H_5OH$:$CH_3OH$ | 2:8 | 6:24 | |
| 30 | $C_2H_5OH$: $CH_3OH$ | 1:9 | 3:27 | |
| 31 | $CH_3OH$ | 100% | 30 | MOM-31 |
| 32 | $CH_3OH$:$H_2O$ | 9:1 | 27:3 | |

The 32 fractions each checked for the presence of color on TLC. From the fractions, first six fractions were colorless and showed no spot on TLC of different solvent system. The rest are colored.Fr-18 obtained with a solvent system EtOAC: $C_2H_5OH$ in the ratio (9:21), Fr-19 $C_2H_5OH$: $CH_3OH$ (6:24), Fr-25 $C_2H_5OH$: $CH_3OH$ (18:12), Fr-28 $C_2H_5OH$: $CH_3OH$ (9:21), Fr-31 $CH_3OH$ (100%) were colored. From these fractions, Fr-18, Fr-19, Fr-25 and Fr-31 were taken to spectroscopic analysis due to their amount and level of purity. From the spectral data Fr-19 has clear, and visible spectra that leads to further characterization. Due to the absence of characteristic colored spot on TLC of other fractions Fr-19 is selected for characterization

## Results and discussion

Firstly, we extracted air dried leaf powder of *Maerua oblongifolia* with methanol at room temperature. Phytochemical investigation of the plant crude extracts showed the presence of different compounds. This is checked by subjecting to column chromatography on silica gel while 32 fractions were collected with increasing polarity of solvent system and y checking with TLC. This study has resulted in the isolation and characterization of MOM-19.

## Spectral data

Compound MOM19 Yellowish solid, $^{13}$CNMR12.09, 14.12,14.27, 18/18, 19.74, 20.53, 21.50, 22.68, 23.04, 24.73, 25.51, 25.60, 27.19, 27.96, 29.08, 29.14, 29.25, 29.35, 29.44, 29.59, 29.68, 30.91, 31.20, 31.91, 32.76, 34.02, 37.27, 39.35, 58.29, 127.10, 127.72, 128.23, 128.26, 129.73, 130.01, 130.24, 131.94 $^1$H NMR (400 MHZ, $CDCl_3$) δ in ppm, 1.0, 1.3, 1.6,2.3, 5.5.

## Characterization of compound MOM-19

The compound MOM-19 is a yellow crystalline solid obtained from Methanol extract. From TLC plate it shows a yellow color, which is a characteristic of terpenes. Its characterization was determined using spectroscopic techniques. This compound has Rf value 0.69 using EtOAC: $C_2H_5OH$ (8:2) as a solvent system.

$^1$H NMR (400MHZ: $CDCl_3$) spectrum showed a multiplate peak at δ 5.5 is for proton of CH group bonded with hydroxyl group and methine group. A singlet peak at δ 2.3 indicates hydrogen bonded with C=C. The triplet peak at 1.6 indicates hydrogen bonded with $CH_2$=$CH_2$The triplet peak at 1.3 indicates the $CH_2$=$CH_2$ group. The multiplet peak at 1.0 indicates the $CH_2$ group that is bonded with $CH_3$ and $CH_2$ (Table 2). The $^{13}$C NMR spectrum of the compound showed well resolved resonance of 38 carbon atoms (Table 3).

**Table 2.** $^1$H NMR data of compound MOM-19.

| δ ( ppm) | Multiplicity |
|---|---|
| 1.0 | M |
| 1.3 | T |
| 1.6 | T |
| 2.3 | S |
| 5.5 | M |

**Table 3.** $^{13}$C NMR data of compound MOM-19.

| Carbon No. | $^{13}$C NMR data, δ ppm | DEPT-135 spectra | Type of carbon |
|---|---|---|---|
| 1 | 178.91 | | Quaternary |
| 2 | 131.94 | CH | Methine |
| 3 | 130.24 | CH | Methine |
| 4 | 130.01 | | Quaternary |
| 5 | 129.73 | CH | Methine |
| 6 | 128.26 | | Quaternary |
| 7 | 128.23 | CH | Methine |
| 8 | 127.72 | CH | Methine |
| 9 | 127.10 | | Quaternary |
| 10 | 58.29 | CH | Methine |
| 11 | 39.35 | $CH_2$ | Methylene |
| 12 | 37.27 | $CH_2$ | Methylene |
| 13 | 34.02 | $CH_2$ | Methylene |
| 14 | 32.76 | $CH_2$ | Methylene |
| 15 | 31.91 | $CH_2$ | Methylene |
| 16 | 31.20 | $CH_2$ | Methylene |
| 17 | 30.91 | $CH_2$ | Methylene |
| 18 | 29.68 | | Quaternary |
| 19 | 29.59 | $CH_3$ | Methyl |
| 20 | 29.44 | | Quaternary |
| 21 | 29.35 | $CH_3$ | Methyl |
| 22 | 29.25 | | Quaternary |

| Carbon No. | $^{13}$C NMR data, δ ppm | DEPT-135 spectra | Type of carbon |
|---|---|---|---|
| 23 | 29.14 | $CH_2$ | Methylene |
| 24 | 29.08 | $CH_2$ | Methylene |
| 25 | 27.96 | $CH_2$ | Methylene |
| 26 | 27.19 | $CH_2$ | Methylene |
| 27 | 25.60 | $CH_2$ | Methylene |
| 28 | 25.51 | $CH_2$ | Methylene |
| 29 | 24.73 | $CH_3$ | Methyl |
| 30 | 23.04 | $CH_3$ | Methyl |
| 31 | 22.68 | $CH_3$ | Methyl |
| 32 | 21.50 | $CH_3$ | Methyl |
| 33 | 20.53 | $CH_3$ | Methyl |
| 34 | 19.74 | $CH_3$ | Methyl |
| 35 | 18.18 | $CH_3$ | Methyl |
| 36 | 14.27 | $CH_3$ | Methyl |
| 37 | 14.12 | $CH_3$ | Methyl |
| 38 | 12.09 | $CH_3$ | Methyl |

The number of carbon atom indicates that it is a triterpene.

## Conclusion

Here, the leaves extracts of *Maerua oblongifolia* has been analyzed. Conclusions drawn are based on several different analyses as described in our paper. The present study is important as the search for new pharmacologically active compounds from plant extracts has led to the discovery of many clinically useful drugs. Nonetheless, the efficacy of this plant extracts needs to be validated in vivo. Importantly, as these extracts contain many compounds along with the active compounds. Hence future directions should be focused on the isolation and identification of active compounds with antimicrobial activity rather than simply screening the plant crude extracts.

## Conflict of interest statement

Authors declare that they have no conflict of interest.

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