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Research Article

Characterization of some compounds isolated from Sweet basil (*Ocimum basilicum* L.) leaf extract

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ABSTRACT

Background: For thousands of years, several people depend on traditional medicines from flowers, bark, leaves and fruits of plants. Sweet Basil (*Ocimum basilicum L.*) is a medicinal herb which belongs to the family of Labiatae and it is the most abundant of the genus *Ocimum.* The present study aimed to isolate pure compounds from chloroform/methanol (1:1) crude extract of the leaves of *O. basilicum* herb and characterize them using infra-red (IR) and nuclear magnetic resonance (NMR) spectroscopic techniques.
Methods: The sample of powdered leaves of *O. basilicum* was extracted using a mixture of chloroform and methanol (1:1) and the crude extract was investigated for its chemical composition using spectroscopic techniques.
Results: Only one fraction designated as *O. basilicum* -14 (OB-14) displayed a single spot on TLC plate. Results showed that two isomeric compounds named *(1-(2-vinylcyclohexa-1,4-dienyl) propan-2-ol and 2-(2-vinylcyclohexa-1,5-dienyl) propan-1-ol)* were isolated as a mixtures using column chromatography over silica gel. The structures of these compounds were identified using IR and one dimensional NMR spectroscopic techniques such as proton NMR (^{1}H NMR), carbon-13 NMR (^{13}C NMR) and distortionless enhancement by polarization transfer (DEPT).
Conclusions: So, more compounds can be isolated from the plant using different chromatographic techniques.

Keywords: Crude extract, Isomeric compounds, *Ocimum basilicum*, Spectroscopic techniques

INTRODUCTION

Plants comprise the largest component of the diverse therapeutic elements of traditional health care practices both in human and animal. Nearly all cultures and civilizations from ancient times to the present day have used herbal medicines which are medicinal sources to cure infections.[1]

Medicinal plants have many advantages to the health of individual and the communities. The medicinal advantages of some plants fall in the presence of some chemical compounds that produce fixed physiological actions in the human body. Some of these biologically active ingredients are tannins, flavonoids, alkaloids and phenolic compounds.[2]

Bioactive ingredients of medicinal plants can be identified through extraction and characterization techniques. The mode of action of the plant extracts producing the therapeutic effect can also be better investigated, if the active ingredients are characterized and understood which have led to a continuous search for potent antimicrobial agents. Natural products are believed to play vital roles in the physiology and ecology of the plants that produce them, particularly as defense elements against pests and pathogens or as attractants for beneficial organisms such as insect pollinators. Because of their

biological activities, some plant natural products have been exploited by human beings as pharmaceuticals, stimulants, and poisons.[3]

Natural products are organic compounds that are formed by living systems. The elucidation of their structures and their chemistry, synthesis and biosynthesis are major areas of organic chemistry. The biologically active constituents of medicinal, commercial and poisonous plants have been studied throughout the development of organic chemistry. Many of these compounds are secondary metabolites.[4]

It has been estimated that over 50% of medicines have their origins in these natural products. A number of screening programmes for bioactive compounds exist and have led to new drugs, for example taxol, which is used for the treatment of various cancers. Natural products often have an ecological role in regulating the interactions between plants, microorganisms, insects and animals. They can be defensive substances, antifeedants, attractants and pheromones.[5]

Medicinal plants based drugs owe the advantage of being simple, effective and exhibit broad spectral activity. The revival of interest in the use and importance of African medicinal plants by WHO and many developing countries has led to intensified efforts on the documentation of ethno medical data of medicinal efforts. This is because most traditional healers keep no records and their information is passed on mainly verbally from generation to generation. Researchers are increasingly turning their attention to natural products looking for new leads to develop better drugs against cancer, as well as viral and microbial infections.[6]

The Labiatae is a large family of aromatic herbs and shrubs having flowers resembling the lips of a mouth and four-lobed ovaries yielding four one-seeded nuts lets and including mints. The mints, taxonomically known as Lamiaceae or Labiatae, are a family of flowering plants. They have traditionally been considered closely related to Verbenaceae.[6] But in the 1990s, phylogenetic studies suggested that many genera classified in Verbenaceae belong instead in Lamiaceae. The currently accepted version of Verbenaceae may not be more closely related to Lamiaceae than some of the other families in the order Lamiales. It is not yet known which of the families in Lamiales is closest to Lamiaceae.[7]

Sweet Basil (*Ocimum basilicum L.*) belongs to the family of Labiatae and it is the most abundant of the genus *Ocimum.*[8] Basil has shown antioxidant and antimicrobial activities due to its phenolic and aromatic compounds The main phenolics reported in basil are phenolic acids and flavonol- glycosides.[9]

Based on the international code of botanical nomenclature, the present taxonomic position of sweet basil is as follows: Domain: Eukaryota, Kingdom: Plantae, Phylum: Tracheophyta, Class: Magnoliopsida, Order: Lamiales, Family: Labiatae, Genus: *Ocimum* and Botanical name: *Ocimum basilicum L.*[10]

There are many varieties of *O. basilicum,* as well as several related species or species hybrids also called basil. The type used in Italian food is typically called sweet basil, as opposed to Thai basil (*O. basilicum* var. *thyrsiflora*), lemon basil (*O.* × *citriodorum*) and holy basil (*O. tenuiflorum*), which are used in Asia. While most common varieties of basil are treated as annuals, some are perennial in warm, tropical climates, including holy basil and a cultivar known as 'African Blue'. Basil is originally native to India and other tropical regions of Asia, having been cultivated there for more than 5,000 years.[11]

The basil comes in many different varieties, each with its own unique chemical composition and characteristic flavor. The main use of the herb is culinary. The flavour and character of any particular variety of basil is affected to a great extent by many external environmental factors, including factors such as temperature, the type of soil, the geographic location, and even the amount of rainfall received by the individual plant. Basil extract has various chemical compounds that include α-pinene, camphene, β-pinene, myrcene, limonene, cis-ocimene, camphor, linalool, methyl chavicol, γ-terpineol, citronellol, geraniol, methyl cinnamate and eugenol and other terpenes.[12]

Methyl eugenol [1,2-dimethoxy-4-(2-propenyl) benzene, found in sweet basil is a member of a family of chemicals known as allyl alkoxy-benzenes, which include other naturally occurring materials such as isoeugenol, eugenol, estragole, and safrole. All these compounds typically enter the diet via a variety of different food sources, including spices (nutmeg, all spices), herbs (basil, tarragon), bananas and oranges. Many of these compounds are also found as components of natural oils used in perfume.[13-15]

3,7-Dimethyl-1,5-octadien-3,7-diol is a volatile secondary alcohol found in wine and produced during fermentation and was also reported as a constituent of sweet basil.[16]

Linalool is also one of the major components of sweet basil. It is a naturally occurring terpene alcohol chemical found in many flowers and spice plants.[17]

So, the main objective of this research was to isolate pure compounds from chloroform/methanol (1:1) crude extract of the leaves of sweet basil herb and characterize them

using infra-red (IR) spectroscopy and nuclear magnetic resonance (NMR).

MATERIALS AND METHODS

Materials, apparatus and chemicals

The apparatus and instruments used for the study were: column, separating funnel, thin layer chromatography (TLC) plate, filter paper (Whattman No.1 filter paper), electric blender, pipettes (different size), water bath, UV lamp, beakers (different size), electronic balance, conical flask, flasks (different size), measuring cylinder, Rota vapor, chromatographic chamber, polyethylene bag, ruler, pencil, spatula, shaker, refrigerator, holder, fourier transform infra-red (FTIR) spectrometer, nuclear magnetic resonance (NMR) spectrometer.

Chemicals used were distilled water, silica gel (60-120 mesh, ASTM Germany), solvents (chloroform, n-hexane, ethyl acetate and methanol which were with analytical grades), iodine vapor.

Plant material

O. basilicum leaflets were collected in 2014. The identification of the plant was done at the Herbarium of the department of plant science, Haramaya University, Ethiopia. After collection the leaves were washed repeatedly first with tap water and then with distilled water and air dried completely for 3 days at room temperature.

Isolation of the chemical components of the leaflet extracts

The sample of powdered leaves (100 g) was first soaked with 300 ml of chloroform and methanol (1:1) for 24 hours with continuous stirring at room temperature and this procedure was repeated twice (2x). Then the extracts were filtered using Whatman No. 1 filter paper. Finally, the filtrates collected as such were concentrated at 40°C by rota vapor under reduced pressure, and the crude extract was stored in a refrigerator at 4°C for further analysis.

A glass column 18 inches in height and 1.0 inch internal diameter was used for the chromatography of the crude extracts. About 5 g of the crude extract was chromatographed in a column using 40 g of silica gel (60-120 mesh) as stationary phase. The extract was eluted with increasing polarity of n-hexane, chloroform and methanol mixtures successively using them as eluents (10:0, 9:1, 8:2, 7:3 up to 0:10) to choose the appropriate solvent for the complete resolution of the spots.

Thirty milliliter fractions were collected. All fractions were monitored by TLC. Fractions with spots of the same retention factor (*Rf)* values were combined and rechromatographed in appropriate solvent systems until pure isolates were obtained. Spots on the TLC plate were visualized using Ultra Violet (UV) lamp (254 nm and 365 nm) with some amount of iodine vapor. A glass column 12 inches in height and 0.5 inch internal diameter was used for the rechromatography of smaller fractions from the first column. Ten milliliter fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. Two milliliter fractions were collected.

Finally, IR and one dimensional Nuclear Magnetic Resonance (^{1}H NMR, ^{13}C NMR) and DEPT spectroscopic techniques were used to characterize the isolated compounds.

RESULTS

Yield of the crude extract

The air dried leaves of *O. basilicum* soaked with chloroform and methanol (1:1) mixture of solvents yielded a dark green crude extract of a better yield (23.5 g, 11.75% w/w).

DISCUSSION

Characterization of the isolated compounds

From a total of 22 fractions after column chromatography only fraction 14 (OB-14) displayed a single spot on TLC. The fraction was characterized using IR and 1D NMR, 400 MHz spectroscopic techniques.

IR interpretation of OB-14

IR frequencies of the compound OB-14 is shown in (Table 1).

NMR interpretation of the isolated compounds

Due to similarity in polarity two isomeric compounds were isolated together from the crude extract of the plant and were collected as OB-14. Based on the intensity of signals of the NMR spectra, one compound was a major and the other was a minor components. These two components were analyzed using one dimensional NMR (400 MHz) techniques (^{1}H NMR, ^{13}C NMR and DEPT). The NMR spectrum of the fraction (OB-14) was elucidated as two isomeric compounds coded as OB-14 M (major) and OB-14 m (minor).

Nuclear magnetic resonance (NMR) data of OB-14 M

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) =1.27 (3H, d, J=7 Hz, H-1), δ (ppm) =2.30 (2H, d, J=5.6 Hz, H-3), δ (ppm) =2.32 (1H, d, J=5.6 Hz, OH proton), δ (ppm) =2.75 (2H, d, J=4 Hz, H-5, H-8), δ (ppm) =3.72 (1H, m, J=14.4 Hz, 7 Hz, 6.2 Hz, H-2), δ (ppm) =5.06 (1H, t, J= 6.8 Hz, H-11a), δ (ppm)= 5.10 (1H, t, J=6.8 Hz, H-11b), δ (ppm) =[5.1, 5.38] (1H, q, J=12 Hz, 6.8 Hz, H-6, H-7), δ (ppm)

=6.71 (1H, t, J=5.6 Hz, H-10). The multiplicity of the two hydrogens attached to C-11 is triplet; this is because the two protons are at different chemical environment. So, splitting occurs between these two geminal protons. (MHz: Mega Hertz, $CDCl_3$: Deutrioted Chloroform, ppm: parts per million, H: Hydrogen, d: doublet, J: coupling constant, Hz: Hertz, δ: chemical shift, m: multiplet, t: triplet, q: quartet).

The coupling constant (J) can be calculated by multiplying the chemical shift (ppm) of the individual peaks in a specified multiplicity with the frequency of NMR machine (in this case 400 MHz). For the simple case of a doublet, the coupling constant is the difference between two peaks. The trick is that J is measured in Hz, not ppm.

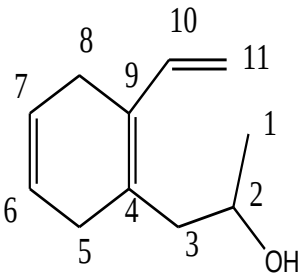
Table 1: Typical infra-red (IR) absorption frequencies of the compound OB-14.

Observed frequency (cm^{-1})	Possible Frequency range (cm^{-1})	Assignments
3409	3200-3600	O-H stretching of alcohol
3019	3000-3100	=C-H stretching in alkenes
2977	2850-3000	Asymmetric -C-H stretching of alkanes
2896	2850-2950	Symmetrical -C-H stretching of alkanes
1630	1620-1680	-C=C- stretching of substituted alkenes
1523	1500-1600	-C=C- stretching of unsubstituted olefins
1423	1350-1470	-C-H bending for alkanes
1218, 1046	1000-1260	-C-O stretching of alcoholic region
772	675-1000	-C-H bending (substituted alkenes)
669	665-1000	-C-H bending (unsubstituted alkenes)

The ^{13}C NMR data of compound OB-14 showed a resonance of the 11 carbon atoms of the compound OB-14 M.^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm)=29.82 (-CH_3, C-1), δ (ppm) =64.65 (-CH-, C-2), δ (ppm) =39.86 (-CH_2-, C-3), δ (ppm) =139.50 (Quaternary carbon, C-4), δ (ppm) =34.82 (-CH_2-, C-5), δ (ppm) =127.11 (-CH=, C-6), δ (ppm) =127.75 (= CH-, C-7), δ (ppm) =29.69 (-CH_2, C-8), δ (ppm) =130.21 (Quaternary carbon, C-9), δ (ppm) =137.24 (-CH=, C-10), δ (ppm) =115.99 (=CH_2, C-11). Therefore, ^{13}C NMR data showed the presence of aliphatic, alcoholic and olefinic carbons which exactly matched with the IR functional groups previously identified.

The distortionless enhancement by polarization transfer (DEPT) data of the compound OB-14 also showed the presence of one methyl carbon, three saturated methylene carbons, one unsaturated methylene carbon, one saturated methine carbon, three unsaturated methine carbons, and two quaternary carbon atoms for the compound OB-14 M. DEPT NMR (400 MHz, $CDCl_3$): δ (ppm)= 29.82 (-CH_3, C-1), δ (ppm) =64.65 (-CH-, C-2), δ (ppm) =39.86 (-CH_2-, C-3), δ (ppm) =34.82 (-CH_2-, C-5), δ (ppm) =127.11 (-CH=, C-6), δ (ppm) =127.75 (= CH-, C-7), δ (ppm) =29.69 (-CH_2-, C-8), δ (ppm) =137.24 (-CH=, C-10), δ (ppm) =115.99 (=CH_2, C-11).

The difference in the number of carbon atoms between ^{13}C NMR and DEPT NMR spectra gives the number of quaternary carbons which do not appear in DEPT because of the fact that the technique is based on C-H bond polarization transfer. Therefore, OB-14 m has two quaternary carbons at 139.50 ppm and 130.21 ppm. Based on the above evidences the structure of OB-14 m was determined to be 1-(2-vinylcyclohexa-1, 4-dienyl) propan-2-ol and has the following structure.



1- (2-vinylcyclohexa-1, 4-dienyl) propan-2-ol

Figure 1: Proposed structure of compound OB-14 m.

NMR data of OB-14 m

OB-14 m is an isomer of the compound identified above (i.e. OB-14 m). This is because the two compounds are eluted together using different solvent systems and due to similarity in polarity during column chromatography and possess the same retention factor on TLC plate. Compounds that have exactly similar polarity are difficult to separate as they elute together with the same RF values. So, NMR results of this minor isomeric compound were displayed together with the major component.

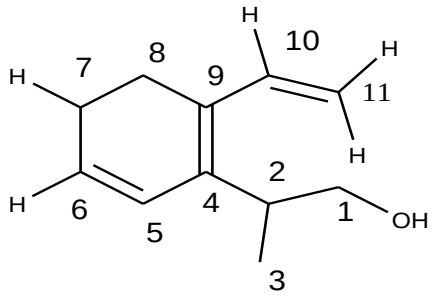
^1H NMR (400 MHz, $CDCl_3$): δ (ppm) =0.966 (3H, d, J=6.4 Hz, H-3), δ (ppm) =2.06 (1H, t, J=6 Hz, H-8), δ (ppm) =2.10 (1H, t, J=6 Hz, OH proton), δ (ppm) =[2.06, 2.30] (1H, m, J=12 Hz, 7 Hz, 5.6 Hz, H-2), δ (ppm) =3.44 (2H, t, J=11.6 Hz, H-1), δ (ppm) =5.06 (1H, t, J=6.8 Hz, H-11a), δ (ppm)= 5.10 (1H, t, J=6.8 Hz, H-11b), δ (ppm) =[5.1, 5.38] (1H, q, J=12 Hz, 6.8 Hz, H-6), δ (ppm) =5.91 (1H, d, J=6.8 Hz, H-5), δ (ppm) =6.95 (1H, t, J=5.6 Hz, H-10). Protons with exactly similar chemical shift values in both the major and the minor compounds showed equivalent protons. (MHz: Mega Hertz, $CDCl_3$:

Deutrioted Chloroform, ppm: parts per million, H: Hydrogen, d: doublet, J: coupling constant, Hz: Hertz, δ: chemical shift, t: triplet, q: quartet).

The ^{13}C NMR data of OB-14 showed a total of 11 carbon atoms at different regions for the compound OB-14 m. ^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm) =55.94 (-CH_2-, C-1), δ (ppm) =31.62 (-CH-, C-2), δ (ppm) =14.12 (-CH_3, C-3), δ (ppm) =139.50 (Quaternary carbon, C-4), δ (ppm) =128.32 (-CH=, C-5), δ (ppm) =130.19 (=CH-, C-6), δ (ppm) =29.36(-CH2-, C-7), δ (ppm) =31.92 (-CH_2-, C-8), δ (ppm) =131.96 (Quaternary carbon, C-9), δ (ppm) =137.18 (-CH=, C-10), δ (ppm) =115.99 (=CH_2, C-11). Carbons with exactly similar chemical shift values in both the major and the minor compounds showed overlapped signals.

The Distortionless Enhancement by Polarization Transfer (DEPT) data of the compound OB-14 m indicated the presence of one methyl, 4 Methylene, 4 methine and 2 quaternary carbons. DEPT NMR (400 MHz, $CDCl_3$): δ (ppm) =55.94 (-CH_2-, C-1), δ (ppm) =31.62 (-CH-, C-2), δ (ppm) =14.12 -CH_3, C-3), δ (ppm) =128.32 (-CH=, C-5), δ (ppm) =130.19 (=CH-, C-6), δ (ppm) =29.36 (-CH2-, C-7), δ (ppm) =31.92 (-CH_2-, C-8), δ (ppm) =137.18 (-CH=, C-10), δ (ppm) =115.99 (=CH_2, C-11).

Based on the above evidences the structure of OB-14 m was determined to be 2-(2-vinylcyclohexa-1, 5-dienyl) propan-1-ol and has the following structure.



2-(2-vinylcyclohexa-1, 5-dienyl) propan-1-ol

Figure 2: Proposed structure of compound OB-14 m.

The two isomeric compounds were isolated for the first time from the leaves of *O. basilicum.* The two compounds mainly differ on the position of one of the double bonds and the methyl group in which the double bond is flanked one carbon far (between C-6 and C-7) from the position of the first π-bond in the major compound while it is between C-5 and C-6 in the minor one. On the other hand, the methyl group and the hydroxyl groups are attached to the same carbon in the major compound whereas in the minor compound the methyl and the hydroxyl groups are attached to different carbons.

So, compound two (OB-14 m) might be biosynthesized by rearrangement reactions of the first compound (OB-14 m). Beyond these two newly identified isomeric alcohols, other compounds with hydroxyl functional group were also isolated from the crude extract of the leaves of *O. basilicum*. Linalool, geraniol, eugenol, methylchavicol, nerol, carvacrol, 1-octanol and 3-hexene-1-ol are a few compounds among the alcohols isolated from the extracts of *O. basilicum.*[18]

CONCLUSION

To the best of our knowledge, this is the first paper to identify the presence of 1- (2-vinylcyclohexa-1,4-dienyl) propan-2-ol and 2-(2-vinylcyclohexa-1,5-dienyl) propan-1-ol as two isomeric compounds in *O. basilicum L.* So, the results of the present study may suggest that *O. basilicum* extracts possesses compounds of different functional groups and more compounds can be isolated from the plant using different chromatographic techniques.

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