EXTRACTION OF BIOACTIVE COMPOUND FROM STAR ANISE (*ILLICIUM VERUM)* USING SOXHLET EXTRACTION

Nurulfarhana binti Mat Amin and Dr NorHuda Ismail

Faculty of Chemical Engineering, Universiti Teknologi Mara

***Abstract*— Day by day, people around the world are demanding natural constituents in food and pharmaceutical product due to its medical value within the plant such as antimicrobial and antifungal properties. Star anise or *Illicium verum* is star shaped fruit which normally used as a flavor in cooking and also as healing herbs where its shows medicinal properties that serve notable health benefits. In this research, *Illicium verum* is used to study the highest oil content in it by using three different solvents, to determine its major phytochemical compound by using GCMS and to identify its chemical structure by using FTIR. The sample of *Illicium verum* is prepared and the oil content is obtained using soxhlet extraction method. Solvents used in this research are distilled water, methanol and ethanol where the ratio of solvent to sample is 10: 1. The essential oil obtained from the extaction is characterized using GCMS to determine its chemical compound and FTIR to determine its chemical structure. Methanol acts as the best solvent since it extracts the highest amount of oil from *illicium verum.* From GCMS, estragole is detected as the major compound found in the *illicum verum* in both methanol and ethanol solvent while from FTIR analysis, chemical compound presence in the extracted oil are hydroxyl group, alkyl halides, carboxylic acid, alkanes and aldehydes.**

Keywords— FTIR Analysis GCMS Analysis Soxhlet Extraction, Solvent ethanol, Solvent methanol, Solvent distilled water.

I. INTRODUCTION

Illicium verum which is the scientific name for star anise is the star shaped fruit originating in southern China. It has an anise- or licorice like flavor, even though it is not correlated to the true anise plants family to the Mediterranean basin and Middle East. It is significant to differentiate between Japanese star anise, which is high in toxic. Illicium verum belong to magnolia family, a small to medium evergreen tree and it can reach up to 8m (26 ft). The leaves of illicium verum are narrow and tapering to a point at each end, while the axiallary flowers colored in yellow. The fruits are harvested before it matures, then dried under the sun (Star anise, n.d).

Illicium verum usually used as spice and also as a healing herbs, it shows medicinal properties that provide significant health benefits. Illicium verum give benefits to human health where it is containing a lot of Shikimic Acid, a plant based compound that is parent to oseltamivir, an antiviral medification that is widely known as Tmiflu. Even though shikimic acid also occurs naturally in gingko and sweetgum fruit, illicium verum has higher concentration compared to them. Besides, Illicium verum also have antifungal properties, antibacterial properties and antioxidant properties (Hendricks, 2015)

Illicum verum is one of the five spices in Chinese five spice powder where the other four of it are cloves, Sichuan pepper, and cinnamon and fennel seeds. Commonly, whole star anise used to sweeten soups and meat stews where normally one or two pieces of it are enough to flavor a large bowl as the taste can be very strong. In addition, the spice is also used in breads, pastries and other types of desserts due to it certain sweetness. Illicium verum can give unique flavor when it is added in the recipe of pudding, strudels and custards. Like most therapeutic preparations for herbs and spices, it can be boiled in water and made into a tea where the tea can help to relieve a few ailments like bronchial cough, indigestion and the lower abdominal pain related with pre-menstrual cycle (PMS) (Ben, 2012)

One of the biggest obstacles facing by scientist is to search natural alternatives for the artificial food additives in order to ensure it is safe, healthy and nutritious to be consumed for the customers. Currently, consumers are demandingly opting for natural preservatives in food and plant extract which are widely explored for their bioactives properties that can act as natural antioxidant and antimicrobials in food preservation system (Ahmad & Youssef, 2015)

According to Aly, Sabry, Shaheen, and Hathout (2014) in such location, such as Egypt, harvested grain is infected by a variety of fungi like Aspergillus flavus, Aspergillus parasiticus and Fusarium verticillioides which lead to deterioration and mycotoxin production. The production of mycotoxin attracts global attention because of the vital economic losses associated with their consequences on human health, animal productivity and trade. The most important mycotoxin is aflatoxin (AFs) and Fumonisins (FUM). AFs activate mutagenic, teratogenic and carcinogenic effects, while FUM can cause a few animal diseases like leukoencephalomalacia in horses, pulmonary edema in pigs, and for humans, the probability of having esophageal cancer and liver cancer is high. Nowadays, protective measures really depend on the chemical control pathogen, with dangerous and unessential environmental consequences. Due to the problem occurred; demand for safe and organic food without chemical preservatives is increasing.

II. METHODOLOGY

A. Materials and Chemicals

The only raw material used in this research is Illicium Verum. The raw materials are bought from a local store or shop around Shah Alam zone. In the extraction methods, ethanol, methanol and distilled water are chemical that used. The apparatus used to assist the whole research are electric blender, soxhlet extractor and rotary evaporator. To accomplish the extraction process, beakers, filter paper, weighing balance, tubes and spatula are used. For the analysis part of the research, GC-MS is used to determine the phytochemical content in the Illicium Verum while FTIR is used to identify the chemical structure.

B. Raw Material Preparation

The raw material preparation of Illicium verum is referred to procedure used by (Ahmad & Youssef, 2015). The fruits of Illicium verum are purchased from local market located in the area of Section 7, Shah Alam. The Illicium verum is grinded by using electric blender. Powdered Illicium verum is put in a plastic bag and then stored in desiccator for further experiment

C. *Soxhlet Extraction*

The first step for the extraction process is by getting the extract using soxhlet extractor. 5g of powdered Illicium verum is extracted using different type of solvents which are distilled water, ethanol and methanol at temperature varies between 100°C to 120°C. The ratio of extract to solvent is 1 : 10 (Ahmad & Youseeef,2015) . The extract is then left to cold, before its solvent being evaporated in the rotary evaporator. Next, the solvent is removed using rotary evaporator at 50°C. The removal of solvent is continued until the solvent is removed completely from the solution.

D. *Analysis of the Extracts*

Analysis part is vital in every research in order to achieve the objectives of the research. After the extraction of raw material is done, the process continues with the analysis. In this research, GC-MS is used to reveal the major bioactives ingredients while FTIR is used to determine the chemical structure in Illicium verum.

According to Adinew (2014), the identification of chemical compound by GC-MS using HP 5890 series GC equipped with mass selective detector (MSD), HP 5972 series (German) in Addis Ababa University, helium is used as carrier gas at constant flow of 1 ml/ min and 1µl injection volume, injector temperature at 250 °C and temperature of ion source at 280°C. The temperature of oven was set from 50°C which is isothermal for 4 minutes, with an increase of 3°C/min to 280 °C and held for 10 minutes. Isothermal temperature is at 280°C. The GC running time was 90.67 minutes.

III. RESULTS AND DISCUSSION

A. *Percentage Yield of Illicium Verum Oil via Soxhlet Extraction*

In this research, soxhlet extraction was used to extract illicium verum essential oil. The experiment was conducted for 6 hours extraction time by using three different types of solvents which are Methanol, Ethanol and distilled water with ration raw material to solvent 1 : 10. Referring to Kasim, Ismail, Masdar, Ab Hamid, and Nawawi (2014), percentage yield of essential oil was calculated using the following formula.

$$\text{Percentage of essential oil (\%)} = \frac{\text{Essential oil weight}}{\text{sample weight}} \times 100$$

Figure 4.1 indicates the essential oil yields of *illicium verum* using different kind of solvents. The solvent used in the experiment were Methanol, Ethanol and distilled water. The results showed that the highest percentage yield of *illicium verum* essential oil was by using methanol followed by ethanol and distilled water were 18.3849%, 15.1767% and 12.5391%, respectively. The extraction yield depends on the types of solvent with different polarity, temperature, Ph, composition of the sample and extraction time. From figure 4.1, it can be seen that the yield of extraction of pure methanol is higher than pure ethanol. This indicates that the yield of extraction increases with the increasing of solvent's polarity used in the extraction. Even though water is normally addressed as "universal solvent" and the polarity of water is higher than methanol and ethanol, the oil yield extracted is the lowest. This situation might be happened due to its properties where water is poor at dissolving non polar molecules.

The operating temperature used in this extraction process is varied between 100°C to 120°C. From the experiment, there was *illicium verum* essential oil collected at temperature 100°C. After the first hour of extraction, *illicium verum* oil can be seen extracted from the process for solvent methanol and ethanol. Using water solvent, it took about 1 hour and half to extract oil from the *illicium verum*. It was also discovered that nearly all of the oil was distilled out and the solvent is recovered for recycle. From the result of the soxhlet extraction, methanol is the best solvent extractor and was supported by the fact that the color of the extracted oil was the darkest compared to solvent ethanol and distilled water which is also means that methanol extracts more oil from the *illicium verum*.

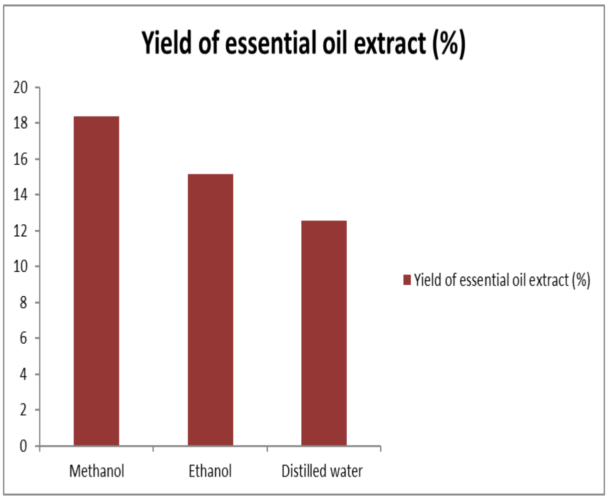

Fig 4.1: Percentage yield of illicium verum essential oil via different solvent

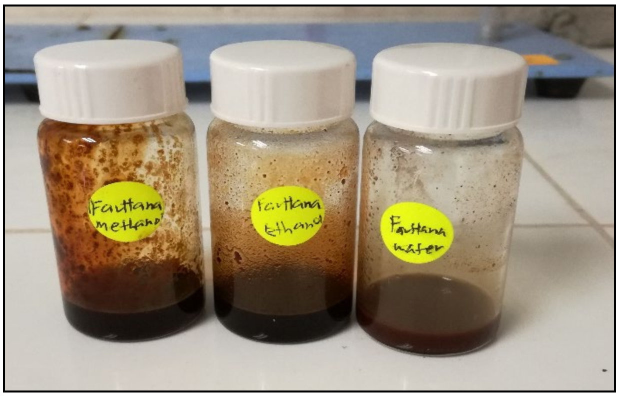

Fig 4.2: color difference of oil extracted from star anise. (from left: methanol, ethanol and water)

B. *Chemical composition of Illicium Verum extracts (GC-MS)*

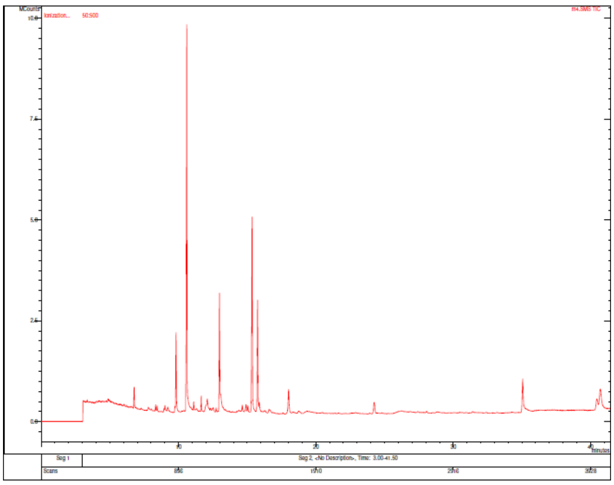

Figure 4.3: Essential oil of *illicium verum* GCMS result

Table 4.2 display the major chemical compounds identified in Illicium verum extracted by using solvent ethanol and methanol, respectively as well as its chemical and/or bioactives properties.

From table 4.2, result from GC-MS analysis of the methanol extracts shows that the highest major compound was Estragole (43.409 %), followed by Camphene (1.712%) and Caryophyllene (0.192). In table 4.3, GC-MS analysis of ethanol extracts revealed that major compounds exist in Illicium verum are also Estragole (49.081%) followed by Camphene (4.612%) and Limonene oxide (0.164%). In line with this research, Ahmad and Youssef (2015) reported the presence of estragole and caryophyllene while researched by Qin et al. stated the existence of limonene, estragole, á-Pinene and à-Phellandrene, the same major compound recognized in the six sample of GC-MS star anise profile. Estragole have similar functional group as anethole. Thus, it is proven from researched by Qin et al., Ahmad and Youssef that the result is match and acceptable.

Table 4.2: Major chemical compound in *illicium verum* extracted using methanol

Chemical component	**Peak area (%)**
6-Octenal, 3,7-dimethyl-	0.918
á-Pinene	1.184
Limonene oxide, cis-	0.720
Camphene	1.712
Ecgonine	3.005
Estragole	43.409
Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methyle	0.386
Caryophyllene	0.192
à-Caryophyllene	0.439
1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-	0.353
1,9-Dihydropyrene	0.495
Phenanthrene, 2-nitro-	1.691
6-Octen-1-ol, 3,7-dimethyl-, propanoate	0.629

Table 4.3: Major chemical compound in *illicium verum* extracted using ethanol

Chemical component	Peak area (%)
Ecgonine	1.243
Camphene	4.612
Limonene oxide, cis-	0.164
6-Octenal, 3,7-dimethyl-	0.773
à-Phellandrene	0.164
Estragole	49.081
6-Octenal, 3,7-dimethyl-	0.086
(valencene) Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7	0.138
Caryophyllene	1.086
à-Farnesene	0.094
Phenanthrene, 2-nitro-	1.691
6-Octen-1-ol, 3,7-dimethyl-, propanoate	0.629

C. *Medicinal Porperties of Illicium Verum*

From the GCMS analysis, there are many compound can be obtained from the extraction of *illicium verum* and each of the compound carries its own medicinal properties which make the *illicium verum* good to human health consumption.

In the soxhlet extraction by using methanol and ethanol, GCMS result shows that the major compound consists in *illicium verum* is estragole. In the research by Leal-Cardoso et al. (2004), he mentioned that estragole have antimicrobial, antispasmodic, neurotopic and immunostimulant properties. In addition, it is also reported to have sedative and anticonvulsant activity linked to its neutropic property. According to Ahmad and Youssef (2015), the medicinal values of estragole is its provide blocks for the transmission of neuromuscular and rise the myoplasmic calcium while Silva-Alves et al. (2013) mentiones in their research estragole has antioxidant and antimicrobial activities. Pharmacological activities of *illicium verum* is documented to be anxiolytic and have anti-inflammatory activity (Silva-Alves et al., 2013).

Other than estragole, Caryophyllene also found in the essential oil of *illicium verum*. Amiel et al. (2012) explained in a research that caryophyllene have antifungal, anti-inflammatory and local anaesthetic properties which can be found in flavor of citrue, spice blends, detergent, soap and lotion. Besides, it is also categorized as strong toxic compound over a variety of cell lines. Magwa, Gundidza, Gweru, and Humphrey (2006), also stated that caryophyllene has antifungal activity Plus, in the researched also mentioned that is has anti-oxidant activity (Magwa et al., 2006). The facts that anti-oxidant properties of caryophyllene is strengthen from the researched by Ahmad and Youssef (2015).

Limonene oxide is also one of the compound contained in the *illicium verum* essential oil. According to Magwa et al. (2006), limonene has antifungal and anti-oxidant activity while Soković, Glamočlija, Marin, Brkić, and van Griensven (2010) mentioned the present of antibacterial properties in the limonene oxide. The antibacterial properties of limonene oxide also mentioned in the researched by Iacobellis, Lo Cantore, Capasso, and Senatore (2005).

In the essential oil of illicium verum, camphene was also found as one of the major compound. From research by Quintans-Júnior et al. (2013), camphene is a monoterpene that is usually found as major compound in the essential oil of aromatic plant. It may contain significant pharmalogical properties like antimicrobial, analgesic, antioxidant and antitumor. In addition, camphene also provides important effects on control nervous system (CNS) and cardiovascular system (Quintans-Júnior et al., 2013). Camphene which is sometimes known as camphor also produces meaningful antinociceptive effects (Milovanović et al., 2016).

D. *Chemical Structure of Illicium Verum Using FTIR*

Fourier Transform Infrared Spectroscopy (FTIR) are generally used to determine the functional group from each sample of the result. It can detect sample in gas, liquid and solid state. Sample will be put inside the FTIR and the monitor will display the functional group detected from the instrument and revealed result in spectra figure. From table 4.2 and 4.3, estragole is found as the major compound obtained from the illicium verum essential oil.

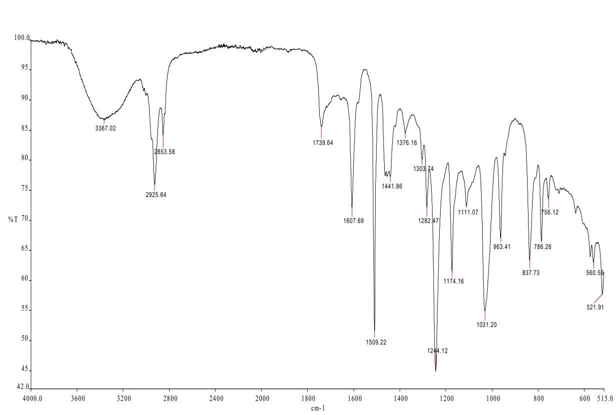

Figure 4.4: Essential oil of *illicium verum* FTIR result

Table 4.8: FTIR summary using ethanol as solvent

Wavenumber	Assignment	Compound class

3200 - 3400	O-H stretch	Hydroxyl group
2800 – 3000	C-H stretch	Carboxylic acid
1600 – 1700	C=O stretch	Aldehyde
1320 - 1000	C-O stretch	Carboxylic acid
515 – 800	C-Br stretch	Alkyl halides

Table 4.9: FTIR summary using methanol as solvent

Wavenumber	Assignment	Compound class
3200 - 3400	O-H stretch	Hydroxyl group
2800 – 3000	C-H stretch	Carboxylic acid
1600 – 1750	C=O stretch	Aldehyde
1440 – 1450	C-C stretch	Aromatics amines
1335 – 1250	C-N stretch	Aromatics amines
1000 -1240	C-O stretch	Carboxylic acid
750- 840	C-Cl stretch	Alkyl halides
500 – 570	C-Br stretch	alkyl halides

Table 4.10: FTIR summary using distilled water as solvent

Wavenumber	Assignment	Compound class
3200 - 3400	O-H stretch	Hydroxyl group
1600 – 1750	C=O stretch	Aldehyde
1335 – 1250	C-N stretch	Aromatics amines

Table 4.8, table 4.9 and table 4.10 were display the summary of FTIR analysis of illicium verum essential oil using ethanol, methanol and distilled water as solvent. Most of functional group were detected in the sample that used methanol as solvent which are hydroxyl group, carboxylic acid, Aldehyde, Aromatics amines, Carboxylic acid, and Alkyl Halides compared to ethanol and distilled water. Using Distilled water as extraction solvent, FTIR results show the least amount of functional group which are Hydroxyl group, aldehyde and Aromatic amine where it justifies that it is not the ideal kind of solvent in the extraction of essential oil of illicium verum.

From the analysis of FTIR, hydroxyl group is found at wavelength range between 3200 to 3400 cm-1 in the essential oil extracted using ethanol, methanol and distilled water as solvents. The larger the wavenumber, the higher the energy increase within the sample that is needed to cause molecular vibrations. Energy used to cause molecular vibrations is parallel to the infrared energy level where the total amount of light absorbed will determine the types of functional group exist in the sample. Besides, O-H bond is classified as stiff bonds and its involve the bonding of hydrogen, therefore it has higher vibrational frequency and the peak is broader compared to other functional group.

Three important factors to be considered in determining the functional group using IR spectrum are wavenumber, absorbance intensity and absorbance shape (sharp or broad peaks). Essential oil that used methanol as solvent shows there are C=O stretch Aldehyde at wavenumber range between 1600 – 1750 cm-1 and C-O at wavelength range between 1000 -1240 cm-1 of stretch carboxylic acid. Every functional group have its own criteria of absorbance frequency such as when the number of bonds between two atoms is increase, the bond stiffness also increases. Thus, it makes the bond difficult to stretch. This phenomenon explains why C=O stretch Aldehyde have double bond while C-O stretch carboxylic acid have only single bond between these two atoms.

Region lower than 1500 cm-1 is called fingerprint region. Within this region, vibration is normally complex and difficult to determine the exact functional group for each peaks

IV. CONCLUSION

Illicium verum or commonly known as star anise one of spices that contains medicinal properties that serve important health benefits to human. Essential oil from iilicium verum can be obtained by soxhlet extraction process at temperature varies between 100 to 120 °C for 6 hours. The solvent used to help the extraction process were methanol, ethanol and distilled water. Result showed that different types of solvent had significant impact on the amount of oil yield of illicium verum. The result was supported by the analysis using Fourier Transform Infrared Spectroscopy (FTIR) spectra and Chromatography Mass Spectrometry (GCMS).

Methanol is the best solvent used in the extraction of illicium verum using soxhlet extraction compared to ethanol and distilled water since it has high polarity and effective at the temperature between 100 to 120°C. Besides, methanol also extracts more oil from the illicium verum. This can be seen from the color of oil extracted which is the darkest compared to ethanol and distilled water.

Estragole which has antimicrobial, antispasmodic, antioxidant antivities acts as the major compound contained in the illicium verum in both solvent, methanol and ethanol. Besides, estragole also blocks for the transmission of neuromuscular and rise the myoplasmic calcium The result was obtained from the analysis by using GCMS. Other types of compound obtained in illicium verum were Camphene, Caryophyllene, limonene oxide, á-Pinene and à-Phellandrene.

FTIR spectroscopy revealed the existence of numerous group of compound including (OH) hydroxyl group, carboxylic acid, alkyl halides, aldehydes and alkanes.

Furthermore, these chemical compound and chemical structure obtained from the result needs additional assessment before it can be reported as drug to cure general human disease.

ACKNOWLEDGMENT

Thank you to my supervisor, Dr NorHuda Ismail for the invaluable guidance, continuous encouragementand constant support and Universiti Teknologi Mara for allowing me complete this research study.

REFERENCES

[1] Star Anise - The Epicentre. (n.d.). Retrieved October 09, 2016, from http://theepicentre.com/spice/star-anise/H. Poor, (2013). An Introduction to Signal Detection and Estimation. Chapter 4. New York: Springer-Verlag.

[2] Hendricks, J. (2015, June 09). Health Benefits of Star Anise. Retrieved November 20, 2016, from http://www.livestrong.com/article/399013-health-benefits-of-star-anise/

[3] . Ahmad, A. F., & Youssef, M. S. (2015). Chemical composition and bioactive properties of Illicium verum (star-anise) extracts Prepared by different methods. Journal of Chemical, Biological and Physical Sciences (JCBPS), 5(2), 1160.

[4] Aly, S. E., Sabry, B. A., Shaheen, M. S., & Hathout, A. S. (2014). Assessment of antimycotoxigenic and antioxidant activity of star anise (Illicium verum) in vitro. Journal of the Saudi Society of Agricultural Sciences.

[5] Adinew, B. (2014). GC-MS and FT-IR analysis of constituents of essential oil from Cinnamon bark growing in South-west of Ethiopia. International Journal of Herbal Medicine, 1(6), 22-31.

[6] Kasim, N. N., Ismail, S. N. A. S., Masdar, N., Ab Hamid, F., & Nawawi, W. (2014). Extraction and Potential of Cinnamon Essential Oil towards Repellency and Insecticidal Activity. International Journal of Scientific and Research Publications, 4(7).

[7] Leal-Cardoso, J., Matos-Brito, B., Lopes-Junior, J., Viana-Cardoso, K., Sampaio-Freitas, A., Brasil, R., . . . Albuquerque, A. (2004). Effects of estragole on the compound action potential of the rat sciatic nerve. Brazilian Journal of Medical and Biological Research, 37(8), 1193-1198.

[8] Silva-Alves, K., Ferreira-da-Silva, F., Peixoto-Neves, D., Viana-Cardoso, K., Moreira-Junior, L., Oquendo, M., . . . Leal-Cardoso, J. (2013). Estragole blocks neuronal excitability by direct inhibition of Na+ channels. Brazilian Journal of Medical and Biological Research, 46(12), 1056-1063.

[9] Amiel, E., Ofir, R., Dudai, N., Soloway, E., Rabinsky, T., & Rachmilevitch, S. (2012). β-Caryophyllene, a compound isolated from the biblical balm of gilead (Commiphora gileadensis), is a selective apoptosis inducer for tumor cell lines.

[10] Magwa, M. L., Gundidza, M., Gweru, N., & Humphrey, G. (2006). Chemical composition and biological activities of essential oil from the leaves of Sesuvium portulacastrum. Journal of Ethnopharmacology, 103(1), 85-89.

[11] Soković, M., Glamočlija, J., Marin, P. D., Brkić, D., & van Griensven, L. J. (2010). Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an in vitro model. Molecules, 15(11), 7532-7546