Comparison of Chemical Composition and Toxicity of Essential Oils from Lemongrass (Cymbopogon Citratus) Extracted with Microwave-Assisted Hydrodistillation (MAH) and Conventional Hydrodistillation (HD) Methods

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Abstract

The demand of essential oil in current industry has increased due to its bioactive compound that shows various therapeutic effects. Therefore, the qualities of essential oil mainly depend on its constituents. This study was carried out to determine the effects of Microwave-assisted hydrodistillation (MAHD) and hydrodistillation (HD) methods on yield, chemical composition and toxicity of essential oil from Lemongrass (*Cymbopogon citratus*). A pale yellow oil yield of 1.46 % (90 min) and 1.38% (180 min) were obtained from MAHD and HD methods, respectively. GC-MS results shows a total of twenty compounds in MAHD oil and seven compound in HD oil. MAHD oil contained slightly higher amount of citral, which is the key component of Lemongrass (*Cymbopogon citratus*), compared to HD oil, 86.48% and 83.15%, respectively. However, hydrodistilled oil showed the presence of higher β-Myrcene, 9.91%, and only 3.96% were found in the MAHD oil. Other identified constituents, include Linalool, 1,6-Octadiene,3,5-dimethyl-,trans, Pinane, Geranic acid and 1,5-Octadiene. Cytotoxicity study was also carried out by using Artemia Salina (Brine shrimp) lethality bioassay. The Lemongrass (*Cymbopogon citratus*) oil extracted using MAHD and HD gives LC_{50} value of 0.35µg/ml and 0.29µg/ml, respectively. A minor variation in the total chemical composition of essential oil may contribute for different level of biological activity expression.

Index Terms— cytotoxicity; essential oil; hydrodistillation Lemongrass; Microwave-assisted hydrodistillation.

INTRODUCTION

Essential oils are botanical extracts of various plant materials, and do not only originate from flowers, but from herbs, trees and various other plant material. It is estimated that the global number of plants is of the order of 300,000 and about 10% of these contains essential oils and could be used as a source for their production [1]. Their application in pharmaceutical and food industry gain growing attention due its biological activity, antioxidant, antibacterial and antifungal properties. Physically, essential oils are made up primarily of the elements; carbon, hydrogen and oxygen, and these primary elements form a wide variety of compounds that fall basically into two family groups which are hydrocarbons and oxygenated compounds. However, within essential oil chemistry, each functional family of compounds is known to have specific therapeutic effects.

Lemongrass (*Cymbopogon citratus*), a perennial plant with long, thin leaves, is one of the largely cultivated medicinal plants for its essential oils in parts of tropical and subtropical areas of Asia, Africa and America [2]. It contains 1-2% of essential oil on dry basis [3] and the chemical composition of Lemongrass (*Cymbopogon citratus*) essential oil is varying widely upon genetic diversity, habitat and agronomic treatment of the culture [4].The leaves of Lemongrass (*Cymbopogon citratus*) present lemony characteristic flavor due to its main content, citral which present great importance to the industry. Citral, a combination of neral and geranial isomers, is used as a raw material for the production of ionone, vitamin A and beta-carotene [3]. It possesses a high cytotoxic activity [5]. There were a number of studies carried out to prove the anti-oxidant, anti-microbial and anti-fungal activities of Lemongrass (*Cymbopogon citratus*) [6], [7], [8]. In industry lemongrass (Cymbopogon citratus) oil has many other uses, such as perfume in soaps, flavoring agent for tea and medicine to treat various health ailments, including acne, athlete's foot, flatulence, muscle aches and scabies [9].

The common methods to extract essential oil from medicinal plant, including for Lemongrass (*Cymbopogon citratus*), are hydrodistillation (HD), steam distillation, steam and water distillation, maceration, empyreumatic distillation and expression [10]. Previous researches, [10], [11], [12], had proved the quality of essential oil mainly depends on their constituent which is primarily influenced by their extraction procedures. In contrast, these common methods can induce thermal degradation, hydrolysis and water solubililization of some fragrance constituents. According to [13], steam distillation caused susceptible chemical changes on monoterpenes compound. In addition, the oil obtained through solvent aided extraction contains residues that pollute the foods fragrances to which they are added and also leads to losses of more volatile compound during the removal of its solvent.

Recently, microwave-assisted hydrodistillaton (MAHD) procedures for isolating essential oils have become attractive for use in laboratories and industry due to its effective heating, fast energy transfer and also an environmental friendly extraction technique. Its acceptance as potential and powerful alternative for conventional extraction techniques has been proved through several research [1], [4], [14]. Although, several studies has reported the benefits of MAHD over HD on several plant, but there are no reports of the simultaneous comparison of MAHD and HD extraction procedures on extraction of essential oil from Lemongrass (*Cymbopogon citratus*). Besides, earlier studies were mostly focused on the comparison of the yield through advance and conventional extraction method. For gain the clear knowledge of a method's efficiency, it is important to carry out the constituents as well as the toxicity comparison of the extracts so that their real clinical benefits can be studied. Therefore, the present investigation was designed to evaluate the yield and chemical composition of the essential extracted from MAHD and HD method. GC-MS was used for the analysis of chemical composition. Then, the extracts were examined for their toxicity using brine shrimp lethality test (BST) which based on the ability to kill laboratory cultured brine shrimp (Artemia nauplii). BST appears to be the predictive of cytotoxicity and pesticidal activity [15].

MATERIALS AND METHOD

A. Plant Materials

Lemongrass (*Cymbopogon citratus*) leaves were collected from home garden in northwest of Malaysia. The plant sample was freshly cut, 10cm from the root, in the morning of the day they were collected. According to [7], for Lemongrass (*Cymbopogon citratus*), the percentage essential oil yield for the partially dried leaves was found to be higher than that of the fresh leaves. Thus, once collected, the plant material were dried at room temperature for a week then kept in a sealed plastic bag at ambient temperature and protected from the light. The samples were ground using a kitchen grinder (Super Blender,Panasonic,Tokyo, Japan) at room temperature prior to extraction. This is because, extraction yield increase by decreasing the particle size due to the higher amount of oil released as the leave cells are destroyed by milling. In order to improve the collection efficiency, the plant material was soaked in its distilled water for 30min before the extraction performed.

B. Extraction of Essential Oil

A modified domestic microwave oven model Samsung MW71E connected to the Clevenger apparatus was modified for MAHD operation. The Samsung MW71E has 1150 Watt power consumption, 800 Watt output power with 250v-50Hz power source; 2450MHz. The cavity dimensions of the microwave oven were 306 x 211 x 320mm. The flask containing 50 g of Lemongrass (*Cymbopogon citratus*) with its distilled water was placed within the microwave oven cavity. A condenser which has been set on the top, outside the oven, was used to collect the extracted essential oils. The essential was decanted from its condenser after 90 minutes since this period was sufficient enough to achieve exhaustive extraction of its essential oil through MAHD [14].

For hydrodistillation (HD), 50g of fresh Lemongrass (*Cymbopogon citratus*) leaves were placed in a 1L flask containing

400ml of distilled water and hydrodistilled for 3h using a Clevenger-type apparatus. The essential were decanted from the condensate in 30 min interval. The system was operated at a fixed power of 500W and under atmospheric pressure [11].

C. Analysis of Sample

To remove the water, the decanted essential oil were dried over anhydrous sodium sulfate, weighed and stored in vial at 4^{o}C prior to analysis. The amount of yield obtained from the extraction was analyzed to evaluate the performance of MAHD in Lemongrass (*Cymbopogon citratus*) oil extraction. Yield of oil that obtained for every run was calculated by using Equation (1):

$$Yield\ of\ essential\ oil = \frac{amount\ of\ essential\ oil\ (g)\ obtained}{amount\ of\ raw\ materials\ (g) used} \quad (1)$$

A GC-MS instrument (5973N, Agilent Technologies, Wilmington, DE, UAS) equipped with a mass selective detector operating in the electron impact mode (70eV) was used to study the composition of the essential oil at extracted various group of parameter condition to analyze its quality. The GC part (6890N, Agilent Technologies, Palo Alto, CA, USA) was equipped with an HP-5MS (Agilent BTechnologies) capillary column (30 m long, 0.25 mm id and 0.25 lm film thickness). Temperature-programming of the oven included an initial hold at 50 °C for 5 min and a rise to 240 °C at 3 °C min-1 followed by additional rise to 300 °C at 5 °C min-1. A final hold for 3 min was allowed for a complete column clean-up. The injector was set at 280 °C. The samples were diluted with n-hexane (1/10, v/v) and a volume of 1.0 µl was injected to the GC with the injector in the split mode (split ratio: 1/10). Carrier gas, He, was adjusted to a linear velocity of 1 ml min-1 [12]. The compounds of the extracted essential oils were identified by comparing their mass spectral fragmentation patterns with those of similar compounds from a database (Wiley/NBS library) or with published mass spectra (Massada 1976; Adams, 2001). The components were quantified based on the comparison of compound's retention period, which were similar in both techniques. The normalization method was used; the value of total peak areas is considered 100% and the percentage of each component was calculated using the area of each peak.

D. Brine Shrimp Lethality Assay

In vitro lethality assay of Artemia Salina (Brine shrimp) was used to evaluate the cytotoxicity of the compound as described by [16], with some modifications. This analysis has been used to monitor the biological activity of the extracts. Brine shrimp eggs were hatched in a vessel containing artificial sea water prepared by dissolving 65g artificial sea salt in 2.5L of distilled water. The vessel was kept under illumination and facilitated with good aeration for 48h at room temperature.

Each extract solutions were tested at a concentration level of 0.1, 0.2, 0.5, 1.0 and 2.0µg/ml and in triplicate. Dimethyl sulphoxide (DMSO) was used as a solvent since it has been observed as almost zero mortality on brine shrimp by [17]. Firstly, stock solution was prepared by emulsifying 2mg of the essential oils separately in 0.3ml of DMSO and then the volume adjusted to 2ml by adding artificial seawater to gain 1000ppm concentration. From this stock solution, serial dilution was done to obtain the test concentrations where the final volume of each test tube will be 5ml. For positive control, potassium dichromate dissolved in artificial sea water to attain the concentrations from 0.1 to 0.9mg/ml [18]. While, the mixture of DMSO and artificial seawater only served as the negative control. Ten shrimp larvae was added to each test tube and incubated for 24 h. The number of survived larvae in each test tube was counted after 24 h under a lighted background.

Results and Discussion

*Current research is the first report on the simultaneous comparison of MAHD and HD effect on cytotoxicity activity of Lemongrass (*Cymbopogon citratus*) oil. This comparison has been supported by the result of GC-MS analysis which provides the quantitative and qualitative information of Lemongrass (*Cymbopogon citratus*) oil content. HD is an accepted method that is used as reference for the quantification of essential oils [12].*

*The MAHD and HD of Lemongrass (*Cymbopogon citratus*) gave a pale yellow essential oil with yield of 1.46% and 1.38%, respectively. The induction time for MAHD was only 12 minutes whereas for HD it was 30min. This show, 60% of the total oil can be extracted using MAHD by the time the extraction of essential oil by HD started. It is interesting to note that, the amount of yield extracted by MAHD after 90 min almost similar as the oil resulted after 180 min by HD. These results showed a significant saving of time and energy as well as the cost in the extraction procedure. The same configuration of results was also obtained by previous researchers in the activity of comparing effect of MAHD and HD on different oil bearing plants [19], [20], [21].*

*Table 1 lists the composition of Lemongrass (*Cymbopogon citratus*) oil obtained by MAHD and HD methods based on the results of GC-MS. It shows that twenty compounds were found for MAHD and seven compounds for HD. Thermal degradation may take place during HD as the number of compound found through this method was significantly small. Both of the oils were characterized by monoterpenoids with oxygenated monoterpene which is isomers of citral dominating their compositions [22], [23], [8]. Citral is the key compound to evaluate the quality of Lemongrass (*Cymbopogon citratus*) oil [8]. [22], province on the same oil by Clevenger found the similar major components but in different percentage as well as dissimilarity in its minor components. This variability may due to the different climatic and soil growing conditions [24].*

The application of medicinal plants, such as Lemongrass (*Cymbopogon citratus*), has increases especially for culinary and pharmaceutical purposes. Therefore, experimental screening of toxicity on such plant is essential to assure their safety and effectiveness. Brine shrimp bioassay is a simple model applied for test cytotoxicity which use to determine the lethal

concentration of active compound in brine medium [18]. Brine shrimp lethality results of the crude extracts of Lemongrass (*Cymbopogon citratus*) using MAHD and HD are shown in Figure 1. It is reported that LC50 value lower than 1000µg/ml is considered significantly active [16], thus it suggest the Lemongrass (*Cymbopogon citratus*) oil extracted through MAHD and HD possess high toxicity effect with LC_{50} value of 0.35 µg/ml and 0.29 µg/ml, respectively. This value is in agreement with the finding of [23]. The dominance of citral, most important member of acyclic monoterpenoids, in the Lemongrass (*Cymbopogon citratus*) oil probably accounted for its high efficacy rate and this has been supported by a number of studies [5], [25], [26]. No mortality was detected in the negative control tubes signifying that the Lemongrass (*Cymbopogon citratus*) oil were accountable for the observed mortality. In the comparison of LC_{50} value of HD and MAHD, oil extracted using HD possess slightly higher toxicity compared to MAHD. This result could be explained considering the differences in their chemical composition where HD oil contained higher amount of β-Myrcene (9.91%) compared to MAHD (3.96%). β-Myrcene is monoterpene hydrocarbon which hold cytotoxic effect [27]. According to [28], β-Myrcene itself shows LC_{50} value of 39.2µg/ml on brine shrimp and this verified that it be one of the active substances that responsible for this event. As the oil extracted using MAHD was less toxic, it is more suitable for use for the culinary and pharmaceutical purposes. Oil extracted oil HD should be avoided in folk medicine due its high toxicity level [13].

Fig.1 Brine shrimp lethality of Lemongrass (Cymbopogon citratus) oil extract using MAHD and HD method

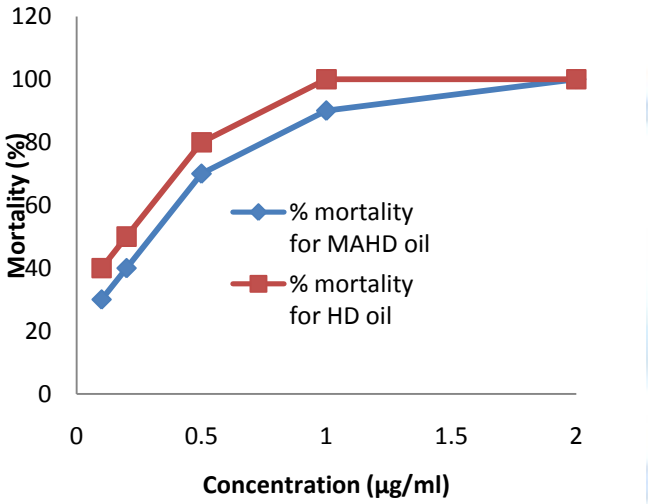

TABLE I: THE CHEMICAL COMPOSITION OF LEMONGRASS (*CYMBOPOGON CITRATUS*) OIL EXTRACTED BY MAHD AND HD METHODS

No.	Compound	Retention Time (min)	Percentage Composition (%)	
			MAHD	HD
1	β-Myrcene	3.82	3.96	9.91
2	Linalool	6.04	0.48	-
3	3,5-Dimethyl-2-cyclohexen-1-one	6.43	0.17	
4	1,6-Octadiene, 3,5-dimethyl-, trans	7.08	0.95	1.01
5	Pinane	7.65	1.77	
6	Cyclohexane	8.18	0.14	1.48
7	Neral	10.13	35.67	33.69
8	Geranial	11.22	50.81	49.46
9	Furane	13.10	0.17	-
10	1,5-Octadiene	13.57	0.40	-
11	Geranic acid	15.05	1.33	-
12	Nerolidol	17.16	0.48	-
13	2-Norpinene	17.94	0.51	-
14	2-Ethylcyclohexanone	19.73	0.22	-
15	Caryophylene oxide	22.71	0.24	-
16	Selina-6-en-4-ol	24.06	0.35	-
17	1,19-Eicosadiene	34.23	0.13	-
18	Palmitic acid	35.09	0.24	-
19	Elaidic acid	40.34	0.42	-
20	R-citronellol	42.98	0.50	
21	4-Tetradecyne	49.87	-	0.18
22	Olealdehyde	50.10	-	0.30

CONCLUSIONS

In current study, the advantage of MAHD over HD in extraction of essential from Lemongrass (*Cymbopogon citratus*) has been studied where by using MAHD shorter extraction time was achieved. In addition, GC-MS results proved that MAHD yield oil with higher main abundant compounds. The low LC_{50} value of oil obtained by MAHD compared to HD oil makes it to be safe for use this plant for pharmaceutical industry. The high toxicity level of Lemongrass (*Cymbopogon citratus*) oil was mainly contributed by its two key components which were citral and β-Myrcene. LC_{50} values of Lemongrass oil (*Cymbopogon Citratus*) suggest that the oil may have insecticidal compound and contain medicinally important secondary plant metabolites. Due to the substantial saving of time, cost and energy with no significant changes in its constituents, MAHD process is a good alternative in the extraction processes of essential oil from Lemongrass (*Cymbopogon Citratus*). The result obtained from this study encourage applying the MAHD for the extraction of the essential oil of some other plant materials with the combination of various biological activity studies.

References

[1] K. C. B. Husnu, & B. Gerhard, Handbook of Essential Oils: *Science, Technology, and Application*s; NW:Taylor & Francis Group, 2010

[2] N. E. Tajidin, S. H. Ahmad, A. B. Azimah and M. Munirah, " Chemical Composition and Citral Content in Lemongrass (Cymbopogon Citratus) essential oil at three maturity stages," *International Journal of Biotechnology*, vol.11(11), pp.2685-2693, February 2012.

[3] L. H. C. Carlson, C. B. S. Machad, L. K. Pereira and A. Bolzan, "Extraction of lemongrass essential oil with dense carbon dioxide," *Journal of Supercritical Fluids*, vol. 21, pp. 33–39, 2001.

[4] L. Paviani, S. B. C. Pergher, and C. Dariva, "Application of molecular sieves in the fractionation of lemongrass oil from high-pressure carbon dioxide extraction," *Brazilian Journal of Chemical Engineering*, vol.23,pp.219−22, 2006

[5] A.C. Mesa-Arango, J. Montiel-Ramos, B. Zapata, C. Durán, L. Betancur-Galvis L. & E. Stashenko, "Citral and Carvone Chemotypes from the Essential Oils of Colombian Lippia Alba (Mill.) N.E. Brown: Composition, cytotoxicity and antifungal activity," *PubMed*, vol.104(6), pp.878-884, 2009

[6] G. K. Oloyede, I. A. Oladosu and A .F. Shodia , "Chemical Composition and Cytotoxicity of the essential oils of Crinum ornatum (Ait) Bury," *African Journal of Pure and Applied Chemistry*, vol. 4 (3), pp. 35-37, 2010.

[7] J. Behboud, E. Amirreza, M. A. Babak and H. Zarifeh, " Antibacterial Activities of Lemon Grass Methanol Extract and Essence on Pathogenic Bacteria," *Journal of Agricultural and Environmental Science*, vol. 12(8), pp. 1042-1046, 2012

[8] G. T. Nikos & D. E. Costas, "Antifungal activity of lemongrass (Cympopogon citratus L.) essential oil against key postharvest pathogens," *Innovative Food Science Emerging Tec*hnology, vol.8, pp. 253-25, 2007

[9] W. R. L Masamba, J. F.M. Kamanula, M. T. Elizabeth & G. K. C. Nyirenda, "Extraction and Analysis of Lemongrass (Cymbopogon citratus) Oil: An essential oil with potential to control the Larger Grain Borer (Prostephanus truncates) in stored products in Malawi," *Journal of Agricultural Science*s, vol.2(1), pp.56-64, 2003

[10] M. Dhobi, V. Mandal and S. Hemalatha, "Optimization of microwave assisted extraction of bioactive flavonolignan-silybinin," *Journal of chemical metrology*,vol. 3(1), pp. 13-23, 2009

[11] J. Ashgari, C. K. Touli and M. Mazaheritehrani, "Microwave-assisted hydrodistillation of essential oils from Echinophora platyloba DC," *Journal of Medicinal Plants Research*, vol. 6(28), pp. 4475-4480, 2010

[12] K. Sibel, N. Sedef, K. Nural, S. Sahin, S. Gulum and B. Beste, " Microwave-assaited hydrodistillation of essential oil from rosemary," *Journal of Food Science Technology*, 2012.

[13] O. O. Okoh, & A. J. Afolayan, " The Effects of Hydrodistillation and Solvent Free Microwave Extraction Methods on the Chemical Composition and Toxicity of Essential Oils from the Leaves of Mentha longifolia L. subsp. Capensis," *African Journal of Pharmacy and Pharmacology*, vol.5(22), pp. 2474-2478, 2011

[14] H. Wang, Y. Liu, S. Wei and Z. Yan, "Comparative seasonal variation and chemical composition of essential oils from the leaves and stems of Schefflera heptaphylla using microwave-assisted and conventional hydrodistillation," *Journal of Indutrial Crops and Product*, vol. 36, pp. 229-237, 2010

[15] I. A. Owokotomo, O. Ekundayo, & O. Dina , "Ocimim Gratissium : The Brine Shrimps Lethality of a New Chemotype Grown in South Western Nigeria," *Global Journal of Science Frontier Research*, vol. 12(6):, pp. 45-49, 2012

[16] B. N. Meyer, N.R Ferrigni, J. E. Putnam, L. B. Jacobsen, D. E. Nichols, D. S. Moore & J. L. McLaughlin, " Brine shrimp: a convenient general bioassay for active plants constituents,," *Planta Med*, vol.45, pp.31-34, 1982

[17] M. Al-Amin, & M. R. Islam, "Synthesis of some bis-triazole derivatives as probes for cytotoxicity study," *Bangladesh Journal of Pharmacology*, vol.1, pp. 21-26, 2006

[18] S. Sasidharan , O. Jinxua, L. L. Yoga & S. Amutha, "In Vivo Toxicity Study of Ganoderma boninse," *African Journal of Pharmacy and Pharmacology*, vol. 5(16), pp. 1819-1823, 2011

[19] O. Gulmira,, D. Fatih, O. Temel, T. Nurhayat, E. W. David, I.K. Shabana, H. C. B. Kemal, D. Ahmet, & H. Ergin, "Gas Chromatographic –Mass Spectrometric Analysis of Volatiles Obtained by Four Different Techniques from Salvia rosifolia Sm., and Evaluation for Biological Activity," *Journal of Chromatography* A, vol.1217, pp. 741-748, 2010

[20] A. B. L. Djouahri. & Meklati.Y.B, "Effect of extraction method on chemical composition, antioxidant and anti-inflammatory activities of essential oil from the leaves of Algerian Tetraclinis articulate Masters," *Journal of Industrial Crops and Products*, vol. 44, pp.32-36, 2013

[21] G. Mohammad-Taghi and R. Karamatollah, "Comparison of microwave-assisted hydrodistillation with the traditional htdrodistillation method in the extraction of essential oil from Thymus vulgaris L.," *Journal of Food Chemistry*, vol. 109, pp. 925-930, 2008

[22] A. R. Mohamed, Y. I. Sallam, A. S. El-Leithy, & E.A Safaa, "Lemongrass (Cymbopogon citratus) Essential Oil as Affected by Drying Methods," *Journal of Annal of Agricultural Science*, vol.57(2), pp.113-116, 2012

[23] C. Veeraphant, V. Mahikittiku & N. Soonthornchareonnon, " Acarcidal Effects of Thai Herbal Essential Oils against Dermatophagoides pteronyssinus," *Journal of Pharmaceutical Science*, vol.38(3-4), pp. 1-12, 2011

[24] A.P Murray, M. A. Frontera, M. A. Tomas, & M. C. Mulet, "Gas Chromatography-Mass Spectrometry Study of the Essential Oils of Schinus longifolia (Lindl.) speg., Schinus fasciculata (Griseb.) I. M. Johnst., and Schinus areira L.," *Journal of biosciences*, vol.60(1-2), pp. 25-9, 2005

[25] S. I. Rabbani, D. Kshama & N. Zahra, "Anti-Clastogenic Effects of Citral," *Iranian Journal Of Pharmacology & Therapeutics*, vol.4, pp.28-31,2004

[26] L. P. Sharlene, M. M. André, T. S. Roberto, A. C. K. Maria & Z. S. Gisel, "Vasodilator Activity of the Essential Oil from Aerial Parts of Pectis brevipedunculata and Its Main Constituent Citral in Rat Aorta," *Molecules*, vol.18, pp.3072-3085, 2013

[27] M. Majnooni, K. Mansouri, M. Gholivand, A. Mostafaie, H. Motlagh, N. Afnanzade, M. Abolghasemi & M. Piriyaei, "Chemical Composition, Cytotoxicity and Antioxidant Activities of the Essential Oil from the Leaves of Citrus aurantium L," *African Journal Biotechnology*, vol.11, pp. 498-503, 2012

[28] M. M. Saleh , F. A. Hashem & K.A Glombitza, "Cytotoxicity And In Vitro Effects On Human Cancer Cell Lines Of Volatiles Of Apium Graveolens Var. Filicinum," *Pharmaceutical Pharmacological Letters*, vol.8(2), pp. 97-99, 1998

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