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Extraction of Bio-Active Compounds Extracted from *Inula Helenium* Roots by Leaching Process.

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ABSTRACT

Leaching process applied for the extraction of bio active compounds from dried roots of (Elecampane) *Inula helenium*. Ethanol, hexane and distillated water were used as solvents. Roots were soaked with ethanol (5% w/v) with various concentration of ethanol (30 to 98%) at one day to know effect concentration of the solvent with concentration of bio active compound in *Inula helenium*. The same procedure was done using hexane as solvent. Also distilled water was used as solvent for extraction 5% (w/v) where plant material was soaked in water at different temperatures (25, 40, 65, 80, and 90) ˚C. In all solvents undertaken, the effect of time duration on active ingredient (Thymol, Isoalatolactone, Alatolactone, 10-isobutyryl-oxy 8-9-epoxy thymol isobutyrate, 10-isobutyryl-6-methoxy 8-9-epoxy thymol isobutyrate) was studied. HPLC analysis revealed that the extract contains several active constituents such a (Thymol, Isoalatolactone, Alatolactone, 10-isobutyryl-oxy 8-9-epoxy thymol isobutyrate, 10-isobutyryl-6-methoxy 8-9-epoxy thymol isobutyrate). The process provided an almost complete exhaustion of herbal mass and highly enriched final extract. The experimental results have shown that the greatest separation were obtained when using distillated water at 65˚C for one day, hexane at 98% concentration after 10 min from leaching process with mixing and when using ethanol at 70% concentration for one day.

Keywords: *Inula Helenium*, alantolactone, anti-tumor, HPLC analysis.

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INTRODUCTION

Elecampane *Inula helenium* (Compositae) is a widely occurring perennial herb in Europe and East Asia [1], but now grows in many high temperature regions of the world [2]. The pharmacological activity of elecmpane is related to the content of mainly sesquiterpene lactones such as alantolactone, isoalantolactone, dihydroalantolactone, etc. [3] and the roots contain up to 5% of essential oil, thymol derivatives, triterpenes, sterols and polysaccharide [4,5]. Inula helenium L. (Compositae) and its active components have been widely used as anti-inflammatory, anti-microbial, and anti-cancer agents [6].

The roots have been traditionally used as an expectorant, antitussive, diaphoretic and bactericidal agent in folk medicine [7]. It is known that herb is used for cold, flu, pain, skin infections, parasite infections, anti-ulcer, anti-inflammatory and anti-tumor functions it is rich in inulin and helenin (alantolactone) components with potentials of anticancer effects , it contains chemical alantolactone with allergic response that restricts its use even in skin dermatitis However, examining whether the extract of *Inula helenium* could inhibit the growth of human oral cancer cells [8-10] extract of the roots of this plant showed antiproliferative activities against three tumor cell lines: human gastric adenocarcinoma cells, human uterus carcinoma and mouse melanoma [1] and therefore, that it may have potential properties for anti-tumor drug discovery [9,10].

Using ethanol by ultrasound-assisted extraction (UAE) of alantolactone and isoalantolactone from the roots of *Inula helenium* L. and Gas chromatographic (GC) method was used for determination the medical contents in the investigated extracts [11]. Bioassay-guided fractionation of the *I. helenium* hexane extract resulted in the isolation of alantolactone, isoalantolactone, and 11αH, 13-dihydroisoalantolactone. Activities of these three isolated constituents, as well as, those of synthetic isomers are reported [12]. Dried water extracts from *Inula helenium* roots obtained using ultrasound contained biologically active substances. It has been demonstrated that the major active low-molecular components of roots of *I. helenium* L. are alantolactone and isoalantolactone [4]. It uses sound waves at frequencies above the range audible to humans to disrupt the plant cell wall, thereby enhancing solvent penetration into the plant material and facilitating the release of extracts [13]. In recent years, the application for the isolation of various biologically active compounds from plant materials has been reported [14].

Nowadays, renewed interest has grown in the use of medicinal plants as a source of naturally bioactive compounds. Extraction is the first key step in isolation of biologically active compounds so we applied that on extraction of *Inula helenium*.

MATERIALS AND METHODS

Equipments

The separation occurred on liquid chromatography Shimadzu 10 AV-LC equipped with binary delivery pump model LC-10A Shimadzu; the eluted peaks were monitored by UV-Vis 10 A- SPD spectrophotometer. Hot plate magnetic stirrer (FINETECH) Korea made and Ultrasonic bath.

Reagents

Hexane, ethanol and distilled water were used as extracting solvents of active compounds.

Acetic acid, acetonitrile, deionized water, methanol HPLC and liquid N_2 were used for HPLC analysis. Plant material – dried aerial parts (roots) of *Inula helenium* was ground, homogenized, and stored in dark place. Pure *Inula helenium* standard was obtained from Malaysia.

Preparation of plant samples and bioactive compounds extraction

Inula helenium roots were obtained from herbal drugstore, dried in the shade either at room temperature or at 50˚C. Dried and grounded *Inula helenium* was used as the starting material. The prepared sample, with an approximately 5% moisture content, was stored in a dry container for subsequent use. Leaching process was performed at room temperature with continuous stirring by magnetic stirrer at 10 min to homogenate the solution; this was followed by leaching operation but without stirring. Ethanol, hexane and distilled water, were used as solvent for bio -active extraction. The liquid solutions obtained at the end of each

experiment were filtered through a cloth to remove large root pieces and then through a filter paper to remove smaller particles. The extracts were stored in capped dark glass bottles either at room temperature or at 4˚C. For routine testing, extracts were stored at room temperature a reduction of activity was not observed when compared with cooled extracts [15]. It is obvious from HPLC analysis that the extract contains several active constituents such a (Thymol, Isoalatolactone, Alatolactone, 10-isobutyryl-oxy 8-9-epoxy thymol isobutyrate and 10-isobutyryl-6-methoxy 8-9-epoxy thymol isobutyrate). The retention time of these compounds was presented in Table (1). For primary extract purification and concentration, ethanol 5% (w/v) with various concentration of ethanol (30-98%), hexane and distilled water were used as solvents for extraction of plant material. The water treatment was done at different temperatures (25, 40, 65, 80, and 90 ˚C) and the solvents were tested with the gradual time increase (1-3 days).

Table 1: Eluted material of the standard

Subjects	Retention time (min)
Thymol	0.98
Isoalatolactone	1.80
Alatolactone	2.97
10-isobutyryl-oxy 8-9-epoxy thymol isobutyrate	3.97
10-isobutyryl-6-methoxy 8-9-epoxy thymol isobutyrate	4.63

HPLC Analysis

The active ingredients of *Inula helenium* (Areek har) was analyzed by HPLC. The alcoholic extract in closed tube was separated on FLC (Fast Liquid Chromatographic) column under the optimum condition (column 3µm particle size, 50*4.6 mm I.D, C-18 DB column). The mobile phase was 0.1% acetic acid in deionized water: acetonitrile (20:80 v/v) detection UV set at 264 nm and the flow rate was 0.8 ml/min.

For preparation of sample, 0.5 g of each sample was weighted, then dissolved in 10 ml methanol for HPLC, the sample was shacked in Ultrasonic bath for 10 minutes, then concentrated by evaporating the solvent with a stream of liquid N_2 until it reaches nearly 0.5 ml, then adding some of the mobile phase to reach 1 ml. Twenty µl were injected into HPLC column. The concentration for each compound was quantitatively determined by comparison with the peak area of the standard sample.

$$Concentration\ of\ sample\ (\mu g/ml) = \frac{area\ of\ sample}{area\ of\ standard} \times conc.of\ standard \times dilution\ factor$$

RESULTS AND DISCUSSIONS

In the present work, three different methods for extraction of bioactive compounds from *Inula helenium* were used. Extraction methods were ethanol, hexane, and water extraction. All extraction methods were used in these experiments produced the same kind of chemicals with different concentrations. They were Thymol, Isoalatolactone, Alatolactone, 10-isobutyryl-oxy 8-9-epoxy thymol isobutyrate and 10-isobutyryl-6-methoxy 8-9-epoxy thymol isobutyrate. The sequences of the eluted material of the standard were as shown in Fig. (1), each standard was 25µg/ml.

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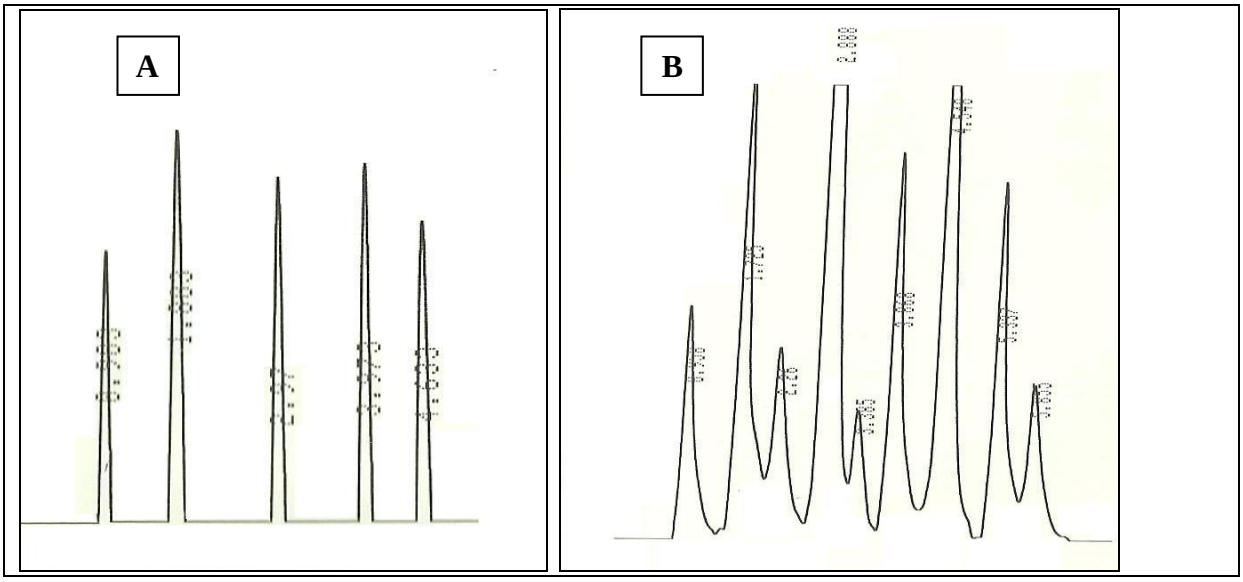

Figure 1: Dynamics of bio-active concentration in liquid phase extraction (A) standard and (B) sample.

Ethanol extraction method

Effect of extraction time

Fig. (2) shows the influence of extraction time (1-3 day) on the chemicals concentration at constant weight of roots powder (5% w/v) and constant ethanol concentration at 98%. It was found that the chemicals concentration increased with increasing of extraction time up to one day. After that, the increasing of chemicals concentration became not significant between 1 to 3 days for all ingredients. This could be due to the fact that higher extraction percentage of chemicals in *Inula helenium* powder was obtained on the first day. Similar results were obtained by Jansa *et al.* (16).

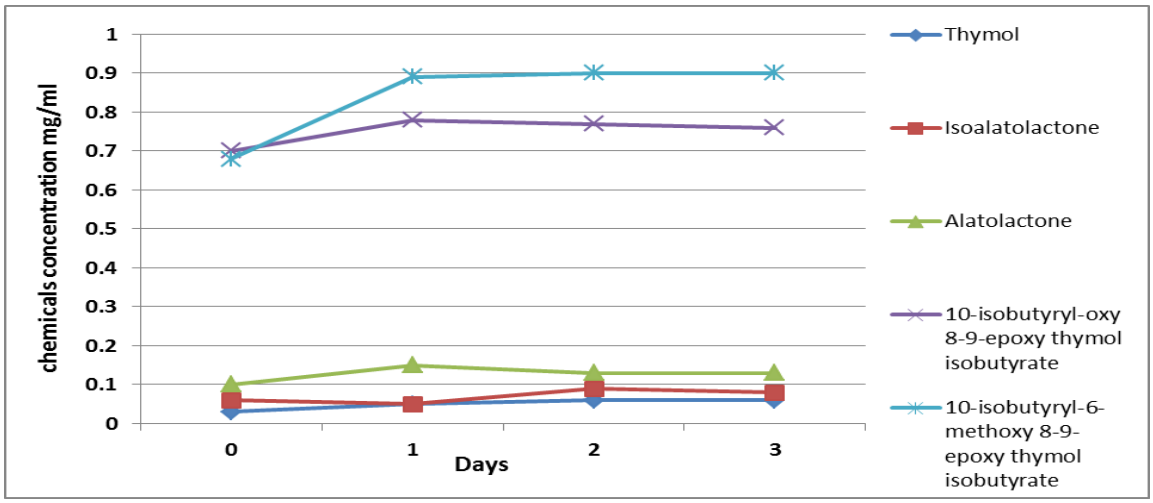

Figure 2: Effect of different extraction time on chemicals concentration using 98% ethanol and 5% w/v of *Inula helenium*).

Effect of ethanol concentration

The effect of ethanol concentrations on bioactive compounds extraction from *Inula helenium* at constant weight of roots powder 5% (w/v) and one day time of extraction was presented in Figure (3). It was found that the concentrations of the chemical compounds increased with increasing of ethanol concentration from 30 to 70%. And then, they were reached lower concentration at 98% ethanol concentration (Fig. 3). That

was because ethanol helped to exhausting the active compounds from roots but if the ethanol concentration increased above 70%, a decrease in the concentration of compounds happened because some of the compounds in the roots are water soluble. So that a better extraction was obtained if the ratio of ethanol to water as (70:30) (17).

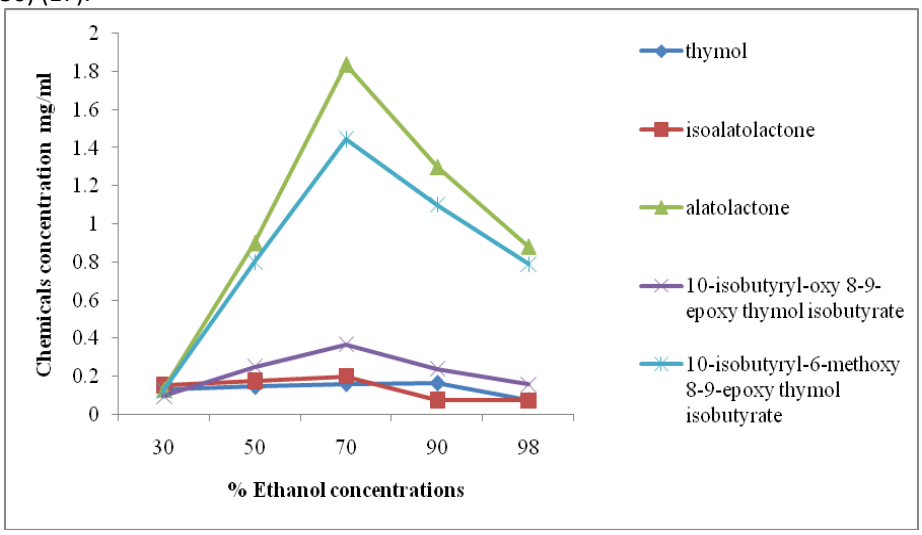

Figure 3: Effect of different ethanol concentrations on bioactive compounds concentration at one day and 5% w/v of *Inula helenium*.

Hexane extraction method

Effect of extraction time

Fig. (4) shows the influence of extraction time (1-3 day) on the bioactive compounds concentration at constant weight of roots powder 5% (w/v) and constant hexane concentration 98%. It was found that the chemicals concentration decreased with increasing of extraction time. So, there is no need to continuous with the leaching process if was hexane as solvent (18).

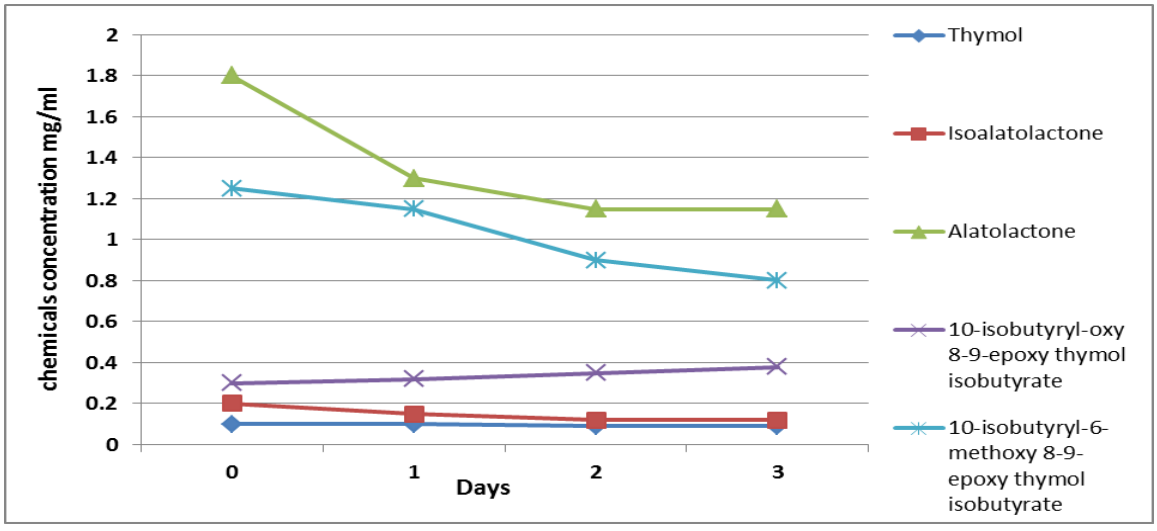

Figure 4: Effect of different extraction time on bioactive compounds concentration using 98% hexane and 5% w/v of *Inula helenium*.

Effect of hexane concentration

Fig. (5) show the effect of hexane concentration on the extraction of bioactive compounds at constant weight of roots powder (5% wt/v) and extraction time (1 day). It was found that the concentrations of the

bioactive compounds decreased with increasing of hexane concentration. This is may be due to inhibitory effect of hexane on extraction of compounds. Similar finding was reported by Spiridon *et al.* (18).

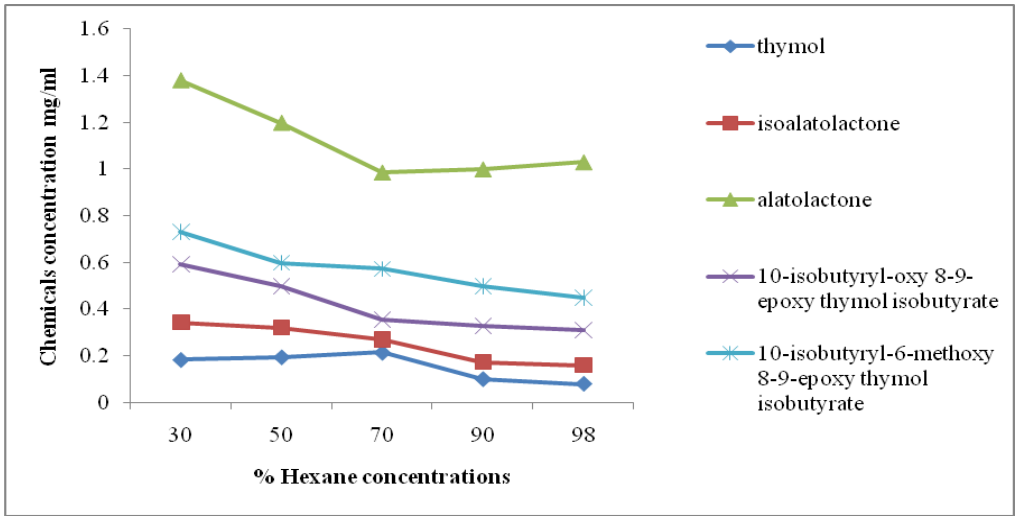

Figure 5: Effect of different hexane concentration on bioactive compounds concentration at one day and 5% w/v of *Inula helenium*.

Water extraction method

Effect of extraction time

Fig. (6) shows the influence of extraction time (1-3 days) on the bioactive compounds concentration at constant weight of roots powder 5% (w/v) and at room temperature. It was found that the bioactive compounds concentration after one day was nearly constant with extraction time in leaching process. Therefore; the use of one day in the process of extraction is the best time in order to have the highest concentrations of active compounds in the plant Elecampane. From that, we can conclude increase the length of extraction time for one day is considered a negative factor in the leaching process and all solvents (19).

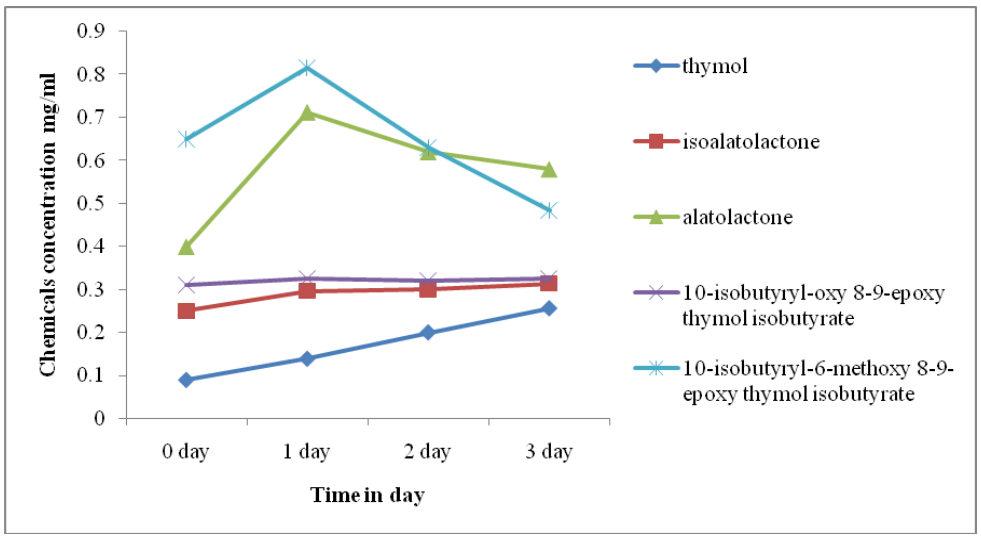

Figure 6: Effect of different extraction time on bioactive compounds concentration using water at room temperature and 5% w/v of *Inula helenium*.

Effect of temperature on the extraction

Fig. (7) shows the influence of temperature effect on the bioactive compounds concentration at constant weight of roots powder 5(% w/v) and at 1 day. It was found that the concentration was increased with increasing of temperature that occurred due to the fact that hot water lead to exhausting actives compounds, but higher temperatures break the bonds of the ring of the active ingredient in the plant roots of Elecampane (20).

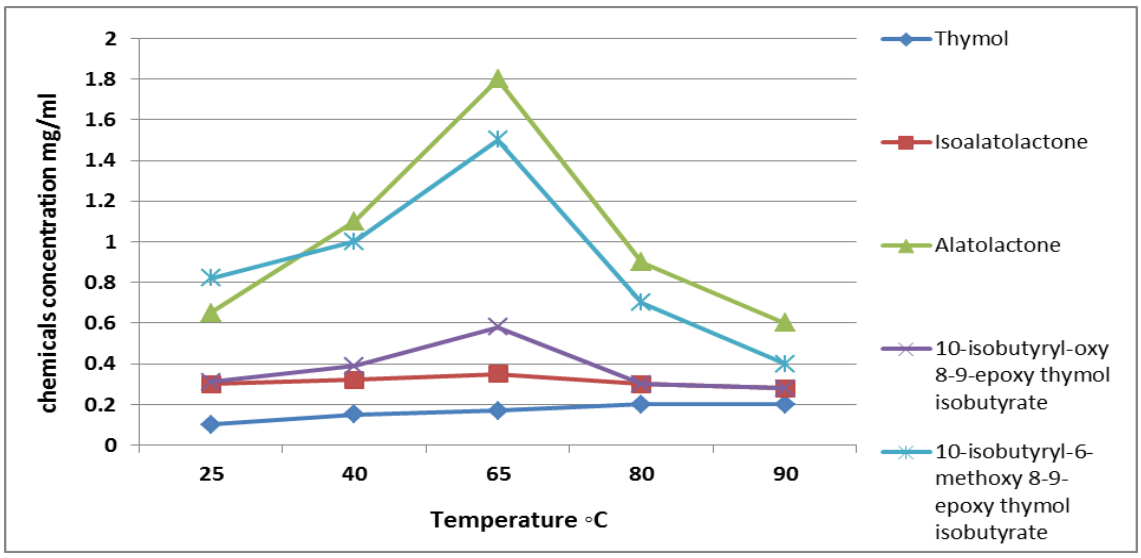

Figure 7: Effect of different temperature on bioactive compounds concentration at one day and 5% w/v of *Inula helenium*.

CONCLUSION

Results of the present work show that the active compounds concentration in batch and leaching process for the extraction of Thymol, Isoalatolactone , Alatolactone, 10-isobutyryl-oxy 8-9-epoxy thymol isobutyrate, 10-isobutyryl-6-methoxy 8-9-epoxy thymol isobutyrate. The process provided an almost complete exhaustion of herbal mass and highly enriched final extract. The experimental results have shown that the greatest separation was obtained when using distillated water at 65˚C for one day, hexane without leaching process and when using ethanol at 70% concentration.

REFERENCES

[1] Tenji Konishi, Yasuo Shimada, Tsuneatsu Nagao, Hikaru Okabe, and Takao Konoshima. Biol Pharm Bull 2002;25 (10): 1370-1372.
[2] Steven H. An Electronic Journal for NSP Distribution 2003;19:11.
[3] Jasna M Canadanovic-Brunet, Sonja M Dilas, Gordana S Cetkovic, Vesna T Tumbas and Zoranka N Malesevic 2002; 33: 127-134.
[4] Anna Stojakowska, Janusz Malarz, and Wanda Kisiel Z. Naturforsch. C. J Biosci 2004;59c: 606-608.
[5] Batbayar ND, Banzragch KT Inngjerdingen, R Naran, TE Michaelsena and BS Paulsen. Asian J Trad Med 2008;3(1): 33-41.
[6] Eun Jung Park, Young Min Kim, Sang Won Park, Hye Jung Kim, Jae Heun Lee, Dong-Ung Lee, and Ki Churl Chang. Food Chem Toxicol J 2013;55: 386- 395.
[7] Yong-Ming Zhao, Yu-jin Wang, Mei Dong, Man-Li Zhang, Chang-Hong Huo, Yu-Cheng Gu and Qing-Wen Shi; Published in Khimiya Prirodnykh Soedinenii, (2010), 3: 315–317.
[8] Rakesh Sharma. The Open Nutraceuticals J 2010;3: 129-140.
[9] Lee M, et al. Pharmacol Ther Toxicol 2011.

[10] Dorn DC, Alexenizer M, Hengstler JG, Dorn A., Source Laboratory of Developmental Hematopoiesis, Cell Biology Program, Memorial Sloan-Kettering Cancer Center, 2006;20(11): 970-980.
[11] Antoaneta Trendafilova, Christo Chanev, Milka Todorova. 2010;6(23): 234-237.
[12] Charles L Cantrell, Leonid K Mamonov, Natalja Ryabushkina, Tatyana S Kustova, Nikolaus H Fischer, and Kevin K Schrader. ARKIVOC 2007:65.
[13] Mason TJ, Paniwnyk L, Lorimer JP. Ultrason Sonochem 1996;3 (3): 253–260
[14] Huie CW A. Anal Bioanal Chem 2002;373: 23–30.
[15] Maja Alexenizer and August Dorn. 2007;80: 205-215.
[16] Jasna M, et al. APTEFF 2002;33(1-174): 127- 134.
[17] Batbayar N, et al. Asian J Trad Med 2008;3(1): 33- 41.
[18] Spiridon I, Bodirlau R and Teaca C. Cent Eur J Biol 2011;6(3): 388-396.
[19] Spiridon I, et al. Cent. Eur J Chem 2013;11(10): 1699-1709.
[20] Zheng LL, Wang D, Li YY, Peng HY, Yuan MY, Gao F. Phcog Mag 2014;10:(S1):141-146.