LJAST
Libyan Journal of Applied Science and Technology
مجلة ليبيا للعلوم التطبيقية والتقنية

Volume11 Issue 02
December 2023
ISSN 2958-6119

Estimation and stability study of oleuropein from olive leaves extract (Olea europaea L.)

Amna S. Elgubbi[a,*] , Rabia M.Elburki[b], A.L.Alajtal[a]
a.elgubbi@sci.misuratau.edu.ly, rabia92elburky@gmsil.com, alajtal6@yahoo.com
a Chemistry Department, Faculty of Science, Misurata University, 2478, Misurata, Libya
b pharmacognosy Department, faculty of pharmacy, Misurata University, 2478, Misurata,Libya
E-mail *a.elgubbi@sci.misuratau.edu.ly
http://orcid.org/0009-0009-0450-1447
Received 3 July 2023; revised 12 August 2023; accepted 18 August 2023

Abstract

This study included extraction and estimation of oleuropein concentration in different types of olive leaves, it is the most important medicinal plant that is widely distributed in Libya and is used in floclore medicine. The effects of different parameters (solvent, temperature, pH, extraction method and time) during and after extraction were investigated. The obtained results showed that the maximum amount of Oleuropein is found in olive leaves from chemlali and Corantina. Furthermore, the soxhlet approach outperforms the maceration method for extraction, and the optimal conditions for preserving the compound's stability when employing dioxane as a solvent were dioxane 60% at 25° C and pH3

Keywords: Oleuropein, dioxane, olive leaves, UV-Spectrophotometer, Soxhlet

1. Introduction

The olive leaves contain significant amounts of phytochemicals, which are correlated with an array of health effects [1]. The extract of olive leaves helps to lower blood pressure in cases of hypertension, reduces levels of bad cholesterol (LDL), stimulates immune system, and fights the cold and flu. It also has antifungal and antibacterial prooerties, and the natural antioxidants present help to fight free radicals and detoxify of carcinogens and harmful chemicals [2].

polyphenols are the most known group of bioactive compounds in olive leaf extracts. Oleuropein and its derivatives, such as oleuropein aglycone, oleuropein-aglycone di-aldehyde, ligstroside, and hydoxytyrosol are the most characteristic phenolic compounds in olive leaves [1]. Oleuropein concentration can reach up to 140mg/g on a dry matter basis in young olives [3], and 60-90mg/g of dry matter in the leaves [4].

There are several factors affecting Oleuropein extraction, such as enzyme effect, solvent, effect of pH and temperature. In addition, the qualitative and quantitative composition of olive leaves depends on several conditions, such as climatic and storage conditions, geographical origin, the drying and extraction methods [1].

There are several studies that have showed that Oleuropein contents are dependent on varieties of olive leaves, Geographic origin, time of cultivation, type of extraction method, temperature, pH and solvent type.

Himours et al,2017. Studied oleuropein content on olive leaves extract from two varieties of Olea Europea L (Chemlali and Dathier) [5], Hadeel Homssi, Muberra Kosar* 2019. Determined oleuropein amounts of olive leaves from different regions of Northern Cyprus [6]. Chaleb TayouB,Huda Sulaima et al 2012. Studied determination of Oleuropein in leaves and fruits of some Syrian Olive varieties [7]. Ibraheem AFaneh et al., 2015. studied the effect of olive leaves drying on the content of oleuropein and found that the highest Oleuropein content with olive leaves dried at ambient temperature and extraction performed with 20% acetonitrile [8]. Yateem, H et al. (2014) Studied optimum conditions for oleuropein extraction from olive leaves and found that the highest Oleuropein yield with ethanol 80%. In addition, there was an increase in Oleuropein content with an increase in temperature, the ideal temperature was 60c◦, while pH 3 was ideal pH, where soxhlet extraction was given the highest Oleuropein content [9]. Nasirs.S.A.Malik, and Joe M Bradford,2008. Studied the recovery and stability of oleuropein and other phenolic compounds and showed that oleuropein and other polyphenols in methanol extracts were quite stable for 30 days [10]. Mohammad Shah Faisal et al. (2015). Studied the development and validation of the UV Spectrophotometer method for the quick estimation of olive leaf extract in pharmaceutical formulations. The study showed that the methods were accurate and precise, as indicated by the good recoveries of the drugs and low % RSD values. The proposed methods could be applied for routine analysis for quantitative determination of the olive leaf extract in both pure and dosage forms [11]. Therefore, there was no literature review to estimate Oleuropein content by the UV Spectrophotometer method, there was also no scientific literature to estimate the Oleuropein content from olive leaves in Libya, and there were no studies dealing with dioxane solvent for Oleuropein extraction. The object of current work to estimate Oleuropein content of different types of olive leaves in Misurata, Libya. The study also aims to find the best conditions to extract the highest amount of Oleuropein, and study the stability of different extracts.

2.Materials and methods:

2.1. Sample collection:

Different types of green, fresh Olive leaves (chemlali, Arbequina, Corantina) were randomly collected from different areas of Misurata, Libya, in middle of November, And from different positions from olive trees.

2.2.sample drying:

Fresh green olive leaves were dried at ambient temperature (25C°) for 7-8 days. The dried samples were then ground to obtain powder, which was stored in the dark container at room temperature until extraction.

2.3. chemicales:

Oleuropein standard 75%(Pro Health, USA), Dioxane (MRS SCIENTIFIC UK), Ethanol (CARLOERBA.France).

2.4. preparation of Standard stock solution:

The accurate amount of 10 mg oleuropein standard was weighted and dissolved in 10ml (Ethanol 80%) and then diluted to 100ml.

2.5. Preparation of calibration curve for oleuropein for UV-spectroscopy:

Oleuropein standards were prepared at 1mg/ml concentration for stock solution and the five dilutions (0.005,0.01,0.02,0.03,0.05mg/ml) were prepared for the calibration curve. The absorbance of solution was measured at 280 nm with a UV- spectrophotometer (ALigent).

2.6. Extraction of olive leaves with 80%Ethanol and 60% Dioxane:

15 grams of Arbequina olive leaves powder were placed in the thimble of a soxhelt apparatus and then extracted with 300ml of solvent mixture for 4 hours, with Ethanol 80% at 60c◦ and/or with Dioxane 60% at 75c◦. The extract was cooled at room temperature and then filtered off. The filtrate extracts were then evaporated in a rotatory evaporator at room temperature under vacuum for 2hours. The concentrated extracts were stored in a refrigerator at 2c◦ to 8c◦ until analyzed.

2.7. Extraction of chemlali olive leaves with maceration with different temperature:

5 grams of olive leaves powder were macerated with 50 ml Dioxane 60% at different temperature 25c◦, 40c°,75c◦ for 4hours, the extract was filtrated off, then the filtrate was evaporated to get a concentrated extrac,t which was stored in the refrigerator at 2-8c◦ until analyzed.

2.8. Extraction of chemlali olive leaves with different PH (Dioxane 60%):

5 grams of chemlali olive leaves were macerated with 50 ml (Dioxane 60%) at PH (3, 5,7,9) for 4 hours at room temperature. The pH was adjusted with 0.1N HCL and/or 0.1N NaOH. The extract was filtrated off, then the filtrate was evaporated to get concentrated extract, which was stored in the refrigerator at 2-8c◦ until analyzed.

2.9. Extraction of chemlali olive leaves with different PH (Dioxane 60%) at 60c°:

5 grams of chemlali olive leaves were macerated with 50 ml (Dioxane 60%), at PH (3,9) for 4hours at 60c◦, the extract was filtrated, and the filtrate was evaporated to get concentrated extract which was stored in the refrigerator at 2-8c◦ until analyzed.

2.10. Preparation of sample solution for UV- spectrophotometer analysis:

0.1ml of each extract was diluted to 10 ml with ethanol 80% and/or Dioxane60%. The absorbance of the solution was measured by a UV- spectrophotometer at 280nm against a blank.

2.11. Stability study of the analyte:

Known concentrations of oleuropein for Ethanol, Dioxane, and Dioxane extract with pH (3,5,7,9) were prepared, and in order to minimise the possible degradation of the analyte, they were had stored in dark colored vials and kept refrigerated at 2-8c. At room temperature, samples were drawn on days 1,7,14,21,30 days, and assayed for their oleuropein concentration.

2.12. statistical analysis:

Data were subjected to statistical analysis using Package for Social Science-21(SPSS).The data were presented as the mean± standard deviation. Difference were considered significant at $P<0.05$. comparing the mean concentration for different solvents and pH at different days by using one-way analysis of variance(ANOVA), followed by Fisher"s Least Significant Difference (LSD) test.

3.Results and discussion:

3.1. Calibration curve for Oleuropein standard:

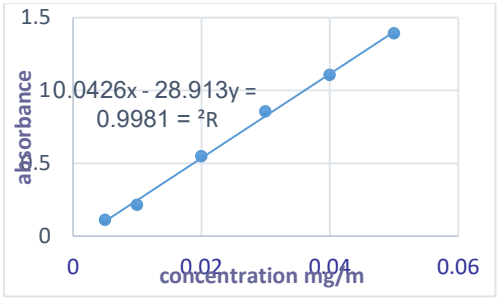

Figure3.1: calibration curve at 280nm by UV-Visible spectrophotometer

The regression equation was found to be y=28.913x-0.0426. The correlation coefficient(r^2) of the standard curve was found to be greater than 0.998 (figure 3.1).

3.2. Extraction of olive leaves with Ethanol 80% and Dioxane 60%:

The results showed that the effect of extracting solvent on oleuropein content by two types of solvent with soxhlet extraction, Oleuropein content for Arbequina olive leaves with ethanol 80% (35.41mg/g) (3.5%) was significantly ($p<0.005$) higher than dioxane 60% (31.01mg/g) (3.1%) (table 3.1).

This may be due to the fact that ethanol 80% has high polarity and the ability to form intermolecular hydrogen bonds with polar components, and dioxane 60% was a good solvent due to its ability to form hydrogen bond with Oleuropein.

Ethanol 80% and dioxane 60% increase solubility of polyphenols because the addition of water to organic solvents weakens hydrogen bonds between polyphenol and protein in cells [12].

Table 3.1: Oleuropein content of Arbequina olive leaves by soxhelt extraction**:**

Solvent type	Oleuropein content (mg/g) 280nm	percentage
Ethanol 80%	35.41 ±0.687[ab]	3.5
Dioxane 60%	31.01 ±0.317[ab]	3.1

[ab]…significant difference, n=3 (P=0.001) t-test=0.001 at 280nm

3.3. Extaction of chemlali and Corantina olive leaves with Dioxane 60%:

The determination of oleuropein from different types of olive leaves were studied by extraction with soxhlet method with the same solvent. The results showed that Chemlali and Corantina olive leaves have higher oleuropein content (54.66mg/g) (5.5%) for chemlali olive leaves and (54.55mg/g)(5.5%) for Corantina olive leaves than Arbequina olive leaves (31.01mg/g) (3.1%) at 280nm (table 3.2).

This difference was probably releated to the genetic features of each variety and their growth under the same climatic and geographical conditions. When genetic difference is great, which resides in genes, the morophology and biochemical diversity for each species are different. They can make a difference in amount or the type of chemical conistuents produced. Whenever such biochemical variation occur, each particular type is known as a physiological variety[13].

Table 3.2: Oleuropein content of olive leaves by soxhelt:

Type of olive leaves	Solvent type	Oleuropein content (mg/g) 280nm	Percentage
Arbequina olive leaves	Dioxane 60%	31.01 ±0.317	3.1
Chemlali olive leaves	Dioxane 60%	54.66±0.306	5.5
Corantina olive leaves	Dioxane 60%	54.55 ±0.234	5.5

t-test=92.474 p=0.000, for Arbequina and chemlali olive leaves, t-test=8.594 p=0.000 for Arbequina and Corantina olive leaves

t-test=1.873 p=0.134, for chemlali and Corantina olive leaves.

3.4. Extraction of chemlali olive leaves with maceration with different temperature:

The eEffect of temperature on Oleuropein content was studied by maceration of olive leave powder with dioxane 60% at different temperature. There was a decrease in oleuropein yield with an increase in temperature by the maceration method, with the highest oleuropein amount at 25c◦(21.68mg/g) (2.2%), followed by (16.50mg/g) (1.7%) at 40c◦ and (13.06mg/g) (1.3%) at 75c◦ (table3.3).

This is due to the use of dioxane 60% as a solvent with a high temperature, which causes rapid oxidation of the hydroxyl group. This is a basic characteristic for solvents, tempertures above 50c◦,70c◦ cause rapid polyphenol degradation[14] and activtion of enzymes responsible for Oleuropein degradation.

Table3.3: Oleuropein content of chemlali olive leaves by maceration with different temperature:

Temperature	Solvent type	Oleuropein content (mg/g)	Percentage
25c°	Dioxane 60%	21.68 ±0.201^{ab}	2.2
40c°	Dioxane 60%	16.50 ±0.234^{ab}	1.7
75c°	Dioxane 60%	13.06 ±0.099^{ab}	1.3

^{ab}…significant difference

(p=0.000) T-test=29.062 at 25c° and 40c°, (p=0.000) T-test=66.644 at 25c° and 75c°,

(p=0.000) T-test 23.459 at40c°and 75c°

3.5:Oleuropein content of chemlali olive leaves by soxhelt and maceration method:

Soxhelt method given significant (p<0.05) higher oleuropein content (54.66mg/g) than Maceration method at different temperatures 25c◦(21.68mg/g), 40c◦(16.50mg/g), 75c◦(13.06mg/g) figure (3.2).

This is because soxhelt extraction is more efficient for a short time than maceration.The maceration method for extraction requires a longer time for extraction at 25c°, because soluble phenolics are present within the plant cell vaculoes. [15]

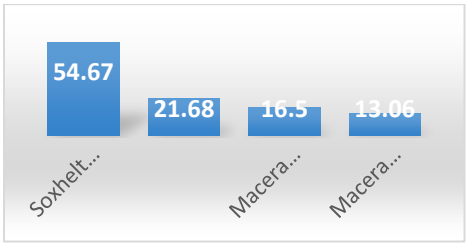

Figure3.2: Oleuropein content with soxhlet and maceration method

3.6. Extraction of chemlali olive leaves with different pH (Dioxane60%):

The extraction of oleuropein was performed with dioxane 60% maceration with different pH. The highest oleuropein content is at pH 3 (38.87mg/g) (3.9%) and pH 9 (38.79mg/g) (3.9%), followed by pH 5 (28.32mg/g) (2.8%), pH 7 (20.30mg/g) (2%) table (3.4).

The highest amount of Oleuropein at pH 3 because polyphenols are weakly acidic and generally more stable at low pH, and acidic condition helps polyphenol to stay neutral, thus to be easily extracted into organic solvent[16].The effect of acid on glycoside bonds helps to breaking down glycoside bonds.

Dioxane is a basic solvent, so extraction occurs more efficiently at an alkaline pH. Extraction with dioxane 60% at pH 5 (2.8%) is relatively quite, due to the fact that Oleuropein is weakly acidic, so it is quite soluble at pH 5. The least amount of Oleuropein extraction is at pH 7 (2%), which may be an unsuitable PH for Oleuropein extraction. In science, Oleuropein Polyphenol (weak acid) is not a suitable PH to maintaining the stability of polyphenols.

Table 3.4: Oleuropein content of chemlali olive leaves by maceration method with different PH(60% dioxane):

pH	Oleuropein content(mg/g)	Percentage
pH 3	38.87 ±0.208^{ab}	3.9
pH 5	28.32 ±0.416^{ab}	2.8
pH 7	20.30 ±0.244^{ab}	2.0
pH 9	38.79 ±0.238^{ab}	3.9

(P=0.817)T-test=0.248 at pH 3 and pH 9, (p=0.000) T-test=39.266 at pH 3 and pH 5

(p=0.000)T-test=100.152 at pH 3 and pH 7, (p=0.000) T-Test=28.776 at pH7and pH 5

(p=0.000)T- test=37.790 at pH 5 and pH 9, (p=0.000) T-test=93.678 at pH 7 and pH 9

3.7. Oleuropein content of chemlali olive leaves by maceration (dioxane60%) with different pH at 60c◦:

Extraction was performed by maceration of olive leaves powder with dioxane 60% (pH 3, pH 9) at 60c◦. Tthe results showed that there was 70% decreasing in Oleuropein content at PH 3 (11.76mg/g) (1.2%) and 60% at PH 9 (16.20mg/g) (1.6%) at 60c◦, compared to extraction by maceration at 25c◦ (table 3.5).

This is due to a high temperture with acidic and alkaline pH that facilitates Oleuropein degradation, first, catalyst hydrolysis of glycoside bonds to Oleuropein aglycon and glucose by acid, and then catalyst ester bond hydrolysis on Oleuropein aglycon to produce hydroxytyrosol and elonic acid. Science temperature at 60c◦ acts as catalyst for this reaction more than assists in extraction.

Table3.5: Oleuropein content of chemlali olive leaves by maceration (60% dioxane) at 25c◦; 60c◦:

pH	Oleuropein content (mg/g) 25c◦	Percentage	Oleuropein content (mg/g) 60c◦	percentage
pH3	38.87 ±0.208[ab]	3.9	11.76 ±0.111[ab]	1.2
pH9	38.79 ±0.238[ab]	3.9	16.20±0.043[ab]	1.6

[ab]:non-significant T-test=199.608 p=0.000 for pH 3 at 25c◦ and 60c◦

T-test=1.55.545 p=0.000 for pH 9 at 25c◦ and 60c◦

3.8. Stability of oleuropein for ethanolic extract:

Stability of oleuropein with the same initial concentration in an ethanol 80% solvent at 25c◦ and 2-8c◦ for 30 days was studied. The results showed that the initial Oleuropein concentration(0.061mg/ml) was stable for ethanolic extract at room temperature and 2-8c° for 30 days table (3.6).

This is due to the ability of ethanol 80% to form hydrogen bonds with polyphenols and act as a preservative to prevent microbial growth.

Microbial growth produces enzymes responsible for oleuropein degradion.

Table 3.6: Oleuropein concentration of ethanol 80% extract for 30 days:

time	concentration (mg/ml) 25c◦	Concentration (mg/ml) 2-8c°
1st day	0.0614±0.0004	0.0614±0.0004
7th day	0.0621±0.0006	0.0666±0.0004
14th day	0.0636±0.0003	0.0605±0.0005
21st day	0.0633±0.0003	0.0633±0.0003
30th day	0.0636±0.0003	0.0696±0.0003

3.9. Stability of oleuropein for dioxane extract:

Stability of oleuropein with the same initial concentration with dioxane 60% solvent at 25c◦ and 2-8c◦ for 30 days was studied. The results showed that there was a non-significant difference in Oleuropein concentration (0.0617-0.0589mg/ml) for dioxane 60% extract from the first to the seventh day at refrigerated and room temperature figure(3.3). There was a decrease (72.6%) from e initial Oleuropein concentration (0.0159mg/ml) at room temperature, (0.0122mg/ml) at 2-8c◦ after 14 days, there was a slight increase in Oleuropein concentration to (0.0227mg/ml) (0.0239mg/ml) at 21st day at room temperature and 2-8c◦ (Table 3.7).

The dioxane 60% was stable for 7days due to the formation of hydrogen bonds. Dioxane 60% is a heterocyclic diether that contains two oxygen atoms with lone pairs of electrons and has the ability to form two hydrogen bonds with polar components. After 7 days hydrolysis occurs with a lose of 74.3% from the initial Oleuropein concentration at 14 days at RT and 2-8c°. This is due to the fact that the dioxane solvent has basic characteristics and acts on the hydrolysis of the ester bond and the ionizion of hydroxyl group for Oleuropein.

Table 3.7: Oleuropein concentration of 60% Dioxane extract for 30 days:

Time	Oleuropein concentration (mg/ml) 25c◦	Oleuropein concentration (mg/ml) 2-8c◦
1st day	0.0617±0.0003	0.0617±0.0003
7th day	0.0589±0.003	0.0589±0.0006
14th day	0.0159±0.0292	0.0122±0.0087
21st day	0.02270±0.003	0.0239±0.0003
30th day	0.02497±0.0006	0.0229±0.0007

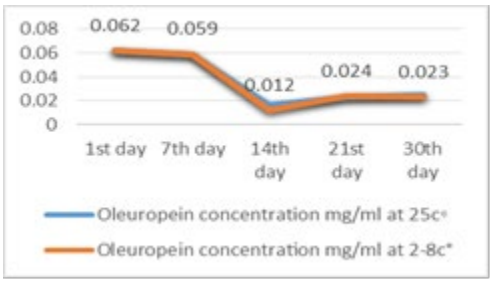

Figure 3.3: Oleuropein concentration for 60% dioxane with time

3.10. Stability of Oleuropein at different pH with 60% dioxane:

The stability of different extracts with different pHs at the same initial concentration was studied for 30 days. Oleuropein concentration is more stable at pH 5 (0.058mg/ml) and pH 3 (0.048mg/ml) than at pH 9 (0.034mg/ml)and at pH 7 (0.025mg/ml) at 30th day figure, (3.5). Oleuropein concentration was highly unstable at pH 3 (0.014mg/ml) after 7 days, followed by pH 9 (0.037mg/ml), pH5 (0.041mg/ml), pH 7 (0.044mg/ml). There was a gradual increase in concentration at pH 3 (0.046mg/ml), pH 5 (0.050mg/ml) at 14th day, pH 3 (0.048mg/ml), pH 5 (0.057,0.058mg/ml) at 21st and 30th days. Oleuropein concentration was nearly stable (P>0.005) at pH 9 (0.037-0.034mg/ml) from 7th to 30th day. There was a significantly gradual decrease in Oleuropein concentration at pH 7 (0.035-0.025mg/ml) from 7th to 30th day table (3.8).

Oleuropein concentration was highly unstable at pH 3 (0.014mg/ml) after 7 days, this is explained acid – catalyzed ester hydrolysis with dilute aqueouse HCL, with less effect by pH5(0.041mg/ml) this is explained that reaction proceed very slowly in absence of strong acids, there was graduall increase in concentration at pH3(0.046mg/ml) and pH5(0.050) at 14 days this Is explain Fisher esterfication, that reach equlibrium by reversible reaction [17].

Oleuropein concentration was more stable at acidic pH 5, pH 3 for 30 days, due to acidic nature of Oleuropein. science Oleuropein contains a number of OH group on Benzene ring, which give it acidity, polyphenols is weakly acidic, so it more stable on at pH 5 (0.058mg/ml) than pH 3 (0.048mg/ml), After 7 days Oleuropein concentratin at pH 9 (0.037mg/ml), this reflux base-promoted hydrolysis is called saponfication (figure3.4). and the concentration was stable from 7 to 30 days. This is reflux base promoted hydolysis of an ester, as the result is an essentially irreversible reaction due to the carboxylate ion being very unreactive toward nuclophile substitution because it is negativelly charged[17]. and ionization of hydroxyl group on polyphenol by alkaline pH.

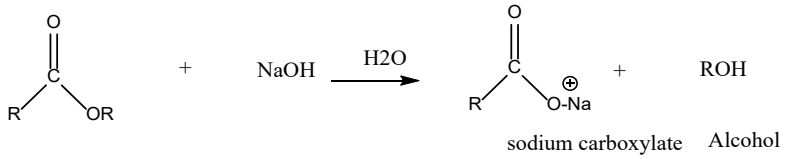

Figure 3.4:hydrolyis of ester bond by base

Table 3.8: **Effect of pH on oleuropein concentration after extraction with 60% dioxane:**

Sample pH	1st day	7th day	14th day	21st day	30th day
pH3	0.0503±0.0002	0.014±0.0003	0.046±0.0016	0.048±0.0002	0.048±0.0002
pH5	0.050±0.0002	0.041±0.001	0.050±0.0002	0.057±0.0003	0.058±0.0001
pH7	0.050±0.0002	0.044±0.0002	0.035±0.0001	0.038±0.0003	0.025±0.0001
pH9	0.050±0.0002	0.037±0.0046	0.039±0.0005	0.036±0.0001	0.034±0.0001

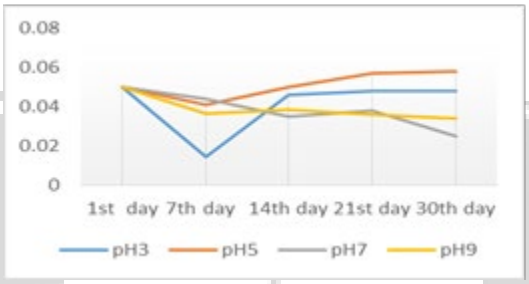

Figure 3.5: Oleuropein concentration at different pH with Time

4. Conclusion:

Oleuropein extraction and maintaining stability were achieved with ethanol 80%. Chemlali and Corantina olive leaves have far higher oleuropein contents than Arbequina olive leaves.

The maximum oleuropein contnt was found in maceration with dioxane 60% at 25c°, at 40c°, oleuropein concentration decreased by (23.9%) and at 75C° decreased by39.85%.

oleuropein concentrations in Soxhlet method extraction ware higher than those in maceration at several temperatures.

Oleuropein content was significantly greater during extraction at acidic pH (pH 3) and alkaline pH (pH 9), , followed by pH 5, with the lowest oleuropein level at neutral pH (pH 7).

When extraction was carried out at 60C° there was a decrease 67.85% in oleuropein concentration and a decreasa 57.1% at pH 3 and pH 9, respectively.

When kept at room temperature and 2-8c° the Initial oleuropein concent for an ethanolic 80% extract remained quite steady for 30 days.

After being kept at room temperature and 2-8c◦ for 7 days the Initial oleuropein concent for dioxane 60% showed a noticeable decline.

, there was a sharp decrease in oleuropein concentration at 14th day when stored

The concentration of oleuropein was quite constant at acidic pH (pH 3, pH 5), while it drastically decreases at neutral and alkaline pH (pH 7, pH 9).

References

[1] Benavente-Garcia J.Castillo J, Lorente A, Ortuno A, DelRio JA. Antioxident activity of phenolic extracted from Olea Europaea L. leaves. Food Chem.2000,68;457-462.

[2] Amay M Basuny and Shaker M Arafat². Olive leaves Healthy Alternative for green tea. Biochemistry Department, Faculity and Agriculture Beni-Suef university india submission: Februray11,2017.

[3] Amiot,M.J,Fleuriet,A,Macheix,J.J.1986. importance and evolution of phenolic compounds in olive during growth and maturation.Journal of Agricultural and food chemistery,34(5);828-826.

[4] Khan,Y,Siddharth,P,NiraJ,V,Amee,B.vimal,K 2007.Olea Europea:A phytopharmacological Reviews,1:114-118.

[5] Himours;Yahia A;Belattar H. Oleuropein and antibacterial activities of Olea europea L.leaf extract. European scientific journal febtuary 2017 edition vol.13.no.6 ISSN:1857-7881(print) e-ISSN 1857-7431. URL:http://dx.doi.org/10.1944/esj.2017 .v13n6p34.

[6] Hadeel Homssi, Muberra Kosar,2019. Oleuropein amounts of olive leaves from different regions of northern Cyprus.ISSN 2651-3587, https:// dergipark org tr/emujphansc.

[7] Ghaleb TAXOUB*[1]Huda SULAIMAN[1],Abdul Hadi HASSAN[2],Malik ALORFI[1],2012. Determination of Oleuropein in leaves and fruits of some Syrian Olive varieties.int.J.Med.Arom.Plants(SSN2245-4340).

[8] Ibraheem Afaneh[1], Hiba Yateem[2], Faud Al-Rimawi 2015.effect of olive leaves drying on the content of oleuropein. American Journal of analytical chemistry,246-252: http://www.scrip.org /journal/ajac .http://dx.doi.org/10.4236/ajac.2015.63023.

[9] Yateem, H, Afanch, I, AL-Rimawi,2014. Optimum conditions for oleuropein extraction from olive leaves. International journal Applied science and technology, vol.4, no.5. October 2014.

[10] Nasir S.A Malik*and Joe M.Bradford 2008. Recovry and stability of oleuropein and other phenolic compounds during extraction and processing of olive (Olea Europea L) leaves. Journal of food, Agriculture and environment Vol.6(2):8-13.2008.

[11] Mohammad Shah Faisal[1],Zainab Mohammed Alhssony et al 2015. Development and Validation of UV Spectophotometer method for the quick estimation of olive leaf extract (OLE) in pharmaceutical formulation. MMSJ VOL.2 ISSUE.2(Winter 2015) www. Misurata.edu.ly

[12] G.Sripad, V. Prakash and M.S.Narasings Rao,J.Biosci, 4(1982).

[13] Mohammed Ali.Textt book of pharmacognosy second edition 1993. 35,94,461.

[14] B.Renoe,American Laboratory,34-40(1994).

[15] L.N.Seetohul,M.Islam,W.T.O Hare and Z.Ali,J.SCi.Food Argi;86(13),2092-2098(2006).

[16] A.Khoddami,M.Wilkes and T.Roberts,molecules,18,2328-2375(2013).

[17] T.W.Graham Solomons,Criage B.Frhle, Scott A.Snyder,copyright 2014,2011.11.th Edition, Organic chemistery international student page 793,790,130,131.