International Conference on Innovations and Research in Agriculture,
Food Science, Forestry, Horticulture, Aquaculture, Animal Sciences,
Biodiversity, Ecology and Climate Change
(AFHABEC-2018)

Preliminary Screening and Comparative analysis of Leaf Samples from *Ex-vitro* and *In-vitro* Grown Cultures of *Olea europaea* L. Barnea

Taramla Raman and Susmita Shukla*

Applied Plant Biotechnology Research Lab, Centre for Plant and Environmental Biotech,
Amity Institute of Biotechnology, Amity University, Noida (U.P), India
E-mail: sshukla3@amity.edu

Abstract–*Olea europaea L. commonly known as Olive is a long lived tree mainly found in the Mediterranean region. Its fruits and oil extracted from seeds have been used worldwide in culinary and other cosmetic as well as remedial applications since time immemorial. Its leaves have been used in formulation of herbal tea and in traditional medicine. Phytochemicals are those chemical compounds produced by plants as a defense mechanism or induced during stress such as drought condition, high soil alkalinity, acidity or salt content. In recent years, the presence of important phytochemicals and potentially bioactive compounds in olive extract has been discovered and analyzed for their application in pharmaceutics and medicine. Oleuroepin, hydroxytyrosol, polyphenols and flavonoids are some of the major active compounds present in olive extract. Tissue culture is an important alternative technique for the production of desirable plant components, increase the production through inducing stress in-vitro and in turn, conserve the plant species from being endangered. Preliminary tests were conducted for various phytochemical constituents by preparing O. europaea leaf extracts from leaf samples of field grown and In-vitro culture and callus in different organic solvents- Chloroform, Ethanol and Methanol. In the present study, leaf extract using Chloroform as organic solvent gave the best result with clear, distinct layers as compared to olive extract using Ethanol and Methanol solvents. In-vitro grown leaf extract and callus extract had more content of flavonoids and steroids which suggests that tissue culture induces increased production of phytochemicals.*

Keywords*: Olea europaea L., Barnea, Phytochemical, Chloroform, Ethanol, Methanol, In-vitro.*

1. INTRODUCTION

The word "phytochemicals" is derived from the Greek word "Phyto" meaning plants. These are bioactive compounds present in plants[1] that are non-nutritive but serve as a protective or disease preventing shield for the survival of the plant[2]. They play an important role in the growth and development of the plant and in providing color as well as flavor to the plants. In addition, they also play a wide role of activity such as prevention of cardiovascular disease, cancer, neurodegenerative diseases, diabetes, and as anti-oxidant supplement. Polyphenols represent the largest phytochemical category among the plant kingdom that are found to be naturally occurring in fruits, vegetables and cereals[3,4]. In plants, polyphenols are involved against pathogen aggression or ultraviolet radiation, whereas, during consumption they attribute to color, bitterness, flavor and oxidative stability[4]. With medical evidences and epidemiological studies that strongly suggests the role of these secondary metabolites in the prevention of diseases, the structural and functional studies of phytochemicals have awaken an interest in the research field which will lead to improvement in human health. The main classes of polyphenols include phenolic acids and flavonoids which is responsible for the different colors in flowers, leaves and fruits.

An important application of plant tissue culture is to enhance the secondary metabolites content by providing stress *in-vitro* against which plants increase the production of secondary metabolites as defense and survival mechanism. This study was carried out to analyze for presence of phytochemicals in leaf and callus extract of *O. europaea* using different organic solvents to find out which one gives the best result, and a qualitative comparison of their contents in *in-vitro* leaf extract and callus with respect to color intensity observed during the different tests performed. The phytochemicals derived from *O. europaea* have a wide medical applicability as potential anti-cancer, lowering of blood pressure[5] and as anti-microbial agent[6]. Earlier studies have shown the presence of important phytochemicals in *O. europaea* tree such as oleuropein, hydroxystyrol[7,8].

2. MATERIALS AND METHODS

Sample preparation: Fresh leaves of *O. europaea* cv Barnea were obtained from field, washed thoroughly with tween 20 and kept to dry in hot air oven for few hours (3-5 hours) or overnight for proper drying to enable easy crushing. The dried leaves were then crushed and dipped in organic solvents (Chloroform, Ethanol and Methanol) (1:6 v/v) and left for 3

ISBN: 978-93-85822-67-4

hours before it was strained using Whatman filter paper. Similar steps were followed with *in-vitro* leaves and callus sample.

The following screening tests were conducted for the presence of different phytochemicals-

(i) Test for steroids: 0.5 ml of the extract was dissolved in 5 ml of the chloroform. Equal volume of sulphuric acid was added on the sides of the test tube and let to stand for few minutes. If the upper layer turns red and sulphuric acid layer showed yellow with green fluorescence, it indicates the presence of steroids [9].

(ii)Fehling's Test for reducing sugar: A mixture of 1ml Fehling's solution A and 1ml of Fehling's solution B was added in an empty test tube. To this, add 3-4 drops of the extract, mix gently and place tube in a water bath at 60 °C. Changing of the blue color to red color indicates the presence of reducing sugars.

(iii) Test for terpenoids: 1ml of extract was placed into the test tube to which 0.4ml of chloroform and 0.6ml of concentrated sulphuric acid was poured gently into the tube at an inclined portion. A reddish brown coloration was indicative of the presence of terpenoids[10].

(iv)Test for phlobatannins: Deposition of a red precipitate when sample aqueous extract was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins[10].

(v) Test for flavonoids: 1ml of 10% NaOH was mixed with equal volume of filtrate and thoroughly mixed. Development of yellow coloration indicates presence of flavonoids[9].

3. RESULTS AND DISCUSSION

Selection of organic solvent for screening of phytochemicals: In the preliminary phytochemical screening for presence of secondary metabolites, the leaf sample obtained from mother plant (field) was extracted using chloroform, ethanol and methanol as extracting solvents.

In the extraction using chloroform, the leaf extract showed positive for the presence of the following phytochemicals-

1) Steroids- a distinct blue upper layer and a brown ring was observed
2) Reducing sugar - Fehling's reagents were reduced from blue color to red precipitate
3) Flavonoids - yellow colored upper layer were distinctly visible on addition of 10% NaOH. However, presence of phlobatannins and terpenoids were negative in the tests.

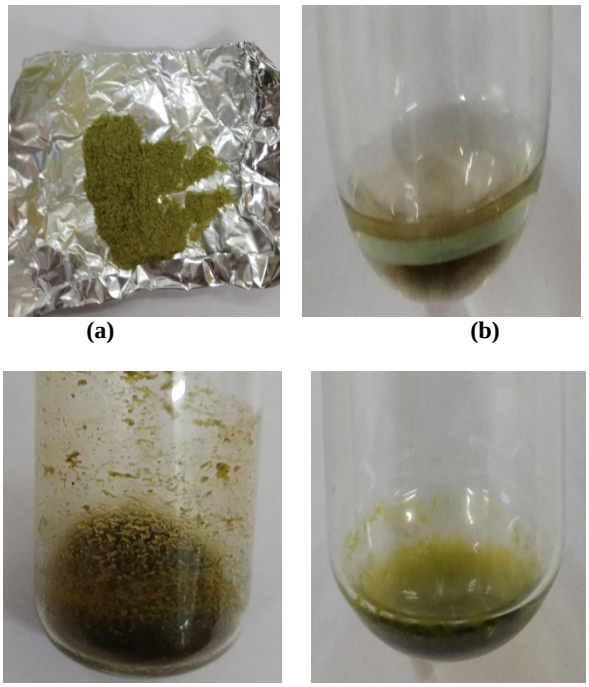


Figure 1. A- Powdered sample from dried leaf; B - Upper blue layer and brown ring observed indicating presence of steroids; C – Red precipitate formed when sample extract and Fehling's solution are mixed indicating presence of reducing sugar; D – upper yellow colored layer indicates presence of flavonoids.

Similarly, sample extracted using ethanol also showed the presence for steroids, reducing sugar and flavonoids. However, in methanol extract, only presence of steroid and flavonoids were observed and test result was negative for reducing sugar as shown in Table 1. The best result was obtained using chloroform as organic solvent as it gave clear, distinct layers as compared to ethanol extract.

Table 1: Phytochemical screening of *Olea europaea* L. leaf extract using different organic solvent extract

Phytochemical	Organic solvent		
	Chloroform	Mathanol	Ethanol
Flavonoids	+	+	+
Reducing sugar	+	-	+
Terpenoids	-	-	-
Steroids	+	+	+
Plobatannins	-	-	-

Taking this into consideration, similar tests were also performed in samples of *in-vitro O. europaea* leaves and callus using Chloroform as organic solvent for extraction. A much more intense red precipitate was observed in callus extract as compared to leaf extracts (both *in-vitro* and *ex-vitro*) which indicates an increased presence of reducing sugar in callus. The *in-vitro* leaf sample also showed increased

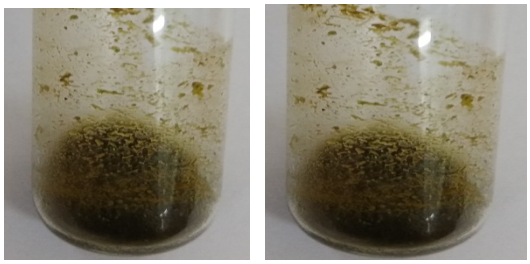
ISBN: 978-93-85822-67-4

intensity in color for flavonoids and steroids tests as compared to field grown leaf samples (Table 2).

Table 2: Phytochemical screening using chloroform as organic solvent extract.

Phytochemical	Leaf extract (Field)	Leaf extract (in vitro)	Callus
Flavonoids	+	++	++
Reducing sugar	+	+	+++
Steroids	+	++	+

[(-) indicates absence; (+) indicates presence; (++) and (+++) indicates intense coloration]



(a) **(b)**

(c)

Figure 2. Fehling's reducing sugar test carried out using Chloroform as extracting solvent.

A- Field grown leaf extract
B- In-vitro grown leaf extract
C- Callus extract showing intense red precipitate

4. CONCLUSION

In tissue cultured cells, the concentration of phytohormones plays an important role in accumulation of secondary metabolites[11, 12]. Jain et al, 2012 also demonstrated similar finding in *Sericostoma pauciflorum* which is in accordance with our result. Plant cell cultures have also been labelled as factories for production of secondary metabolites as they can be induced to produce high value secondary metabolites[14]**.** Plant secondary metabolites has a wide applicability in the food as additives, in cosmetics and drug and pharmaceutical industries.

5. ACKNOWLEDGMENT

The authors are thankful to Amity University Uttar Pradesh for providing the necessary infrastructure and facilities for carrying out the research work.

REFERENCES

[1] Diep CS, Baranowski J, Baranowski T (2015). Chapter 4 – The impact of fruit and vegetable intake on weight management. Managing and Preventing Obesity In: Behavioural Factors and Dietary Interventions, A volume in Woodhead Publishing Series in Food Science, Technology and Nutrition, pp59–78.

[2] Arendt EK, Zannini E (2013). Cereal Grains for the Food and Beverage Industries. A volume in Woodhead Publishing Series in Food Science, Technology and Nutrition. Pp 155-200.

[3] Martinez KB, Mackert JD, McIntosh MK (2017). Chapter 18 – Polyphenols and Intestinal Health. Nutrition and Functional Foods for Healthy Aging. Academic Press.pp 191-210.

[4] Pandey KB and Rizvi SI (2009). Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev. 2(5): 270–278.

[5] Matthias-Heinrich K (2002). Blood pressure lowering effect of an Olive leaf extract (*Olea europaea* L.) in L-NAME Induced hypertension in rats. Arzneim-Forsch. Drug Res. 52(11):797-802.

[6] Sudjana AN, D'Orazio C, Vanessa R, Rasool N, Justin Ng, Islam N, Rile TV, Hammer KA (2009). Antimicrobial activity of commercial *Olea europaea* (olive) leaf extract. International Journal of Antimicrobial Agents 33:461-463.

[7] Goulas V et al (2009). Phytochemicals in Olive leaf extracts-and their antiproliferative activity against cancer and endothelial cells. Mol. Nutr. Food. Res. 53:600-608.

[8] Pereira et al (2007). Phenolic Compounds and Antimicrobial Activity of Olive (*Olea europaea* L. *Cv.*Cobrançosa) Leaves. Molecules 12(5):1153-1162.

[9] Gul R,Syed UJ, Faridullah S, Sherani S, Jahan N (2017). Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from Ephedra intermedia Indigenous to Balochistan. https://doi.org/10.1155/2017/5873648

[10] Ajiboye BO, Ibukun EO, Edobor G, Oja AO, Onikanni SA (2013). Qualitative and quantitative analysis of phytochemicals in *Senecio biafrae* leaf. International Journal of inventions in pharmaceutical sciences. 1(5):428-432.

[11] DiCosmo F, Towers GHN (1984). Stress and secondary metabolism in cultured plant cells. In:Timmermann BN, Steelink. FA, Loewus FA (eds) Recent advances in phytochemistry, vol. 18.New York: Plenum pp. 97-175.

[12] Mantell SH, Smith H (1984). Culture factors that influence secondary metabolite accumulation in plant cell and tissue cultures. In: Mantell SH, Smith H (eds). Plant Biotechnology. Cambridge: Cambridge University Press; pp. 75–108.

[13] Jain C Satish, Boskey Pancholi, Renuka Jain (2012) "*In-Vitro* Callus Propagation and Secondary Metabolite Quantification in *Sericostoma Pauciflorum*." Iranian Journal of Pharmaceutical Research *IJPR,* 11.4: 1103–1109. Print.

[14] Ramachandra SR and Ravishankar GA (2002). Plant cell cultures: Chemical factories for secondary metabolites. Biotechnology Advances, 20(2):101-153.