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# A Review on EXTRACTION AND ANALYSIS OF PHENOLICS IN PLANTS - A CASE STUDY IN BASIL LEAVES

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**Abstract**

Phenolic compounds are abundant in majority of food researches because of having numerous positive effects for health-protecting properties and enhancement. Similarly, it has been reported to possess antioxidant properties in basil leaves (Ocimum basilicum L.). Moreover, its phenolic compounds include simple phenol, hydrobenzonic acid and cinnamic acid derivatives, flavonoids, tannins, among others. The extraction of phenolics from raw materials is the first step for their analysis. Therefore, this paper provides a summary of background information and the extraction methods used for the analysis of phenolics in this plant material, especially in basil leaves, a case study.

## I. Introduction of basil

Basil (*Ocimum basilicum L.*) is a common medicinal and culinary herb. There are more than 30 species of herbs and shrubs from tropical and subtropical regions of Asia, Africa and Central and South America, but the main place of diversity appears to be Africa (Vieira and Simon 2000). The herb has a source of essential oils and aroma compounds, with an attractive fragrant (Morales

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and Simon 1996). One study classified the *O. basilicum* cultivars in seven types (Figure 1):

(a) tall slender types, which include the sweet basil group;

(b) large-leafed, robust types, including 'Lettuce Leaf' also called 'Italian' basil;

(c) dwarf types, which are short and small leafed, such as 'Bush' basil;

(d) compact types, also described, *O. basilicum* var. *thyrsiflora,* commonly called 'Thai' basil;

(e) *purpurascens,* the purple-colored basil types with traditional sweet basil flavor;

(f) purple types such as 'Dark Opal', a possible hybrid between *O. basilicum* and *O. forskolei,* which has lobed-leaves, with a sweet basil plus clove-like aroma; and

(g) *citriodorum* types, which includes lemon-flavored basils (Hussain, Anwar et al. 2008).

Thai basil is a type of basil native to Southeast Asia. It grows up to 45 cm and has shiny green, slightly serrated, narrow leaves with a sweet. Thai basil has a purple stem which is square. The leaves are opposite and decussate. One of the most known uses of this herb is a spice and ingredient in Italian and Southeast Asian cuisines. In addition, this herb also used as traditional medicines.

## II. Chemical composition in basil leaves

By presenting of secondary metabolites such as essentials oils, tannins, phenols, flavonoids, anthocyanins and steroids. Ocimum species have been known for their healthful properties for long time a nd have been used in traditional medicine. The composition of basil leaves shown in table 1.

Basil contained high content of magnesium, potassium and iron (table 1). This element as one of the seven essential macro minerals. Improving the health of cardiovascular system and also protection from number of chronic diseases. In addition, basils are employed a number of vitamin A, vitamin C, calcium and phosphorus. It also contained high concentrations of carotenoids like $\beta$-carotene, and these substances are converted vitamin A in body. Basil is a rich source of polyphenols. Polyphenols include many classes of compounds ranging from phenolic acids, especially, simple or complex flavonoids.

**a. Sweet basil** **b. Italian basil** **c. Bush basil**

**d. Thai basil** **e. Purple – color basil** **f. dark – opal basil**

**g. Lemon – favored basil**

**Figure 1.** Some kind of basil in the world

**Table 1**: Content of nutrition elements in 100g of fresh basil:

| **Nutritive elements** | **content** |
|---|---|
| Fat | 0.64g |
| Protein | 3.15g |
| Water | 92.06g |
| **Vitamins** | |
| Vitamin A | 264µg |
| β-carotene | 3142 µg |
| Thiamin ($B_1$) | 34 µg |
| Riboflavin ($B_2$) | 76 µg |
| Niacin ($B_3$) | 902 µg |
| Panthotenic acid ($B_5$) | 209 µg |
| Vitamin $B_6$ | 155 µg |
| Choline | 68 µg |
| Vitamin C | 11.4 µg |
| Vitamin E | 18 µg |
| Vitamin K | 414.8 µg |
| **Minerals** | |
| Ca | 177 mg |
| Fe | 3.17 mg |
| Mg | 64 mg |
| Mn | 1.148 mg |
| P | 56 mg |
| K | 295 mg |
| Na | 4 mg |
| Zn | 0.81 mg |

## III. Overview phenolics and phenolics in basil

Natural phenolic has been of great interest in the past decades in the studies of nutritional and functional, and antioxidant capability in food. Wide variety of phenolics in food products have been researched and published with numerous positive proclaim for health protecting properties and enhancement. It is noticeable that determinations of total phenolic content (TPC) or specific phenolic components are abundant in majority of food researches and analyses in various processing and experimental constructions.

Phenolic compounds are defined as organic substances that contain the hydroxylated aromatic rings, of which the hydroxyl group is bonded directly to the phenyl, substituted phenyl, or other aryl group (Swanson, 2003). The simplest is phenol ($C_6H_5OH$). Phenolics are responsible for color, flavor, and other quality of foods, especially the plant-based variety. According to Cheynier (2012), the compounds are one of the biggest and widely distributed groups of the secondary metabolites of the plants, which are the result of several biosynthetic pathways, namely shikimic acid and phenylpropanoid pathways, and are generally specified for specific groups of organisms. Though phenolics are not considered as nutrient (Yahia, 2019), they are highly bioactive and known for numerous health benefits.

Due to the presence of hydroxyl groups in the phenol structure, phenolic compounds are soluble in water to some extent, depending on the degree of molecular complex of the substance (Huyskens et al., 1975). This property is proved to be crucial in the extraction of the compounds, for which the solvents are usually of water/ alcohol-based mixtures. Hydroxyl-substituted aromatic rings are also the key feature in phenolic. The rings formation and arrangement result in numerous phenol types from basic structures of simple phenols or phenolic acids to complex polyphenols of flavonoids, lignan and lignin; each of which has its own functional and characteristically properties (Giada, 2013). Another property of phenolic is their ability to bind with other molecules, especially glucoside residues and proteins that help reduce the toxicity of the substances. In the study of Anku et al. (2017), free-formed phenolic compounds are usually toxic and less common in plants although they are considered as one of the defense systems against insects and other organisms. In flavonoids, binding of phenolic to sugar residue also help promote the color of the plant to different gradation (e.g. anthocyanin in strawberries) (Giada, 2013)

There are several ways to classify phenolic compounds. One way is to categorize based on the carbon chain, with which 16 subdivisions are formed (Vuolo et al., 2019). They are simple phenols, benzoquinones, phenolic acids, acetophenones, phenylacetic acids, hydroxycinnamic acids, phenylpropenes, coumarins and isocoumarins, chromones, naphthoquinones, xanthones, stil-

benes, anthraquinones, flavonoids, lignans and neolignanes, and lignins. Based on the natural presence, phenolics can also be shortly distributed (simple phenols, aldehyde derivatives of benzoic acids as essential oils, etc.), widely distributed (flavonoids, phenolic acids, and coumarins), and polymer (such as tannin and lignin) (Giada, 2013). One other common and basic way to identify phenolic compounds is their solubility in water. The unbound low-weighted compounds such as flavonoids and simple phenols belong to the soluble division. These compounds, when ingested into the gastrointestinal tract, can be digested and found in the blood and act as metabolites for the body. Meanwhile, those that are bound with polysaccharides and proteins (e.g. phenolic acids and tannin bounded in the cell walls of the plants) are known as insoluble complexes, which are usually indigestible (Giada, 2013).

In several studies, the total phenolic content is often linked with the antioxidant capacity of the food products. it is common that the determination of free radical scavenging analyses is usually accompanied by the assessment of the TPC. In the research of Minatel et al. (2017), antioxidant property of phenolics can help reduce the lipid oxidation in (plant and animal) tissues, promote antiaging process in human and decrease the inflammation and oxidative stress risk, related with chronic diseases (e.g., cardiovascular diseases, arteriosclerosis, cancer, diabetes, cataract, disorders of the cognitive function, and neurological diseases). The mechanism to scavenge free radicals of the phenolic compounds is subjected to the donation of hydrogens in hydroxyl groups, acidic groups (in phenolic acids), and the double bond of the oxo functional group (-C=O) of some flavonoids. Phenolic can also act as phytochemicals. Phenolic phytochemicals (PPs) is said to be the largest group of phytochemical in plant-based foods (King & Young, 1999). The common present compounds are flavonoids, phenolic acids, and polyphenols. PPs have been reported to contain many disease-preventive properties including cancer or heart diseases. Beside these health-beneficial properties, phenolic in food also play important roles in contribution of food characteristics. These compounds are directly related to sensory characteristics of foods such as flavor, astringency and color (Landete, 2012). The anthocyanin of the flavonoid subgroup is known for its red color in various fruits (e.g. berries family). Phenolic acids have been proposed to be responsible for sour, bitter, and astringent flavors found in vegetable proteins (Huang & Zayas, 1991). Flavor attribution can also be formed by the degradation of amines and amino acids in the presence of phenolic compounds in Strecker-type to produce the carbonyl derivatives (e.g. phenylacetaldehyde) (Delgado et al., 2015).

Phenolic compounds are widely distributed in plant foods. Commonly, broccoli, pepper, shallot, thyme, grapefruit, lemon, orange, cocoa bean, and especially basil leaf are known for containing high amount of phenolics. Red

wines are reported to significate in anthocyanin content (Morton et al., 2000). Thiagarajan et al. (2001) studied on the TPC of green and black teas and revealed that the dry weight content of the products exceeded to 30% of the total flavonoids and polyphenol compounds. Coffee is also rich in these substances, especially chlorogenic acid. It has about 7% of the dry weight of the grains and 15% of the dry instant coffee as phenolic compounds (King & Young, 1999).

Basil has employed high levels of phenolic acid that contribute to its antioxidant capacity (Złotek, Mikulska et al. 2016). The main phenolic acids in basils are rosmarinic, caffeic and chicoric acids. Rosmarinic acid as prevalent phenolic acid in basil extracts, is important phytochemical, due to their antioxidant and pharmacology properties

In basil leaf, large contents of, chicoric, caffeic, and caftaric acids are found. Anthocyanin and other water soluble phenolics are also found in some certain purple basils, of which they are accountable for the color of the plants (Flanigan & Niemeyer, 2014)

## IV. Extraction and characterization of phenolics of basil leaves

Regarding to pharmacological studies, various O. basilicum extracts have been reported for their antibacterial, antifungal and antioxidant activities (Vlase, Benedec et al. 2014). These extracts of leaves and flowering tops have been used widely in food industry such as flavoring foods and beverages, and traditional medicine (Kiferle, Maggini et al. 2012). Phenolic compounds are the major class of basil's secondary metabolites that are contributed to antioxidant and anti-inflammatory activity of its extracts (Kwee and Niemeyer 2011). Specially, type 2 diabetes mellitus (T2DM) is a group of metabolic disorders characterized by hyperglycemia, resulting from resistance to insulin action and inadequate insulin secretion. Hyperglycemia is strongly associated with the increased risk of kidney, eye and heart disease (Du, Hu et al. 2012). One therapeutic approach to decrease this disease especially after eating is to retard the digestion and absorption of ingested carbohydrates through the inhibition of carbohydrate-hydrolyzing enzymes ($\alpha$- amylase and $\alpha$-glucosidase) in the digestive organs. Therefore, these inhibitors could decrease the post-prandial rise in blood glucose concentration. Many research found new hypoglycemic drugs obtained from different sources especially medicinal plants because of their effectiveness, relatively low cost such as Nepalese herb Pakhanbhed (Bhandari, Jong-Anurakkun et al. 2008) and basil (Kwee and Niemeyer 2011).

Therefore, determination of extraction condition of phenolic compounds in plant materials is significantly important because of the influence on their

chemical nature, including the extraction method employed, sample particle size, storage time and conditions. Actually, the chemical nature of plant phenolics varies about simple to highly polymerized substances including different proportions such as phenolic acids, phenylpropanoids, anthocyanins and tannins, others. Moreover, they might also exist other complexes with carbohydrates, protein and other plant components, some high-molecular-weight phenolics. So, phenolic extracts of plant materials are always a mixture of different classes of phenolics and soluble in the solvent system.

Solubility of phenolic compounds is conducted by the type of solvent such as polarity. Methanol, ethanol, acetone, water, ethyl acetate, propanol, dimethylformamide and their combinations are frequently used for extraction of phenolics. In addition, there are variety of method for extract phenolics in basil leaves including microwave-assisted extraction (MAE) (Mushtaq, Choi et al. 2014), ultrasound-assisted extraction (UAE) (Pico 2013) and solvent extraction (Kwee and Niemeyer 2011, Goldsmith, Vuong et al. 2014). However, solvent extraction is more popular than others due to its advantages. Firstly, several extractions can be employed in parallel. Besides, it also required a little in training and can extract more sample mass than other methods. For those reasons, the solvent extraction method was used to extract phenolic and antioxidant compounds (Eskilsson and Bjrklund 2000, Kothari, Gupta et al. 2012).

# V. Conclusion

There are numerous studies suggest that the phenolic containing in foods with a lot of positive effects for health-promoting. Specially, basil leaves have organic phenolic compounds in order to treat diabetic, decrease hyperglycemia, antioxidant, antimicrobial Therefore, the extraction conditions of this leaves are also significantly important because of influence on their bioactive compounds in this plant. Moreover, future work.

# References

[1] Anku, W. W., Mamo, M. A., & Govender, P. P. (2017), *Phenolic Compounds in Water: Sources, Reactivity, Toxicity and Treatment Methods,* Phenolic Compounds - Natural Sources, Importance and Applications. https://doi.org/10.5772/66927

[2] Bhandari, M. R., et al., *$\alpha$-Glucosidase and $\alpha$-amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (Bergenia ciliata, Haw.),* Food Chemistry, **106**(1)(2008), 247-252.

[3] Cheynier, V. , *Phenolic compounds: From plants to foods,* Phytochemistry Reviews, **11**(2012), 153-177.

[4] Delgado, R. M., Zamora, R., & Hidalgo, F. J., *Contribution of phenolic compounds to food flavors: Strecker-type degradation of amines and amino acids produced by o- and p-diphenols,* Journal of Agricultural and Food Chemistry, **63**(2015), 312-318.

[5] Du, Z., et al., *Cultivation of a microalga Chlorella vulgaris using recycled aqueous phase nutrients from hydrothermal carbonization process,* Bioresource Technology **126**(2012), 354-357.

[6] Eskilsson, C. S. and E. Bjrklund, *Analytical-scale microwave-assisted extraction,* Journal of Chromatography A, **902**(1)(2000), 227-250.

[7] Goldsmith, C., et al., *Optimization of the aqueous extraction of phenolic compounds from olive leaves,* Antioxidants **3**(4)(2014), 700-712.

[8] Flanigan, P. M., & Niemeyer, E. D., *Effect of cultivar on phenolic levels, anthocyanin composition, and antioxidant properties in purple basil (Ocimum basilicum L.),* Food Chemistry, **164**(2014), 518-526.

[9] Giada, M. de L. R., *Food Phenolic Compounds: Main Classes, Sources and Their Antioxidant Power. Oxidative Stress and Chronic Degenerative Diseases - A Role for Antioxidants,* (2013), https://doi.org/10.5772/51687

[10] Huang, C. J., & Zayas, J. F., *Phenolic Acid Contributions to Taste Characteristics of Corn Germ Protein Flour Products,* Journal of Food Science, **56**(1991), 1308-1310.

[11] Huyskens, P., Mullens, J., Gomez, A., & Tack, J., *Solubility of Alcohols, Phenols and Anilines in Water,* Bulletin Des Sociétés Chimiques Belges, **84**(1975), 253-262.

[12] Hussain, A. I., et al., *Chemical composition, antioxidant and antimicrobial activities of basil (Ocimum basilicum) essential oils depends on seasonal variations,* Food chemistry, **108**(3)(2008), 986-989.

[13] Kiferle, C., et al., *Influence of root hypoxia and NaCl salinity on sweet basil (Ocimum basilicum L.) grown hydroponically for the production of rosmarinic acid,* Agrochimica **56**(6)(2012), 257-267.

[14] King, A., & Young, G., *Characteristics and occurrence of phenolic phytochemicals,* Journal of the American Dietetic Association, **99**(1999), 213-218.

[15] Kothari, V., et al., *Comparative study of various methods for extraction of antioxidant and antibacterial compounds from plant seeds,*J. of Natural Remedies **12**(2)(2012), 162-173.

[16] Kwee, E. M. and E. D. Niemeyer, *Variations in phenolic composition and antioxidant properties among 15 basil (Ocimum basilicum L.) cultivars,* Food Chemistry **128**(4)(2011), 1044-1050.

[17] Landete, J. M., *Updated knowledge about polyphenols: Functions, bioavailability, metabolism, and health,* Critical Reviews in Food Science and Nutrition, **52**(2012), 936-948.

[18] Minatel, I. O., Borges, C. V., Ferreira, M. I., Gomez, H. A. G., & Lima, C.-Y. O. C. and G. P. P., *Phenolic Compounds: Functional Properties, Impact of Processing and Bioavailability* Phenolic Compounds - Biological Activity (2017) https://doi.org/10.5772/66368

[19] Morton, L. W., Caccetta, R. A.-A., Puddey, I. B., & Croft, K. D., *Chemistry and biological effects of dietary phenolic compounds: Relevance to cardiovascular disease,* Clinical and Experimental Pharmacology and Physiology, **27**(2000), 152-159.

[20] Morales, M. R. and J. E. Simon, *New basil selections with compact inflorescences for the ornamental market,* ASHS Press: Arlington, VA: (1996), 543-546.

[21] Mushtaq, M. Y., et al., *Extraction for metabolomics: access to the metabolome,* Phytochemical Analysis **25**(4)(2014), 291-306.

[22] Pico, Y., *Ultrasound-assisted extraction for food and environmental samples,* TrAC Trends in Analytical Chemistry **43**(2013), 84-99.

[23] Swanson, B. G., "TANNINS AND POLYPHENOLS", In B. Caballero (Ed.), Encyclopedia of Food Sciences and Nutrition (Second Edition), 2003. (pp. 5729-5733), Oxford: Academic Press.

[24] Thiagarajan, G., Chandani, S., Sundari, C. S., Rao, S. H., Kulkarni, A. V., & Balasubramanian, D., *Antioxidant properties of green and black tea, and their potential ability to retard the progression of eye lens cataract,* Experimental Eye Research, **73**(2001), 393-401.

[25] Vuolo, M. M., Lima, V. S., & Marstica Junior, M. R., *Chapter 2 - Phenolic Compounds: Structure, Classification, and Antioxidant Power,* In M. R. S. Campos (Ed.), Bioactive Compounds (pp. 33-50), Woodhead Publishing, 2019,

[26] Yahia, E. M., Postharvest Physiology and Biochemistry of Fruits and Vegetables. Elsevier, 2019.

[27] Vieira, R. F. and J. E. Simon, *Chemical characterization of basil (Ocimum spp.) found in the markets and used in traditional medicine in Brazil,* Economic botany **54**(2)(2000), 207-216.

[28] Vlase, L., et al., *Evaluation of antioxidant and antimicrobial activities and phenolic profile for Hyssopus officinalis, Ocimum basilicum and Teucrium chamaedrys,* Molecules, **19**(5)(2014), 5490-550.

[29] Złotek, U., et al., *The effect of different solvents and number of extraction steps on the polyphenol content and antioxidant capacity of basil leaves (Ocimum basilicum L.) extracts,* Saudi J. of Biological Sciences **23**(5)(2016), 628-633.