



Research article

Organic solvent extraction and spectrophotometric quantification of total phenolic content of soil

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ABSTRACT

Phenolic compounds are regarded as the most abundant plant metabolites that are known to decompose progressively into soils, likened to other soil organic materials. Once assimilated into soils, they can control soil processes, including organic matter decomposition and nutrient cycling. Established that phenolic compounds can influence nutrients availability and soil quality, it becomes crucial to investigate into soil phenolics through the application of appropriate extraction technique and quantification of total phenolic content in soils. This study therefore aimed at utilizing ethanol, hexane and diethyl ether organic solvents to extract and quantify total phenolic content of soil, sampled from a vegetable growing area. Conventional organic solvent extraction method was employed to extract phenolics, while spectrophotometric technique was utilized to quantify total phenolic content. The highest extraction yield of 34.52% was achieved with ethanol followed by diethyl ether (28.23%) and hexane (25.47%). Interestingly, hexane, which had the least extraction yield, rather recorded the highest phenolics concentration of 5.50 ± 0.02 mgGAE/g, with ethanol producing a concentration of 2.04 ± 0.05 mgGAE/g and 3.82 ± 0.01 mgGAE/g for diethyl ether. The percent recovery, limit of detection (LOD) and limit of quantification (LOQ) of phenolic compounds were found to be 102%, 0.8 mg/g and 1.5 mg/g for ethanol; 96%, 0.6 mg/g and 1.2 mg/g for diethyl ether and 94%, 0.4 mg/g and 1.0 mg/g for hexane respectively. These results indicate that for an extraction efficiency and greater yield, the use of ethanol as solvent is preferred whereas extraction using hexane is suitable for total phenolics quantification. The findings of this study have provided a vital insight regarding the influence of organic solvents on the extractability and quantification of total phenolic content of soil.

1. Introduction

Soil is a dynamic ecosystem in which physical, chemical, and biological components interact (Bastida et al., 2009). Soil comprises of four key components including mineral matter, organic matter, soil air and soil water. Mineral matter includes particles such as stones, pebbles, sand, silt and clay while organic matter encompasses animal bodies, dead twigs, roots, leaves and other animal and plant residues as well as living organisms (Saha, 2004). Soil is used as a medium for plant growth and habitat for microorganism. It can as well be used for biomass production, filtering, buffering and transformation action, providing biological habitat and gene reserve (Saha, 2004).

Phenolic compounds refer to a class of organic compounds that have one or more hydroxyl groups directly attached to an aromatic ring (William et al., 2017). They constitutes the most abundant secondary

metabolites in plants and can be categorized into non-soluble compounds such as condensed tannins, lignins, cell-wall bound hydroxycinnamic acids, or soluble compounds including phenolic acids, phenylpropanoids, flavonoids and quinones (Rispaal et al., 2005). Phenolic compounds basically enter soils in a form of liquid or particulate matter, originating from plant materials and industrial wastes (Hattenschwiler and Vitousek, 2000). Following their entry into soils, they influence soil organic matter decomposition and nutrient cycling (Fierer et al., 2001; Toberman et al., 2010). Phenolic compounds in soils may also play a role in controlling many aspects of plant-soil interactions, including the alteration of soil nutrient availability, root and hypocotyl growth inhibition and limitation of water and mineral uptake by plants (Inderjit et al., 2009).

Quantification of phenolic compounds has been achieved through a variety of techniques including the Folin-Ciocalteu assay, CuO oxidation-GC and a high performance liquid chromatography (HPLC) method (Min

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et al., 2015). However, as quantity of phenolic compounds in soils can vary, the Folin-Ciocalteu assay is usually employed to determine the total phenolic content of a soil (Min et al., 2015). This assay is relatively simple and less expensive compared to the CuO oxidation-GC and the HPLC techniques. A study by Bastola et al. (2017) established that the Folin-Ciocalteu assay with gallic acid as standard is best and more appropriate for total phenolic content quantification, compared to other standards including ferulic acid, chlorogenic acid, catechol and vanillic acid.

Extraction represents the initial stage in phenolics analysis, which involves the isolation of the active phenolic compounds from the soil material (Cacace and Mazza, 2003). The extraction yield is mostly dependent on the nature of solvent used with varying polarity, temperature, extraction method, nature of the chemical compound, existence of interfering substances and solvent to sample volume ratio (Koleva et al., 2002). A number of extraction techniques and solvents have been used in previous studies to extract phenolics. Halvorson et al. (2009) extracted phenolics from soil using water, acidified methanol and acetone, and reported that acidified methanol yields more phenolics than water and acetone.

A related study by Santana et al. (2009) on the extraction of phenolic compounds from environmental samples (water, sediments and soils) using various extraction techniques (Soxhlet, ultrasound agitation, microwave assisted extraction, etc.) with different solvents (methanol, acetone, triethylamine, water, etc) reported a significantly higher recoveries of phenolic compounds. Thus, Soxhlet extraction with methanol as solvent gave a percent recovery of 83–97% whereas the ultrasound agitation technique with acetone achieved a percent recovery of 81–99% of phenolic compounds.

Organic solvent extraction has been the main method to effectively extract phenolic compounds from soils (Min et al., 2015). Unfortunately, very few of these organic solvents including methanol, citrate and acetone have been employed in the extraction procedures (Halvorson et al., 2009; Min et al., 2015; Arditoglou and Voutsas, 2008); with the prospect of many others remaining unexploited. Hence, this present study is focused on investigating the probable use of ethanol, hexane and diethyl ether to extract and quantify total phenolic content of soil, sampled from a vegetable growing area. The outcome of this research will provide a great insight concerning the extraction efficiency of the solvents utilized. The study will as well ascertain the total phenolic content of the soil, which is essential for the study of soil organic matter formation and nutrient cycling.

2. Materials and methods

2.1. Study design

This study was solely based on laboratory experimentations to extract and quantify total phenolic content from soil samples, collected from vegetables growing area at the University of Cape Coast Agriculture Farms. Three different organic solvents including ethanol, hexane and diethyl ether were employed in the extraction processes. Following extraction, the extract yield of each solvent was calculated using the appropriate formula. With UV-visible spectrophotometer, the total phenolic content in each extract was determined and the spectral characteristics of the analyte (phenolic compounds) in the UV-visible region studied.

2.2. Sample collection and preparation

The soil sample was collected from vegetable growing area at the University of Cape Coast Agriculture Farms using the traverse soil sampling technique. The surface litters at the sampling spot were cleared by splashing. Soil Auger was drove to plough a depth of 15 cm to draw soil samples and placed in a clean bucket. Foreign materials such as roots, stones, pebbles and gravels were removed and the desired samples were collected into a clean polythene bag and sent to the laboratory for air-drying and analysis. The air-dried samples were sieved with a 2 mm sieve to obtain finely powdered soil materials for extraction and analysis.

The soil used in this study was a loamy soil composed of 40% sand, 40% silt and 20% clay. Other important quality parameters of this soil were determined. This encompassed soil pH (6.0), organic matter content (2.6%), cation exchange capacity (8 meq/100g) and structural stability (0.006 cm^{-1}).

2.3. Sample extraction

A conventional cold maceration solvent extraction technique as described by Handa et al. (2008) with slight modifications was employed to extract phenolic compounds from soil samples. Sample-to-solvent ratio of 10:100 (w/v) was used for the extraction. Ten grams (10 g) of the air-dried soil sample was macerated in a stoppered 250 mL Erlenmeyer flask containing 100 mL of 95% ethanol. The mixture was allowed to stand at room temperature for 72 h with frequent agitation. It was then filtered through whatman No.1 filter paper (125 mm), and the solvent evaporated using a rotary evaporator (Stuart RE 400, UK). Minute traces of solvent left in the extract were desiccated with a vacuum desiccator to obtain whitish dry residues. The dried residue of extract was then weighed to determine the extraction yield. The same procedure was repeated for the other solvents (hexane and diethyl ether), and the extraction yield for each solvent determined using Eq. (1) below;

$$\text{Extraction yield (\%)} = \text{Weight of dry extract} / \text{weight of sample} \times 100 \quad (1)$$

2.4. Spectrophotometric method validation and recovery of phenolic compounds

In order to determine the sensitivity and accuracy of the spectrophotometric analytical technique utilized in the study, the limit of detection (LOD) and limit of quantification (LOQ) together with the spike recovery experiment were performed. The LOD and LOQ were measured based on the standard deviation response of the blank. From the calibration curve of gallic acid standard, the slope was determined, which was used to calculate the LOD and LOQ from the following equations: $\text{LOD} = 3.3(\text{SD}/S)$ and $\text{LOQ} = 10(\text{SD}/S)$. Where; “SD” is the standard deviation of the response of the curve and “S” is the slope of the calibration curve. With the spike recovery test, samples were separated into two portions and a known amount (0.5 mg/L) of analyte added to one portion. The phenolics concentration was then determined for both the spiked (*F*), and unspiked portions (*I*) and the percent recoveries (% R) calculated as; $\% R = F - I/A \times 100$. Where “A” is the concentration of phenolics added to the spiked portion (Adusei et al., 2019).

2.5. Quantification of total phenolic content

The total phenolic content (TPC) in each extract was determined using the Folin-Ciocalteu assay as described by Ainsworth and Gillespie (2007) following slight modifications. Gallic acid was used as a standard. Half a milliliter (0.5 mL) aliquot of 10, 20, 40, 80 and 100 $\mu\text{g}/\text{mL}$ gallic acid solutions were mixed with 2 mL of Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) and was neutralized with 4 mL of sodium carbonate solution (7.5%, w/v). The reaction mixture was incubated at room temperature for 30 min with intermittent shaking for colour development. The absorbance of the resulting blue colour was measured at 765 nm with a UV-visible spectrophotometer (Shimadzu UV mini-1240). The same technique was repeated for all the extracts and the TPC determined from the standard calibration curve of gallic acid and expressed as mg/g gallic acid equivalent (GAE) of the dry extract.

2.6. UV-visible spectroscopic characterization of total phenolic content

Two milliliter (2 mL) of Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) was neutralized with 2 mL of 2% (w/v) sodium carbonate solution. The mixture was scanned in the UV-visible region from 200 to 800 nm and the absorbance determined to serve as a reference. One

milliliter (1 mL) aliquot of each extract was mixed with 2 mL of Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) and was neutralized with 2 mL of 2% (w/v) sodium carbonate solution in separate test tubes. The reaction mixture was incubated at room temperature for 30 min with intermittent shaking for colour development. Mixture of the three extracts was scanned in the UV-visible region from 200 to 800 nm and the absorbance determined and plotted as a function of wavelength (Aleixandre-Tudo and Toit, 2018).

2.7. Statistical analysis

All experimental procedures were performed in triplicate and results presented as mean together with their standard deviations. The obtained data were analyzed with GraphPad Prism 5.01. A one-way ANOVA was employed in the statistical comparisons of mean values among extracts, and P-values less than 0.05 ($P < 0.05$) were regarded as statistically significant. The Pearson correlation analysis was carried out to evaluate the correlation between the extraction yield and TPC of extracts.

3. Results and discussion

3.1. Extraction yield

The most important step prior to quantification of soil phenolics is the extraction stage. This stage involves the isolation of the active phenolic compounds from soil material, which has the capacity to affect the extraction yield, as well as the quantity of phenolics to be extracted. Under the same extraction time and temperature, the composition of sample together with the solvent used for extraction becomes the key significant factor (Koleva et al., 2002). In this present study, ethanol, hexane, and diethyl ether organic solvents were used to obtain the various soil extracts. As presented in Table 1 and Figure 1, the extraction yield ranged from 25.47% for hexane extract to 34.52% for ethanol extract, with diethyl ether producing an intermediary extract yield of 28.23%. The findings of this study displaying differences in extraction yield may be attributed to the disparity in polarity of the extraction solvents. Thus, the polarity of solvents used increase in the following order: hexane < diethyl ether < ethanol. Hence, ethanol being the more polar solvent with higher polarity produced the highest extraction yield compare to the others. Similar findings on the impact of solvents on extraction yield of phenolic compounds was established in a study by Jinshui et al. (2014), on “phenolic compounds determination in apple orchard soil”, where methanol, ethanol, acetone and methylene dichloride were used as extraction solvents. The variation in results of this present study to that of Jinshui et al. (2014) may be due to the differences in extraction techniques (conventional cold maceration and Dionex Accelerated Solvent Extraction System) utilized in the two studies.

The findings of this study also corroborate the statement by Zhao et al. (2006) that the solubility of phenolic compounds is directly related to its molecular structure and polarity of the extraction solvent. Hence, ethanol being the polar solvent with similar molecular structure (the presence of OH group) to that of phenolic compounds, could be

Table 1. Extraction yield and total phenolic content of organic solvent soil extracts.

Solvent	Extraction yield (%)	TPC (mgGAE/g)
Ethanol	34.52 ± 0.15 ^a	2.04 ± 0.05 ^a
Diethyl ether	28.23 ± 0.24 ^b	3.82 ± 0.01 ^b
Hexane	25.47 ± 0.26 ^c	5.50 ± 0.02 ^c
P-value	0.0326	0.0214

TPC= Total Phenolic Content; GAE= Galic Acid Equivalent. Data are presented as mean ± standard deviation. Means in a column with different letter superscripts are significantly different ($P < 0.05$; Tukey: compare all pairs of columns, at 95% confidence level).

responsible for the better solubility and extraction of phenolics than the others. The above result of this study has therefore revealed the influence of solvents on the extraction yield of phenolic compounds in soils.

3.2. Spectrophotometric method validation and recovery of phenolic compounds

The standard gallic acid calibration plot was obtained to be linear with a correlation coefficient (R^2) of 0.9797 and a regression equation of $y = 0.0014x + 0.0957$. This revealed a good linear relationship between the analyte concentrations and the spectrophotometric responses. The LOD of phenolic compounds were 0.8 mg/g, 0.6 mg/g and 0.4 mg/g for ethanol, diethyl ether and hexane extracts respectively, whereas LOQ were found to 1.5 mg/g, 1.2 mg/g and 1.0 mg/g for ethanol, diethyl ether and hexane extracts respectively. With the spike recovery test for phenolic compounds, higher recoveries of 102%, 96% and 94% were obtained for ethanol, diethyl ether and hexane extracts respectively. These results signify the sensitivity, precision and accuracy of the spectrophotometric technique in determining the total phenolic content of the soil extracts.

3.3. Quantification of total phenolic content of soil

The quantitative determination of total phenolic content (TPC) of extracts was performed using UV-visible spectrophotometer. As presented in Table 1 and Figure 1, phenolics concentration was found to be higher in hexane extract than that of ethanol and diethyl ether. Thus, the TPC values were in the order of hexane > diethyl ether > ethanol. This result represents an inverse relationship between solvent polarity or extraction yield and TPC. Thus, the more non-polar solvent extract (hexane extract, with the least extraction yield) was found to be relatively high in TPC, compare to ethanol and diethyl ether extracts. To demonstrate this negative or inverse relationship between the extraction yield and TPC, the Pearson's correlation analysis was performed. A strong inverse non-significant correlation (Pearson $r = -0.9850$, $P = 0.1104$ and $R^2 = 0.9702$) was established among the two parameters (extraction yield and TPC). This correlation result indicates that higher extraction yield does not necessarily infer higher phenolic concentrations, as total phenolic content is dependent on the active phenolic compounds present Min et al. (2015). These results agree with the previous study by Helaleh et al. (2001) who established an inverse relationship between the extraction yield and TPC of soil extracts. The highest TPC obtained by the hexane extract (the more non-polar solvent among the three) also depicts that most of the phenolic compounds in the extracts were either non-polar in nature, or partially methylated (Viacava et al., 2017), and hence extracted effectively with hexane. According to Min et al. (2015), higher phenolic concentrations lower soil organic matter decomposition rate. This is because the high phenolic content is inhibitory to the decomposing microbial community, and phenolics can polymerize or form complexes with other molecules, such as proteins, with a high resistance to decomposition. Hence, higher phenolic concentrations in soils are undesirable, as this can influence organic matter decomposition and soil processes (Min et al., 2015). Previous studies have frequently used solvents such as water, ethanol, methanol and acetone to analyze total phenolics in soils (Jinshui et al., 2014; Halvorson et al., 2009). However, this present study has revealed the efficiency and influence of the understudied organic solvents (ethanol, hexane and diethyl ether) on the extraction yield and TPC of soil, with little or no data in literature.

3.4. UV-visible spectroscopic characterization of total phenolic content

The UV-visible profile of the extracts was taken at 200–800 nm wavelength as shown in Figure 2. The results revealed that all the three soil extracts (ethanol, hexane and diethyl ether extracts) absorbed UV light at 200–300 nm wavelength. The occurrence of absorptions at this wavelength (200–300 nm) reveals the presence of flavanol monomers,

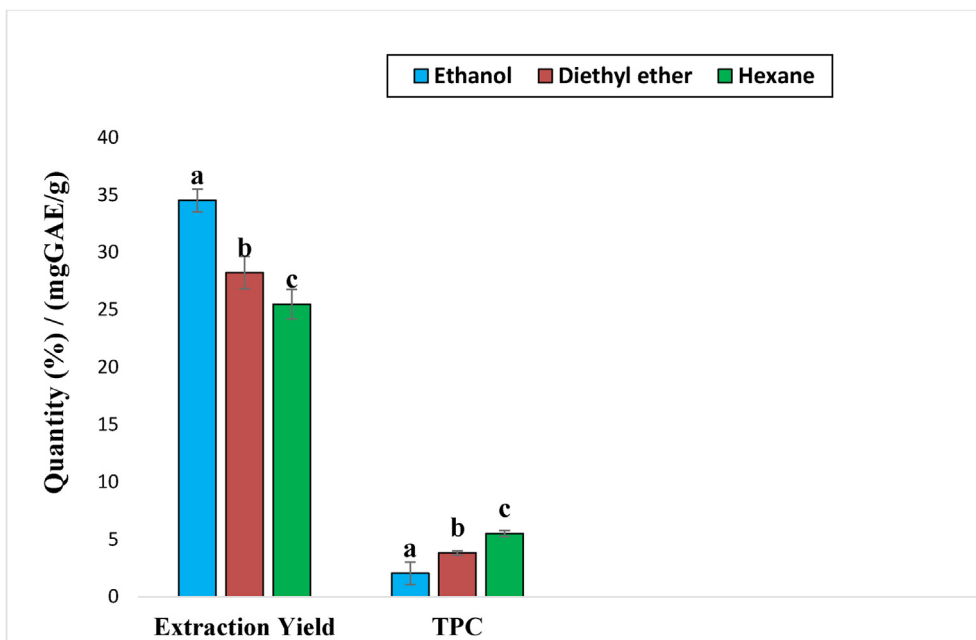


Figure 1. A bar chart representation of the influence of organic solvents on the extraction yield and total phenolic content of soil from vegetable growing area. TPC = Total Phenolic Content. Different letters (a, b and c) indicate statistically significant difference ($P < 0.05$) among solvents. Thus, extraction yield: $P = 0.0326$ and TPC: $P = 0.0214$.

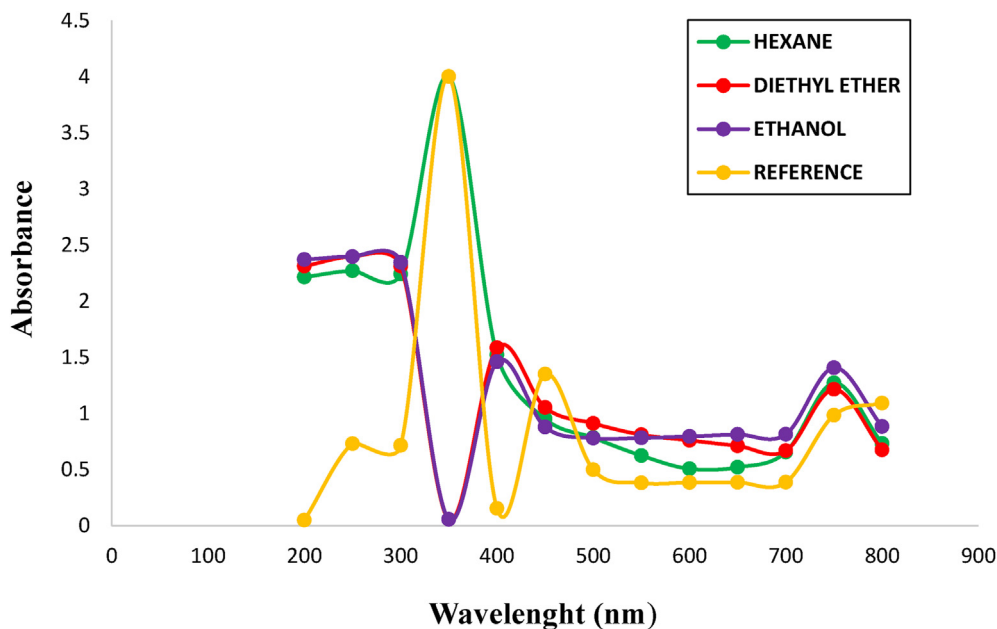


Figure 2. UV-visible spectra of total phenolic content of organic solvent soil extracts.

since monomers of flavanol have an absorption maximum at around 240–290 nm due to their conjugated ring and substitution pattern (Santos-Buelga et al., 2012). However, these colourless compounds did not show absorption features in the visible region of the electromagnetic spectrum. The above result of this study revealed that, there are other phenolic compounds in the extracts that were not detected in the visible region during quantification at 765 nm. This is because some monomers of certain phenolic compounds do not absorb light in the visible region of the electromagnetic spectrum, due to their colourless nature. Hence, there is the need to consider the ultraviolet range of the electromagnetic spectrum when quantifying total phenolic compounds in soil extracts. The highest absorption in the ultraviolet region of the electromagnetic

spectrum for this study was attained by ethanol extract, followed by diethyl ether and hexane, confirming their extraction efficiencies in the visible region of the electromagnetic spectrum.

4. Conclusion

This study has revealed the influence of solvents on the extraction yield and TPC of soil, sampled from a vegetable growing area. Ethanol was established to be more efficient, with an extract yield of 34.52% compare to diethyl ether (28.23%) and hexane (25.47%). In contrast to extraction yield, ethanol which had the highest extract yield rather recorded the least phenolic concentration of 2.04 ± 0.05 mgGAE/g, with

diethyl ether producing 3.82 ± 0.01 mgGAE/g of TPC and 5.50 ± 0.02 mgGAE/g for hexane. A strong inverse correlation was established between the extraction yield and TPC of extracts. In general, extraction using ethanol provides significantly better results, in terms of extraction yield while extraction with hexane is suitable and preferred for total phenolics quantification in soils. Finally, this study recommends future research on identifying and quantifying the individual phenolic compounds in the understudied soil sample.

Declarations

Author contribution statement

Nicholas Akomeng: Conceived and designed the experiments; Performed the experiments; Contributed reagents and materials; Wrote the paper.

Stephen Adusei: Conceived and designed the experiments; Analyzed and interpreted the data.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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References

Adusei, S., Otchere, J.K., Oteng, P., Mensah, Q.R., Tei-Mensah, E., 2019. Phytochemical analysis, antioxidant and metal chelating capacity of *Tetrapleura tetraptera*. *Heliyon* 5 (11), e02762.

- Ainsworth, E.A., Gillespie, K.M., 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nat. Protoc.* 2 (4), 875–877.
- Aleixandre-Tudo, J.L., Toit, W.D., 2018. The role of UV-visible spectroscopy for phenolic compounds quantification in winemaking. In: *Frontiers and New Trends in the Science of Fermented Food and Beverages*, pp. 200–204.
- Arditsoglou, A., Voutsas, D., 2008. Determination of phenolic and steroid endocrine disrupting compounds in environmental matrices. *Environ. Sci. Pollut. Res. Int.* 15 (3), 228–236.
- Bastida, F., Moreno, J.L., Hernandez, T., Garcia, C., 2009. Soil metaproteomics: a review of an emerging environmental science: significance, methodology and perspectives. *Eur. J. Soil Sci.* 60, 845–859.
- Bastola, K.P., Guragain, Y.N., Bhadriraju, V., Vadlani, P.V., 2017. Evaluation of standards and interfering compounds in the determination of phenolics by folin-ciocalteu assay method for effective bioprocessing of biomass. *Am. J. Anal. Chem.* 8 (6), 416–431.
- Cacace, J.E., Mazza, G., 2003. Optimization of extraction of anthocyanins from black currants with aqueous ethanol. *J. Food Sci.* 68 (1), 240–248.
- Fierer, N., Schimel, J.P., Cates, R.G., Zou, J., 2001. Influence of balsam poplar tannin fractions on carbon and nitrogen dynamics in Alaskan taiga floodplain soils. *Soil Biol. Biochem.* 33 (12–13), 1827–1839.
- Halvorson, J.J., Gonzalez, J.M., Hagerman, A.E., Smith, J.L., 2009. Sorption of tannin and related phenolic compounds and effects on soluble-N in soil. *Soil Biol. Biochem.* 41 (9), 2002–2010.
- Handa, S.S., Khanuja, S.P.S., Longo, G., Rakesh, D.D., 2008. *Extraction Technologies for Medicinal and Aromatic Plants*. United Nations Industrial Development Organization and the International Centre for Science and High Technology, Trieste, pp. 22–23.
- Hattenschwiler, S., Vitousek, P.M., 2000. The role of polyphenols in terrestrial ecosystem nutrient cycling. *Trends Ecol. Evol.* 15 (6), 238–243.
- Helaleh, M.L.H., Tanaka, K., Fujii, S., Korenaga, T., 2001. GC/MS determination of phenolic compounds in soil samples using soxhlet extraction and derivatization techniques. *Anal. Sci.* 17 (10), 1225–1227.
- Inderjit, Kaur, R., Kaur, S., Callaway, R.M., 2009. Impact on catechin on soil microbial communities. *Commun. Integr. Biol.* 2 (2), 127–129.
- Jinshui, C., Qun, X., Lina, L., Rohrer, J., 2014. Determination of phenolic compounds in apple orchard soil. *Thermo Sci. Appl. Note* 1077, 1–4.
- Koleva II., van Beek, T.A., Linssen, J.P.H., Groot, A., Evstatieva, L.N., 2002. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. *Phytochem. Anal.: Phytochem. Anal.* 13 (1), 8–17.
- Min, K., Freeman, C., Kang, H., Choi, S., 2015. The regulation by phenolic compounds of soil organic matter dynamics under a changing environment. *BioMed Res. Int.* 1–7.
- Rispaal, N., Morris, P., Webb, K.J., 2005. *Phenolic Compound: Extraction and Analysis*. *Lottus Japonica Handbook*, pp. 349–355.
- Saha, A.K., 2004. *Soil Physics*, first ed. Kalyani publishers, New Delhi, India, pp. 20–23.
- Santana, C.M., Ferrera, Z.S., Padrón, M.E.T., Rodríguez, J.J.S., 2009. Methodologies for the extraction of phenolic compounds from environmental samples: new approaches. *Molecules* 14 (1), 298–320.
- Santos-Buelga, C., Gonzalez-Manzano, S., Duenas, M., Gonzalez-Paramas, A.M., 2012. Extraction and isolation of phenolics compounds. *Methods Mol. Biol.* 864, 427–464.
- Toberman, H., Laiho, R., Evans, C.D., Artz, R.R.E., Fenner, N., Straková, P., Freeman, C., 2010. Long-term drainage for forestry inhibits extracellular phenol oxidase activity in Finnish boreal mire peat. *Eur. J. Soil Sci.* 61 (6), 950–957.
- Viacava, G.E., Roura, S.I., Berrueta, L.A., Iriondo, C., Gallo, B., Alonso-Salces, R.M., 2017. Characterization of phenolic compounds in green and red oak-leaf lettuce cultivars by UHPLC-DAD-ESI-QToF/MS using MS^E scan mode. *J. Mass Spectrom.* 52 (12), 873–902.
- William, W.A., Messai, A.M., Penny, P.G., 2017. Phenolic compounds in water: sources, reactivity, toxicity and treatment methods. In: *Phenolic Compounds- Natural Sources, Importance and Applications*. InTech, pp. 420–443.
- Zhao, M., Yang, B., Wang, J., Li, B., Jiang, Y., 2006. Identification of the major flavonoids from pericarp tissues of lychee fruit in relation to their antioxidant activities. *Food Chem.* 93 (3), 539–554.