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Effect of seasonal changes on the quantity of secondary metabolites from neem and eucalyptus plants in North Central Nigeria

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Abstract

Traditional healthcare system depends majorly on natural medicinal plants from the environment. These plants produce secondary metabolites which confer on them the various medicinal properties; however, Seasonal fluctuations have impact on their availability and quantity hence their therapeutic efficacy. This study was carried out to evaluate the effect of seasonal changes on the quantity of secondary metabolites from Neem and Eucalyptus plants. Leaves and bark of Neem and Eucalyptus plants from SHESTCO, Federal Capital Territory (FCT), Abuja, Nigeria, were collected during the four quarters of the year 2019. Samples were dried, milled into powder, quantity of secondary metabolites was estimated and antioxidant activity was analysed using standard methods and protocols. Results from this study showed a variation in secondary metabolite compositions in response to seasons. In both plants' organs, saponin content peaked and crashed during the second and fourth quarters of the year respectively, while alkaloid had the highest content during the fourth quarter of the year. Highest level of tannin was recorded in the leaf and bark of the plants during the third quarter of the year. Antioxidant activity of both plant extracts showed a regular patterned decrease with increasing concentration, with lowest antioxidant activity for both plants' organs recorded during the third quarter. Findings of the study indicate that the quantity of inherent secondary metabolites in the medicinal plants and their corresponding antioxidant activity varies in specific manners at different times of the year due to seasonal variation.

Keywords: Secondary metabolites; Seasonal changes; Antioxidant; Medicinal plants

1. Introduction

Medicinal plants are highly valuable to human livelihood. WHO estimated that 60% of the world population and 80% of the population of developing countries rely on traditional medicine, mostly plant drugs, for their primary health care needs [1]. Medicinal plants synthesize a number of biological constituents referred to as secondary metabolites or phytochemicals. These are needed for the plant's survival and its protection against predators and insects. The secondary metabolites are an extremely diverse group of compounds not only chemically but also in their functions where they are commonly used in agriculture and drug discovery [2-4]. The medicinal value of these plants lies in their bioactive phytochemical or secondary metabolite constituents that produce definite physiological action on the human body [5].

There is a marked variation in secondary metabolite constituents of medicinal plants due to a number of environmental variables such as temperature, altitude, soil type and change of season/rainfall [6-11]. The production of a variety of phytochemicals is the most successful adaptation of plants while developing various physiological mechanisms by

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which they were able to face both biotic and abiotic stress and threats [12-14]. The production of secondary metabolites, thus, affords plants, unlike other organisms, the ability to survive different seasonal conditions without hibernation [2].

Seasonal changes expose plants to different temperature levels (including extreme levels) that have an effect on their phytochemical compositions, with volatile compounds being the most affected [15]. There are several assumptions regarding the time and season for the collection of various parts of the medicinal plants like spring is suitable for the collection of bark, winter for essential oils etc. [16]. It has been reported by many researchers that during seasonal changes, the content of phenolics and flavonoids varied with the developmental phase of the plants. Djurdjevic and co-workers [17] observed that the maximum content of total phenolics was found in *Conyza Canadensis* L. plants during the flowering and fruiting time in August rather than phenolics which peaks during elongation and excessive plant growth in May and June or in September. In a study on the effects of seasonal variation of the major secondary metabolites present in the extract of *Eremanthus mattogrossensis* (Asteraceae) leaves, a straight correlation between the quantity of metabolites and seasonality was indicated, suggesting that environmental properties elicit important metabolic responses [18].

Eucalyptus globulus and *Azadirachta indica* have been reported to be rich sources of phytochemical compounds such as flavonoids, alkaloids, tannins and propanoids, extracted in the leaf, stem and root of the plants [19-22]. Their antioxidant property has also been widely reported [23-25]. Determination of the seasonal effect on plant phytochemical compositions provides knowledge on the time/season of harvest of individual plant species that afford optimum concentration of active ingredients [26]. Thus, this study aimed at investigating the variation in the yield of *Azadirachta indica* and *Eucalyptus globulus* bark and leaves across the seasons of the year as well as evaluating if the variation of these metabolites modifies the antioxidant activity of the extract from the studied plants' parts.

2. Material and methods

The bark and leaves of *Azadirachta indica* and *Eucalyptus globulus* were collected from the premises of Sheda Science and Technology Complex (SHESTCO) in Sheda village, Kwali, Abuja, located in the North Central region of Nigeria; 8.87^{o} North latitude, 7.01^{o} East longitude and 197 meters elevation above sea level. The collection of samples was done during the dry and rainy seasons. The dry and hot period in march 2019 with average temperature of 29.6°C, average rainfall 20 mm, average humidity 38% and the dry and cold period (harmattan) in December 2019 with average temperature 26.7°C, average rainfall 4 mm, average humidity 26%. The normal rainy period of June 2019 with average temperature 24.5°C, average rainfall 205 mm, average humidity 82% and the heavy rainy period in September 2019 with average temperature 23.6°C, average rainfall 290 mm, average humidity 85%. Temperatures and rainfall levels data were obtained from climatedata online [28] for the period January to December, 2019. The leaf samples were dried at room temperature, ground to powder and stored in the dark until further usage.

2.1. Phytochemical analyses

The bark and leaves of both medicinal plants neem and eucalyptus were analyzed for the quantity of simple phenols, flavonoids, tannins, alkaloids, and saponins, using standard procedures for the quantitative analysis of these phytochemicals.

2.2. Determination of Saponin

Estimation of the quantity of saponin was carried out as described by Obadoni and Ochuko [28]. 0.5g of the sample was added to 20ml of 1N HCl and was boiled for 4h. After cooling, it was filtered and 50 ml of petroleum ether was added to the filtrate and it was evaporated to dryness. 5 ml of acetone-ethanol was added to the residue. 0.4 ml of each was taken into 3 different test tubes, 6 ml of ferric sulphate reagent was added into them followed by 2 ml of conc. H_2SO_4 acid. The mixture was thoroughly mixed after 10 mins and the absorbance was taken at 490nm

2.3. Determination of flavonoids

The determination of flavonoids in the samples was done by acid hydrolysis method as described by Vabkova and Neugebauerova [29]. 0.5g of dried samples was mixed with 5ml of dilute hydrochloric acid and boiled for 10 mins. The boiled extract was allowed to cool and filtered. 1 ml of the filtrate was added to 5 ml of ethyl acetate and 5 ml of 1% ammonium. This was then scanned from 420-520nm for the absorbance.

2.4. Determination of Phenol

The quantity of phenol was determined using the spectrophotometric method as described by AOAC (1995). 0.5g of sample was boiled with 50ml diethylether for 15mins. 5ml of the boiled sample was then pipette into a 50ml flask and

10ml of distilled water is added. 2ml of ammonium hydroxide solution and 5 ml of boiled conc. Butanol was then added to the mixture. The mixture was made up to the mark and left for 30mins to react for colour development and absorbance measured at 505nm wavelength using a spectrophotometer.

2.5. Determination of alkaloid

Determination of alkaloid was done by procedure described by Manjunath and colleagues [31]. 0.5g of leaves sample was macerated with 20ml 96% ethanol and 20% Tetraoxosulphate (VI) acid (1:1v/v). The filtrate (1ml) was added to 5ml of 60% Tetraoxosulphate (VI) acid and allowed to stand for 5 mins. 5 ml of formaldehyde in 60% H_2SO_4 was then added and allowed to stand for 3 hrs. The absorbance was read at 565nm.

2.6. Determination of Tannin

The quantity of tannins in the samples was determined by using the spectrophotometric method as described by Van-Burden and Robinson [32]. 0.5g of the samples was weighed into a conical flask. 50 ml of distilled water was added and stirred for 1 hr. The sample was filtered into a 50 ml volumetric flask and made up to mark. 5 ml of the filtered sample was then pipetted out into a test tube and mixed with 2 ml of 0.1M HCl and 0.008M $K_4Fe(CN)_6.3H_2O$. The absorbance was then measured with a spectrophotometer at 395 nm wavelength within 10 mins.

2.7. Determination of Antioxidant Activity

The antioxidant activity of the samples was evaluated by discolouration of 2, 2-diphenyl-1-picrylhydrozyl radical (DPPH) in methanol by a slightly modified method of Brand-Williams *et al.* [33]. The following concentrations of the extract were tested (0.1, 0.3, 0.5, 0.7, 1.0 mg/ml). The absorbance was monitored at 517nm. Vitamin C was used as the antioxidant standard at concentrations (0.1, 0.3, 0.5, 0.7, 1.0 mg/ml). A blank solution was prepared containing the same amount of methanol and DPPH. The radical scavenging activity was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{A_b - A_s}{A_b} \times 100$$

Where A_b = Absorbance of the blank

A_s = The absorption of the sample extract.

2.8. Statistical analysis

Experimental results were expressed as means ± standard deviations (n = 3, from 3 independent experiments). Differences between phytochemical quantities of the plant leaf and bark samples were determined using analysis of variance (ANOVA, SPSS). The values were regarded as statistically significant at $p < 0.05$.

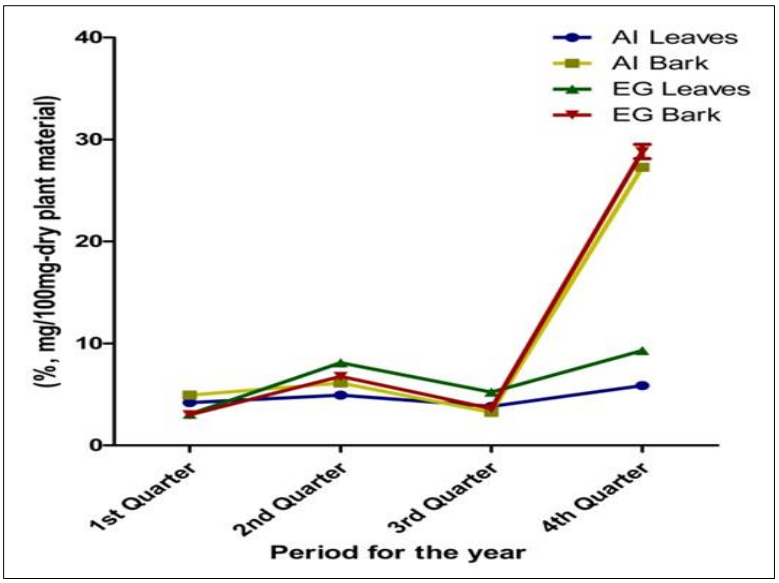
3. Results

3.1. Quantitative Phytochemical Analysis

Determination of the effect of seasonal changes on the quantity of plants phytochemicals is an important step to establish the suitable harvesting season with high amounts of phytochemicals. Seasonal quantitative phytochemical analysis of the bark and leaves of *Azadirachta indica* and *Eucalyptus globulus* showed that these medicinal plants have different alkaloids, flavonoid, phenols, tannin, saponins and tannins content during various seasons of the year. The results are shown in Figures 1-5 for alkaloid, flavonoid, phenol, saponin and tannin respectively for *Azadirachta indica* and *Eucalyptus globulus* bark and leaves.

3.2. Alkaloids Content

The quantity of alkaloid obtained from the leaf and bark of Eucalyptus and Neem plants was significantly high during the cold period, fourth quarter. There was no significant difference in the alkaloid content from the plants' samples during first and third quarter except from leaf of *Eucalyptus globulus*. The result showed that alkaloid quantity from the bark was significantly higher than that obtained from the leaves of both plans (Figure 1).

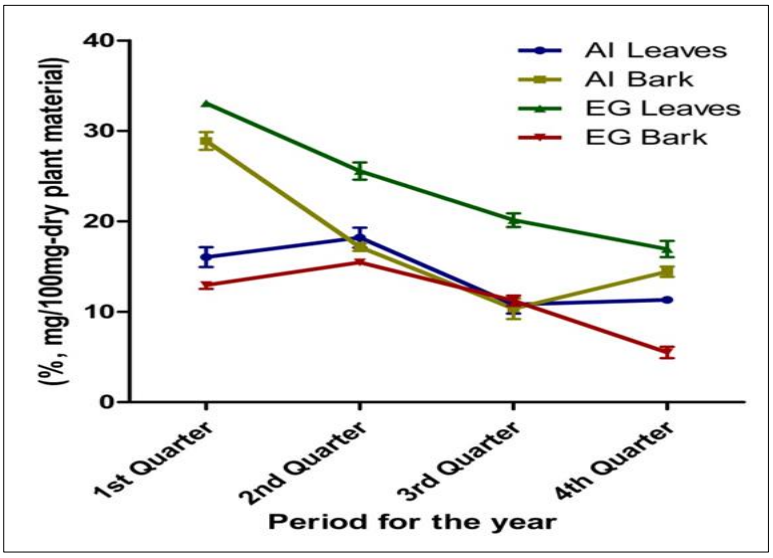


AI = *Azadirachta indica*; EG = *Eucalyptus globulus*

Figure 1 Seasonal quantities (%, mg/100mg-dry plant material) of alkaloid content from the leaves and bark of Neem and Eucalyptus plants

3.3. Flavonoid Content

Figure 2 shows the flavonoid content of the leaves and bark of *Azadirachta indica* and *Eucalyptus globulus* during the four quarters of the year. Results from the study revealed varying concentration of flavonoid across the year. The quantity of flavonoid from neem leaves was significantly high in the first and second quarters which are the hot period of the year while the flavoniod quantity from neem bark was significantly high in the first quarter and the lowest quantity obtained in the third quarter. A significant decrease in flavonoid quantity was observed across the year in *E. globulus* leaf and bark.



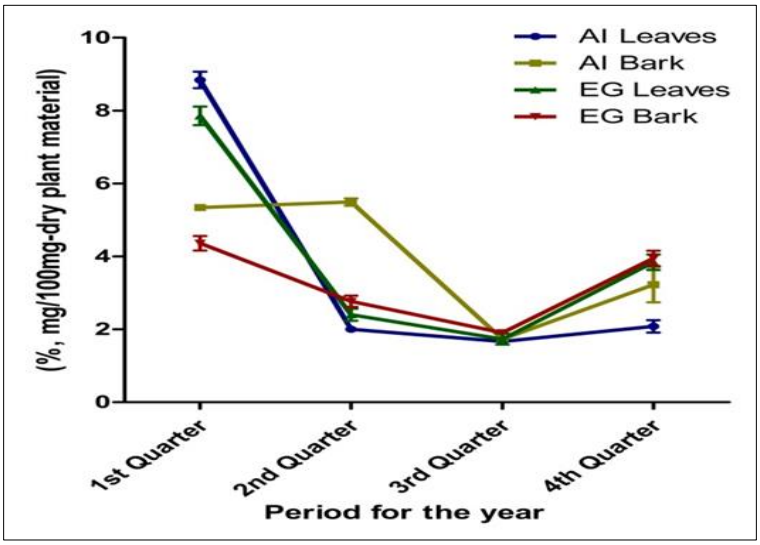
AI = *Azadirachta indica*; EG = *Eucalyptus globulus*

Figure 2 Seasonal quantities (%, mg/100mg-dry plant material) of flavonoid content from the leaves and bark of Neem and Eucalyptus plants

3.4. Phenol content

The phenol quantity obtained from *Azadirachta indica* and *E. globulus* leaves was significantly high during the hot period, first quarter, of the year. A significantly high quantity was obtained during the first and second quarter of year from neem bark while a significant quantity was obtained from the bark of *E. globulus* during the first and fourth quarter.

The lowest quantity of phenol was obtained during the third quarter from the plant samples (Figure 3). The leaves of both planted revealed high quantity of phenol when compared to the bark.

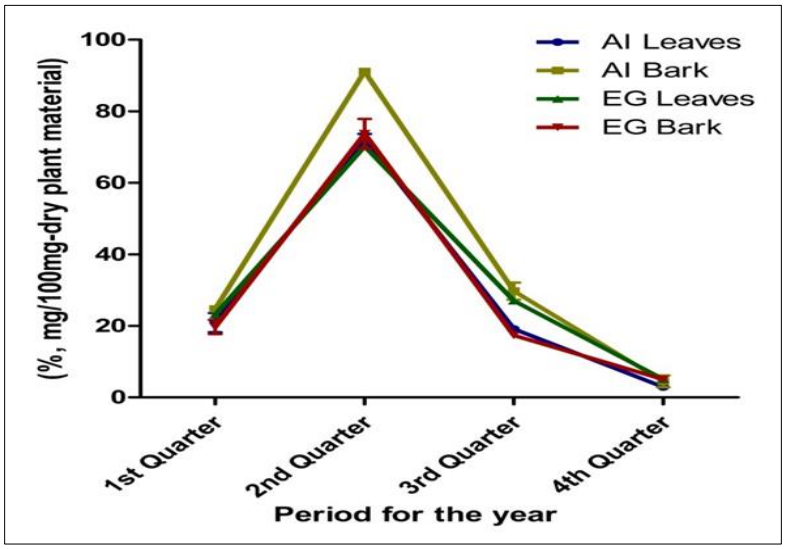


AI = *Azadirachta indica*; EG = *Eucalyptus globulus*

Figure 3 Seasonal quantities (%, mg/100mg-dry plant material) of phenol content from the leaves and bark of Neem and Eucalyptus plants

3.5. Saponin content

A significantly high amount of saponin was obtained during the second quarter of the year from both leaves and bark of *Azadirachta indica* and *E. globulus* while the quantity of phenol was significantly low during the fourth quarter of the year. There was no significant difference in quantity of saponin during the first and third quarters for all plant samples (Figure 4).

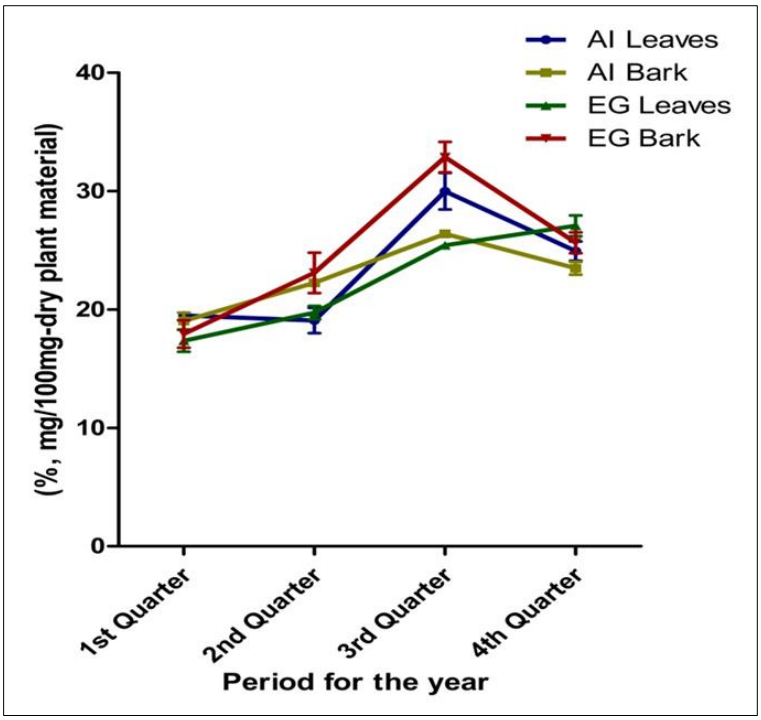


AI = *Azadirachta indica*; EG = *Eucalyptus globulus*

Figure 4 Seasonal quantities (%, mg/100mg-dry plant material) of saponin content from the leaves and bark of Neem and Eucalyptus plants

3.6. Tannin content

A significantly high amount of tannin was obtained during the cold period, third quarter, of the year from both leaf and bark of *Azadirachta indica* and *E. globulus* while a significantly low quantity was observed during the first quarter of the year for all plants' samples.



AI = *Azadirachta indica*; EG = *Eucalyptus globulus*

Figure 5 Seasonal quantities (%, mg/100mg-dry plant material) of tannin content from the leaves and bark of Neem and Eucalyptus plants

3.7. Antioxidant activity

Table 1 Seasonal antioxidant (% Inhibition) activity of Neem plant

Conc. of extract (mg/ml)	1st Quarter		2nd Quarter		3rd Quarter		4th Quarter	
	Leaf	Bark	Leaf	Bark	Leaf	Bark	Leaf	Bark
0.1	82.83	84.44	77.6	82.01	47.48	37.18	79.53	75.61
0.3	82.63	82.57	76.0	72.25	56.72	45.38	79.53	72.91
0.5	80.63	80.22	75.6	59.54	57.35	48.11	77.39	70.47
0.7	78.62	77.96	72.4	44.51	60.71	53.78	78.97	70.67
1.0	76.22	73.42	69.8	26.59	68.49	61.55	78.31	66.04

Table 2 Seasonal antioxidant (% Inhibition) activity of Eucalyptus plant

Conc. of extract (mg/ml)	1st Quarter		2nd Quarter		3rd Quarter		4th Quarter	
	Leaf	Bark	Leaf	Bark	Leaf	Bark	Leaf	Bark
0.1	84.30	83.50	85.8	90.75	55.88	39.71	68.74	77.29
0.3	84.57	83.37	82.0	78.90	60.29	45.80	71.13	75.66
0.5	84.50	83.10	81.8	69.36	62.39	45.62	66.29	75.76
0.7	83.63	82.03	79.2	63.01	65.76	54.62	64.46	72.86
1.0	83.70	80.96	74.4	56.94	67.65	61.34	59.98	72.91

The antioxidant activity results are shown in Table 1 & 2. Our results showed that the antioxidant activity of *Azadirachta indica* leaf and bark ranged from 37.18-84.44% while that of *E. globulus* leaf and bark ranged from 39.71-90.75%. The results revealed a regular patterned decrease with increase in concentration.

4. Discussion

The results from this study indicate that medicinal plants may have high accumulation of different phytochemicals during different seasons, as seen in the case of alkaloids and tannins been accumulated in high amounts during colder seasons while flavonoids were accumulated in high amounts during warmer seasons for the medicinal plants *Azadirachta indica and Eucalyptus globulus.* The results obtained from this study are in agreement with the findings of the previous studies in other plants. Gololo and colleagues reported that high amount of alkaloid is usually obtained from medicinal plants during the colder season [3]; the leaves of *Barleria prionitis, Boerhavia diffusa, Citrillus colocynthis* and *Grewia tenax* were reported to have different yields of total alkaloids and polyphenols in different seasons, with the Indian summer (April-June) showing the highest yield compared to winter (December-February) and rainy season (July-September) [34]. In a separate study, total alkaloids and polyphenols were high during summer season in *Convoliulus microphyllus*, whereas the same phytochemical groups were high during winter in Datura metel [35]. Galolo *et al*. [3] reported that the leaves of *Barleria dinteri, Grewia flava* and *Jatropha lagarinthoides*, collected from Limpopo province in South Africa displayed high yield of simple phenol during Autum (March) in *G. Flava* and during summer in *Jatropha lagarinthoides*. The differences in the seasonal phytochemical quantities of the leaves of medicinal plants were earlier reported to cause seasonal variation in their biological activities [36].

The four seasons during which plant samples were collected are characterized by different factors such as water level in the soil, evapotranspiration rate, light intensity, photosynthetic efficiency, plant water potential and plant stage, directly respond to these variations [3, 37-39]. Ncube *et al*. [40] have found variation in the production of polyphenols in *Tulbaghia violacea, Hypoxis hemerocallidea, Merwilla plumbea* and *Drimia robusta* in different seasons, the explanation lies precisely in the climate differences, biotic and environmental conditions in addition to genetic factors. Other authors [41-47] have also attributed the variation in the production of secondary metabolites to environmental factors.

Both plants' bark and leaves were shown to have lowest antioxidant activity during the cold period, third quarter, of the year while the highest antioxidant activity was observed during the first quarter for both plants' leaves and bark. This report is in agreement with previous reports by Ramírez-Briones *et al.* [48] and Aoussar *et al.* [49] who reported that high antioxidant activity was recorded for plants such as *Pseudevernia furfuracea*, *Evernia prunastri* and *Ramalina farinacea* from Morocco during winter (March) and spring (May). Previous studies by Said *et al.* [24] and Tálos-Nebehaj *et al*. [50] have reported a correlation between the quantity of phenol and antioxidant capacity of plants. They established that polyphenols are primarily responsible for the antioxidant properties of plants. Thus in this study, a low antioxidant capacity and significantly low quantity of phenol was recorded during the cold season for the plants' leaves and bark while a significant high quantity of phenol obtained during the hot period of the year correspond with the high antioxidant capacity recorded during this period as well.

5. Conclusion

Taken together, the findings of the study indicate that the quantity of inherent secondary metabolites of the leaves and bark of *Eucalyptus globulus* and Neem plant and their corresponding antioxidant activity varies in specific manners at different times of the year due to the seasonal variation. Determination of the effect of seasonal changes on the quantity of plants phytochemicals is an important step to establish the suitable harvesting season of the plants in order to obtain high amounts of specific phytochemicals for medicinal purposes. Thus, from our study we recommend the cold period as the optimum period for the use of alkaloids and tannin from *Azadirachta indica and Eucalyptus globulus* for medicinal purpose, due to the high yield during this period.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The Authors declare no conflict of interest.

References

[1] Kosalge SB, Fursule RA. Investigation of Ethno medicinal claims of some plants used by tribals of Satpuda Hills in India. Journal of Ethno Pharmacology. 2009; 121: 456-461.

[2] Yadav RNS, Agarwala M. Phytochemical analysis of some medicinal plants. Journal of Phytology. 2011; 3: 10-14.

[3] Gololo SS, Shai LJ, Agyei NM, Mogale MA. Effect of seasonal changes on the quantity of phytochemicals in the leaves of three medicinal plants from Limpopo province, South Africa. Journal of Pharmacognosy and Phytotherapy. 2016; 8(9): 168-172.

[4] Mohiuddin AK. Effect of Environment on Secondary Metabolism of Medicinal Plants. Open Access Journal of Environmental and Soil Sciences. 2019; 2(1): 145-155.

[5] Akinmoladun AC, Ibukun EO, Dan-Ologe IA. Phytochemical constituents and antioxidant properties of extracts from the leaves of Chromolaena odorata. Scientific Research and Essay. 2007; 2(6): 191-194.

[6] Szakiel A, Paczkowski C, Henry M. Influence of environmental abiotic factors on the content of saponins in plants. Phytochemistry Reviews. 2011; 10(4): 471-491.

[7] Ahmad I, Ahmad MSA, Ashraf M, Hussain M, Ashraf MY. Seasonal variation in some medicinal and biochemical ingredients in Mentha longifolia. Pakistan Journal Botany. 2011; 43: 69-77.

[8] Nchabeleng L, Mudau FN, Mariga IK. Effects of chemical composition of wild bush tea (Athrixia phylicoides DC.) growing at locations differing in altitude, climate and edaphic factors. Journal of Medicinal Plant Research. 2012; 6: 1662-1666.

[9] Jayanthy A, Prakash KU, Remashree AB. Seasonal and Geographical Variations in Cellular Characters and Chemical contents in Desmodium gangeticum (L) DC. - An Ayurvedic Medicinal plant. International Journal of Herbal Medicine. 2013; 1: 34-37.

[10] Odjegba VJ, Alokaloro AA. Simulated Drought and Salinity Modulates the Production of Phytochemicals in Acalypha wilkesiana. Journal of Plant Studies. 2013; 2: 105-112.

[11] Sampaio BL, Edrada-Ebel R, Da Costa FB. Effect of the environment on the secondary metabolic profile of Tithonia diversifolia: A model for environmental metabolomics of plants Scientific Reports. 2016; 6: 29265.

[12] Figueiredo AC, Barroso JG. Pedro LG, Scheffer JC. Factors affecting metabolite production in plants: Volatile compounds and essential oils. Flavour and Fragrance. 2008; 23(4): 213-226.

[13] Mohiuddin AK, Chemistry of secondary metabolites. Annals of Clinical Toxicology. 2019; 2(1): 1014.

[14] Huang W, Bont Z, Hervé MR, Robert CAM, Erb M. Impact of Seasonal and Temperature-Dependent Variation in Root Defense Metabolites on Herbivore Preference in Taraxacum officinale. Journal of Chemical Ecology. 2020; 46: 63–75.

[15] Usano-Alemany J, Pala-Paul J, Herraize-Penalver D. Temperature stress sauses different profiles of volatile compounds of Salvia Iavandulifolia Vahl. Biochemical Systematics and Ecology. 2014; 54: 166-171.

[16] Soni U, Brar S, Gauttam VK. Effect of Seasonal Variation on Secondary Metabolites of Medicinal Plants. International Journal of Pharmaceutical Sciences and Research. 2015; 6(9): 3654-3662.

[17] Djurdjevic L, Gajic G, Kostic O, Jaric S, Pavlovic M, Mitrovic M, Pavlovic P, Flora (Jena). 2012; 207: 812.

[18] Gouvea DR, GobboNeto L, Sakamoto HT, Lopes NP, Lopes JLC, Meloni F, Amaral JG. Quim. Nova. 2012; 35: 2139.

[19] Dixit A, Rohilla A, Singh V. Eucalyptus globulus: A new perspective in therapeutics. International Journal of Pharmaceutical and Chemical Sciences. 2012; 1(4): 1678-1683.

[20] Xu J, Song X, Yin ZQ, Cheng AC, Jia RY, Deng YX. Antiviral activity and mode of action of extracts from neem seed kernel against duck plague virus in vitro. Poultry Science. 2012; 91(11): 2802-2807.

[21] Vecchio MG, Loganes C, Minto C. Beneficial and healthy properties of Eucalptus Plants: A great potential use. The Open Agriculture Journal. 2016; 10(Suppl 1:M3): 52-57.

[22] Khogare BT, Kulal SR, Rangar KK, Kokare BN. Medicinal uses of neem (Azadirachta indica) in human life: A Review. International Journal. of. Life Sciences, Special Issue. 2018; A10: 181-184.

[23] Manikandan P, Anandan R, Nagini S. Evaluation of Azadirachta indica leaf fractions for in vitro antioxidant potential and protective effects against H2O2-induced oxidative damage to pBR322 DNA and red blood cells. Journal of Agricultural and Food Chemistry. 2009; 57(15): 6990-6996.

[24] Said ZB, Slimani S, Remini H, Idir-Himed H, Mazauric J, Madani K, Boulekbache-Makhlouf L. Phytochemical analysis and antioxidant activity of Eucalyptus globulus: A comparative study between fruits and leaves extracts. Journal of Chemical Engineering & Bioanalytical Chemistry. 2016; 1(1): 1-10.

[25] Ganesan D, Al-Sayed E, Albert A, Paul E, Singab ANB, Govindan Sadasivam S, Saso L Antioxidant activity of phenolic compounds from extracts of Eucalyptus *globulus* and Melaleuca styphelioides and their protective role on D-glucose-induced hyperglycaemic stress and oxalate stress in NRK-49Fcells. Natural Product Research. 2018; 32(11): 1274-1280.

[26] Kale VS. Variable Rates of Primary and Secondary Metabolites during Different Seasons and Physiological stages in Convolvulus, Datura and Withania. Asian Journal of Experimental Biological Sciences. 2010; SPL: 50-53.

[27] Climatedata.org [internet] Abuja climate data © 2019 [cited 2019 Dec 31].

[28] Obadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. Global Journal of Pure and Applied Science. 2001; 8: 203-208.

[29] Vabkova J, Neugebauerova J. Determination of total phenoliccontent, total flavonoid content and FRAP in culinary herbs in relation to harvest time. Acta Universitatis Agriculturae Et Silviculturae Mendelianae Brunensis. 2012; LX (1): 167-172.

[30] AOAC. Official methods of analysis (16th edn) Association of Official Analytical Chemists. Arlington, V. A. USA. 1995.

[31] Manjunath A, Mahadev BG, Shradda UN. Estimation of total alkaloid in Chitrakadivati by UV-Spectrophotometer. Ancient Science of Life. 2012; 31(4): 198-201.

[32] Van-Burden TP, Robinson EC. Formation of Complexes between proteins and tannin acid. Journal of Agricultural and Food Chemistry. 1981; 1: 77-82.

[33] Brand-Williams B, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food Science and Technology. 1995; 28: 25-30.

[34] Sahoo KP, Kasera PK, Mohammed S. Secondary metabolites produced during different seasons in some arid medicinal plants. Asian Journal of Plant Science Research. 2012; 2: 650-652.

[35] Kale VS. Variable Rates of Primary and Secondary Metabolites during Different Seasons and Physiological stages in Convolvulus, Datura and Withania. Asian Journal of Experimental Biological Sciences. 2010; SPL: 50-53.

[36] Ncube B, Finnie JF, Van Staden J. Seasonal variation in antimicrobial and phytochemical properties of frequently used medicinal bulbous plants from South Africa. South African Journal of Botany. 2011; 77: 387-396.

[37] Ferri MG. Fisiologia Vegetal, EPU, São Paulo, SP, Brazil. 1986.

[38] Larcher W. Ecofisiologia Vegetal, RiMa, São Carlos, SP, Brazil. 2004.

[39] Taiz L, Zeiger C. Fisiologia Vegetal, Artmed, Porto Alegre, RS, Brazil. 2004.

[40] Ncube B, Finnie JF, Van Staden J. Seasonal variation in antimicrobial and phytochemical properties of frequently used medicinal bulbous plants from South Africa. South African Journal of Botany. 2010; 79: 1- 10.

[41] Ma M, Hong C, An S, Li B. Seasonal, spatial, and interspecific variation in quercetin in Apocynum venetum and Poacynum herdersonii, chinese traditional herbal teas. J. Agric. Food Chem. 2003; 51: 2390-2393.

[42] Brooks JS, Feeny P. Seasonal variation in Daucus carota leafsurface and leaf-tissue chemical profiles. Biochem. Systemat. Ecol. 2004; 32: 769–782.

[43] Ercisli S, Orhan E, Ozdemir O, Sengul M, Gungor N. Seasonal variation of Total Phenolic, Antioxidant Activity, Plant Nutrition Elements, and Fatty Acids in Tea Leaves (Camellia sinensis var sinensis clone Derepazari 7) Grown in Turkey. Pharm. Biol. 2008; 26: 683- 687.

[44] Ruiz-Teran F, Medrano-Martinez A, Navarra-Ocaña A. Antioxidant and free radical scavenging activities of plant extracts used in traditional medicine in Mexico. African Journal of Biotechnology. 2008; 7: 1886-1893.

[45] Santos AO, Kaye O. Composition and chemical-sensorial profile of 'Syrah' cultivated under transient water stress. Rev. Bras. Eng. Agríc. Ambient. 2009; 13: 272-281.

[46] Siatka T, Kašparovà M. Seasonal variation in total phenolic and flavonoid contents and DPPH scavenging activity of Bellis perennis L. flowers. Molecules. 2010; 15: 9450-9461.

[47] Chavarria G, Bergamaschi H, Silva LC, Santos HP, Mandelli F, Guerra CC, Flores CA, Tonietto J. Water relations, yield and phenolic compounds of grapevines cv. Cabernet Sauvignon in three soil types. Bragantia. 2011; 70: 481-487.

[48] Ramírez-Briones E, Rodríguez-Macías R, Salcedo-Pérez E, Ramírez-Chávez E, Molina-Torres J, Tiessen A, Ordaz-Ortiz J, Martínez-Gallardo N, Délano-Frier JP, Zañudo-Hernández J. Seasonal Changes in the Metabolic Profiles and Biological Activity in Leaves of Diospyros digyna and D. rekoi "Zapote" Trees. Plants. 2019; 8: 449.

[49] Aoussar N, Rhallabi N, Mhand R.A, Manzali R, Bouksaim M, Douira A. Mellouki F. Seasonal variation of antioxidant activity and phenolic content of Pseudevernia furfuracea, Evernia prunastri and Ramalina farinacea from Morocco. Journal of the Saudi Society of Agricultural Sciences. 2020; 19: 1-6.

[50] Tálos-Nebehaj E, Hofmann, T, Albert L. Seasonal changes of natural antioxidant content in the leaves of Hungarian forest trees. Industrial Crops and Products. 2017; 98: 53-59.

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