IJBPAS, May, 2021, 10(5): 1616-1629

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

PHYTOCHEMICAL VARIATION IN *DICTAMNUS ALBUS* IN RELATION TO DIFFERENT PARTS: A PERSPECTIVE

NISSAR S[1*], MAJID N[1], RESHI FA[2], RAJA WY[3], NAWCHOO IA[1] AND BHAT ZA[3]

1:Plant Reproductive Biology, Genetic Diversity and Phytochemistry Research Laboratory, Department of Botany, University of Kashmir, Srinagar, 190006, J&K, India

2: Department of School Education, J&K, India

3: Department of Pharmaceutical Sciences, University of Kashmir, Srinagar, 190006, J&K, India

***Corresponding Author: Saduf Nissar: E Mail: sadufnissarnaik@gmail.com**

Received 18th June 2020; Revised 19th July 2020; Accepted 16th Aug. 2020; Available online 1st May 2021

https://doi.org/10.31032/IJBPAS/2021/10.5.5474

ABSTRACT

Dictamnus albus L., commonly known as gas plant has an immense medicinal importance. Although few studies have been carried out regarding the phytochemical constituents of *D. albus* but to date no information is available regarding the partwise variation of phytochemical contents. So, this work aims to investigate the comparative phytochemical profile for different parts of *D. albus* (leaves, stem, root and fruits). The Soxhlet extraction of different parts of *D. albus* was done and the extracts (hydro-alcholic) analyzed for qualitative and quantitative estimation of various phytochemicals. The extracts showed the presence of most promising phytochemicals viz., phenolics, flavonoids, tannins, alkaloids, terpenoids and saponins. The quantitative estimation of the phytochemicals depicted a significant variation among different parts and leaves are considered to possess highest quantity of phytochemicals. These phytochemicals could serve as major source for pharmaceutical products, so the plant species could hold an immense potential to serve as therapy for various chronic diseases. Our results presented a detailed account on the variation of phytochemical constituents in relation different parts of this valuable medicinal plant.

Keywords: *Dictamnus albus*, Partwise, Phytochemical, Soxhlet

INTRODUCTION

The world is blessed with unique medicinal plants and among these, *Dictamnus albus* is considered to be one of the medicinal charms of the nature. *Dictamnus albus*, perennial herb, commonly known as gas plant or burning bush, is distributed throughout south and central Europe, temperate Asia, temperate Himalaya **[1]**. In India, it is distributed in Uttar Pradesh, Jammu and Kashmir and Himachal Pradesh **[1]**. It is regarded as an ornamental and is often grown in gardens of numerous temperate countries for bright and attractive blooms **[1]**. It has a tremendous medicinal importance. In Indian folk medicine, *D.albus* has been used as an emmenagogue and abortive agent **[2, 3]**. The root bark of *D. albus* has been used against jaundice, leprosy, cough, rheumatism, amenorrhea, and some skin diseases **[4]**. In Greek folk medicine it is used as an antispasmodic, tonic, stimulant, and ant-helminthic **[5]**. In folk remedy of Turkey the plant has been used for stomachic, tonic, stimulant and antipyretic activities **[6]**. In Bulgaria and Korea the plant is used as an anticancer, antispasmodic, diuretic and sedative agent **[7]**. In Serbia *D. albus* is used as tea mixture in the treatment of neurasthenia, hysteria, schizophrenia and other mental diseases **[8]**. Decoction of root is applied for scabies and various other skin diseases **[9, 10]**.

Apart from fulfilling the primary need of food by their primary products such as carbohydrates, fats, proteins and vitamins, plants produce a number of secondary metabolites that serve as a potential source of pharmaceutically active substances. A wide range of medicinal plant parts such as root, stem, flower, fruit, twigs, exudates and modified plant organs are used as raw drugs and they possess varied medicinal properties. Although various studies have been carried out on phytochemical constituents in many medicinal plants, not much attention has been paid to the variation of phytochemicals in different parts of these plants. So, the present study seeks to provide a comprehensive investigation on the variation of phytochemical constituents in different parts, so that full phytochemical potential of the plant could be explored.

MATERIAL AND METHODS

Collection of plant material

Healthy and disease free plants of *D.albus* were collected from Sonamarg area of Jammu and Kashmir. The collected specimens were identified and deposited in Kashmir University Herbarium (KASH) under voucher number 2688-KASH. The plant collections were made quite judiciously throughout the course of the present study. The plant materials were fragmented into different parts (root, stem, leaves and fruits) and dried under shade at room temperature for 15-20 days. After shade drying, the plant materials were powdered and stored under proper conditions for future use.

Preparation of Plant Extract

The powdered samples (leaves, stem, root and fruits) of *D. albus* were extracted separately in Soxhlet apparatus using hydro-alcohol (70% ethanol) solvent for 2-3 days. The extracts were concentrated under reduced pressure with rotatory vacuum evaporator to get viscous masses. Finally the extracts were dried, weighed, labeled and stored at 4˚C in storage vials for experimental uses.

Phytochemical Screening

The qualitative phytochemical screening of hydro-alcoholic extracts of different parts of *D. albus* was undertaken following standard methods **(Table 1)**.

Table 1: Qualitative screening tests for different phytochemicals

Phytochemicals	Tests	Ideal observations	References
Carbohydrates	Molisch's: filtrates treated with 2 drops of Molischs reagent	Violet ring at the junction	[11]
	Benedict's: filtrate gently heated in presence of Benedict's reagent	Orange red precipitate	[12]
Anthraquinone glycosides	Borntrager's: sample boiled in presence of 1ml H_2SO_4 for 5 minutes, filtered, cooled and treated with few drops of chloroform, organic layer separated and ammonia added	Pink colour	[13,14]
Cardiac glycosides	Keller Killiani: 1ml glacial acetic acid, a few drops of $FeCl_3$ and 1ml conc. H_2SO_4 added to 2ml filtrate	Brown ring at the interface and violet ring may appear below it. In acetic acid layer greenish ring may form just above the brown ring which gradually spreads throughout this layer	[15]
	Legal's: extract treated with sodium nitroprusside in pyridine and NaOH	Pink to blood red colour	[16]
Proteins and amino acids	Xanthoproteic acid: extracts treated with a few drops of conc. HNO_3	Yellow colour	[17]
	Ninhydrin: extracts treated with ninhydrin reagent (25% w/v) and boiled for a few minutes	Blue colour	[15]
Alkaloids	Dragendroff's: filtrates treated with a few drops of Dragendroff's reagent (iodine in potassium bismuth)	Orange precipitate	[18]
	Wagner's: filtrates treated with 1-3 drops of Wagner's reagent (iodine in potassium iodide)	Brown/reddish precipitate	[19]
	Hager's: extracts treated with 1-3 drops of Hager's reagent (saturated picric acid)	Yellow precipitate	[18]

Phenolics	**Ferric chloride test: extracts treated with a few drops of ferric chloride solution**	**Bluish black**	**[20]**
Tannins	**Ferric chloride test: 2ml extract treated with 2ml of 5% $FeCl_3$**	**Yellow brown**	**[21]**
	Lead acetate: extracts treated with 1-3 drops of lead acetate solution	**White precipitate**	**[15]**
Flavonoids	**Shinoda: 2ml filtrate treated with a few drops of conc. HCl followed by 0.5g of zinc or magnesium turnings**	**Magenta red or pink colour**	**[22]**
	Alkaline reagent: 2ml extract treated with 20% NaOH	**At first intense yellow colouration occurs which becomes colourless on addition of dilute HCl**	**[23]**
Terpenoids	**Salkowski's: 2ml extracts treated with 1ml of chloroform followed by conc. sulphuric acid, shaken and allowed to stand**	**Golden yellow colour**	**[24]**
Saponins	**Foam test: 6ml water added to 2ml extract and shaken vigorously**	**Persistent foam**	**[25]**

Quantitative Estimation

Alkaloid determination

2.5 g of the powdered samples were extracted using 100 ml of 20% acetic acid in ethanol and covered for almost 4 hours. Filtrate was concentrated to 25 ml. Concentrated ammonium hydroxide was added stepwise to achieve precipitation. The whole solution was kept as such so that precipitate will settle. Collected precipitates were washed with dilute ammonium hydroxide and finally filtered. Filtrates were disposed of and pellet obtained were dried and weighed **[26, 27]**.

Saponin determination

10 g of samples were mixed with 100 ml of 20% aqueous ethanol. The mixture was kept at 55°C on water bath shaker for 4 hours. Filtrates were extracted in the same way again. The combined extracts were concentrated over water bath at 90°C to 40 ml. Concentrates were transferred to a separating funnel followed by the addition of 10 ml diethyl ether. After vigorously shaken aqueous layer was recovered and the ether layer discarded. The process was repeated. n-butanol was added to the aqueous layer. The entire mixture was washed 10 ml 5% of aqueous NaCl in a separating funnel twice with. Upper part was retained and heated until evaporation in water bath. Latter it was dried in oven to a constant weight **[26, 28]**.

Terpenoid determination

10 grams of plant powder were soaked in alcohol for 24 hours. Then filtered, the filtrate was extracted with petroleum ether; the ether extract was treated as total terpenoids **[29]**.

Phenolics determination

Total phenolic content was determined by spectrophotometric method. For the analysis 1mg/1ml of the plant extract solutions were prepared. 0.5ml and 1 ml of the solutions were transferred to separate test tubes followed by addition of 1ml of Folin-Ciocalteu reagent (2N) and 4ml of 20% of Na_2CO_3 and ultimately the volume was made up to 6ml with double distilled water. The samples were vigorously shaken and finally allowed to incubate at room temperature for 2 hours after which the absorbance was taken at 765 nm. The samples were prepared in triplicates. Quantification was done using standard calibration curve of gallic acid. The results were expressed as of GAE (gallic acid equivalents) **[30]**.

Flavonoid determination

Total flavonoid content in the plant extracts were quantified using spectrophotometric method. The sample solutions of the extracts were prepared in the concentration of 1 mg/ml. To the test solutions of various concentrations 1 ml of 2% $AlCl_3$ solution (dissolved in methanol) was added. The samples were incubated at room temperature for one hour and finally the absorbance was recorded determined at 415 nm. For each analysis samples were prepared in triplicate and the mean value of absorbance was obtained. The same methodology was followed for the standard solution of rutin and the calibration curve was construed. The flavonoid content in the extracts was expressed in terms of rutin equivalent (mg of RU/g of extract) **[31]**.

Tannin determination

The Folin-Ciocalteu protocol was followed for the determination of total tannin content. The plant extracts were prepared in the 1mg/ml concentrations. A dilution of the sample extracts were taken in test tubes followed by the addition of 7.5 ml of distilled water, 0.5 ml of Folin-Ciocalteu reagent and 1 ml of 35 % Na_2CO_3 solution. Final volume (10 ml) was made with distilled water. The reaction mixtures were vigorously shaken and incubated for 30 minutes at room temperature. The same protocol was followed for standard solutions of gallic acid. Absorbances were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of GAE /g of extract **[32]**.

RESULTS AND DISCUSSION

Qualitative phytochemical analysis

The plant kingdom harbors an enormous pool of biologically active ingredients which differ widely in terms of structure and therapeutic properties. The qualitative phytochemical screening of hydro-alcholic extracts of different parts of *D. albus* revealed the presence of most promising secondary metabolites viz., phenolics, flavonoids, tannins, terpenoids, saponins and alkaloids **(Table 2)**.

Quantitative Estimation of Phytochemicals

The results revealed that the quantity of various phytochemicals showed a significant variation ($p \leq 0.05$) among different parts of the plants. It was clearly evident that the natural products do not show uniform distribution in different parts of the plants. The detailed results are discussed as **(Table 3, Figure 1)**.

Total alkaloid content

The highest total alkaloid content was shown by leaves (189.23±0.586mg) followed by root (117.66±1.528mg), stem (58.67±0.586mg) and fruit (53.0±0.265mg). The higher alkaloid content present in leaves as compared to the other parts could be due to the fact that alkaloids have an important role in anti-herbivory phenomenon. As leaves are the first prey to animals so the highest content was also reported in leaves as a protective measure. In animals alkaloids show a variable mode of action wherein some interferes with components of the nervous system, others affect protein synthesis, membrane transport, and miscellaneous enzyme activities **[33]**.

Total saponin content

The total saponin content does not show any typical trend. It ranges from 16.30±0.173mg (fruit) to 30.50±0.400mg (leaves). The total saponin content was found to be highest in leaf extracts of *D. albus*. The main function of saponins is to provide protection against many pathogens and herbivores. The possible mechanism of action of saponins is membrane perturbation **[34]**. Some studies advocated that in plants, variations in saponin distribution, composition and amounts may influenced by the surrounding environment, growth stage and varying needs for plant protection **[35]**.

Total terpenoid content

The highest total terpenoid content was observed in the leaves and lower in the fruit extracts ranging from 9.433±0.153mg (fruit) to 57.33± 0.493mg (leaves). As per Taiz and Zeiger **[36]**, saponins occur widely in the leaves and fruits of higher plants, conifers, citrus and eucalyptus. Terpenes have a negative impact on herbivores through toxic and deterrent effects **[37, 38]**, wherein toxicity may result from several mechanisms, including inhibition of ATP formation, interference with hormone production, and binding proteins or sterols in the gut **[38]**.

Some plant families are known for their capability to synthesize and emit antimicrobial terpenes as a defensive measure against microbial challenges **[39]** and to attract specific insects for pollination **[40]**.

Total phenolic content

The investigation showed that different parts of *D. albus* possessed more total phenolic content as compared to the other phytochemicals quantified. Among all the extracts studied the highest phenolic content was observed in the leaves (227.71±0.983mg). Phenolics are one of the most ubiquitous groups of secondary metabolites found throughout the plant kingdom **[41]** and act as phytoalexins, phytoanticipins and nematicides against soil-borne pathogens and phytophagous insects **[42,43]**. These exhibit several health beneficial activities such as anti-oxidant, anti-inflammatory, anti-hepatotoxic, anti-tumoral and anti-microbial **[44]**.

Total tannin content

The total tannin content showed a huge variation among different plant parts. The highest content was observed in the leaf (57.67±1.496 mg) extract and least content in stem (9.14±1.720mg) extract of *D. albus*. Tannins are medicinally important because of their astringent properties. These also have a protective role against oxidative stress and degenerative diseases. They are also known as proanthocyanadinis possessing significant properties like antioxidant, anti-apoptosis, anti-aging, anti-carcinogenic, anti-inflammatory and anti-artherosclerosis **[45]**.

Total flavonoid content

The total flavonoid content was found to be more in the leaf extracts (98.75±0.714mg) of *D. albus* as compared to the other parts studied. Flavonoids represent the most common and widely distributed class of plant phenolics **[36]**. The interest in plants rich in flavonoids has tremendously intensified due to their potentially beneficial effects in humans viz., anti-allergic, anti-viral, anti-inflammatory, anti-platelet, anti-tumor, and anti-oxidant activities **[46]**. These are involved in a huge array of physiological processes, such as attracting pollinators and seed dispersal **[47]**, pigmentation for fruits and flowers **[48]**, interactions of plant with microbes and animals **[49]**, auxin transportation **[50]**, and protecting the plant from UV-B damage **[51]**.

Table 2: Qualitative analysis of phytochemicals in hydro-alcholic extract of different parts of *Dictamnus albus*

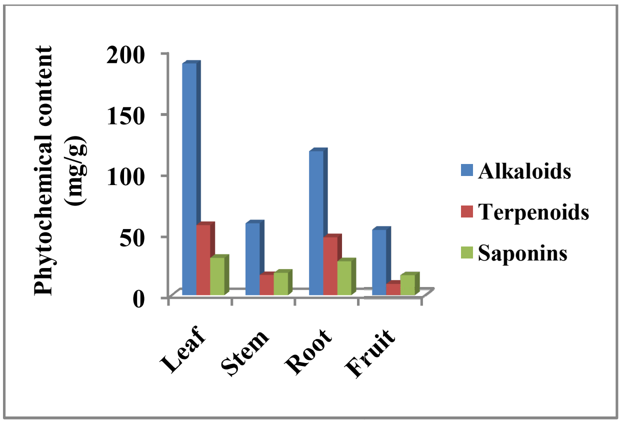
Phytochemicals	Tests	Presence/absence in different parts of the plant			
		Root	Stem	Leaves	Fruit
Carbohydrates	Molisch's	+	+	+	+
	Benedict's	+	+	+	+
Anthraquinone glycosides	Borntrager's	–	–	–	–
Cardiac glycosides	Keller killiani	-	-	-	-
	Legal's	–	–	–	–
Proteins and amino acids	Xanthoproteic acid	+	+	+	+
	Ninhydrin	+	+	+	+
Alkaloids	Dragendroff's	+	+	+	+
	Wagner's	+	+	+	+
	Hager's	+	+	+	+
Phenolics	Ferric chloride	+	+	+	+
Tannins	Ferric chloride	+	+	+	+
	Lead acetate	+	+	+	+
Flavonoids	Shinoda	+	+	+	+
	Alkaline reagent	+	+	+	+
Terpenoids	Salkowski	+	+	+	+
Saponins	Foam	+	+	+	+

+ indicates presence; - indicates absence

Table 3: Quantification of major phytochemicals in different parts of *Dictamnus albus*

Phytochemicals	Parts				**F	***P
	Leaves	Stem	Root	Fruit		
Alkaloids (mg/g)	189.23 ±0.586a*	58.67 ±0.586b	117.66 ±1.528c	53.0 ±0.265d	15671.15	.000
Saponins (mg/g)	30.50 ±0.400a	18.36 ±0.462b	27.73 ±0.208c	16.30 ±0.173d	1296.80	.000
Terpenoids (mg/g)	57.33 ±0.493a	16.46 ±0.351b	47.366 ±0.208c	9.433 ±0.153d	14263.487	.000
Phenolics (mg of *1GAE/g)	227.71 ±0.983a	188.51 ±0.722b	214.93 ±0.718c	175.15 ±1.187d	2014.220	.000
Tannins (mg of GAE/g)	57.67 ±1.496a	9.14 ±1.720b	27.94 ±0.980c	18.29 ±1.020d	738.635	.000
Flavonoids (mg of *2RU/g)	98.75 ±0.714a	54.82 ±0.271b	70.24 ±0.712c	81.30 ±0.465d	3143.251	.000

* Means labeled with the different small letters indicate that they significantly differ from each other among different parts; **F-test (ANOVA); ***Level of significance (p≤0.05); *1Gallic acid equivalent; *2Rutin equivalent



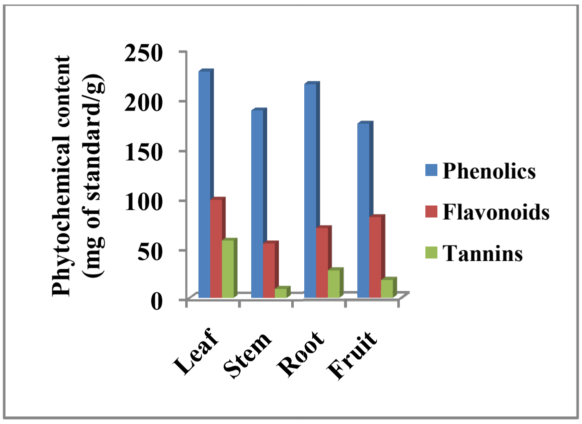


Figure 1: Phytochemical variation in different parts of *Dictamnus albus*

CONCLUSIONS

The present study for the first time provides a comparative phytochemical profile of *D. albus*. The investigation of *D. albus* clearly revealed that all the parts contain natural products but in varied quantities wherein leaves can be considered to serve as a potent source for various pharmaceutical products. Hence leaves could be used as a good source for the isolation of compounds.

ACKNOWLEDEMENT

The authors are highly thankful to the Department of Botany and Department of Pharmaceutical Sciences, University of Kashmir, for providing necessary research facilities. Dr. Gowhar A Shapoo is gratefully acknowledged for helping throughout the research.

REFERENCES

[1] Singh NP, Singh DK, Hajra PK, Sharma BD, *Flora of India*, Introductory Volume, part II. BSI Calcutta, 2000, pp. 201-217.

[2] Saha JC, Savini EC, Kasinathan S, Ecbolic properties of Indian medicinal plants, Part 1, *Indian Journal of Medical Research,* 49, 1961,130-151.

[3] Prakash AO, Biological evaluation of some medicinal plant-extracts for contraceptive efficacy. *Contraceptive delivery systems*, 5(3), 1984, 9-10.

[4] Jung BS, Shin MK, *Encyclopedia of illustrated Korean natural drugs*, Young Lim Sa, Seoul, 1990, pp 785-786.

[5] Souleles C, A new flavonoid glycoside from *Dlctamnus albus*, *Journal* of *Natural Products* 52(6), 1989, 1311-1312

[6] Baytop T, *Therapy with plants in Turkey*, Istanbul University, Istanbul, 1989, p. 418.

[7] Ivanova A, Kostova I, Mikhova B, Vitkova A, Chemical components of *Dictamnus albus* L. *Comptes Rendus de l'Academie Bulgare des Sciences*, 57(11), 2004, 45-48.

[8] Zivotic D, Zivotic D, *Medicinal plants in folk medicine* (in Serbian), Edition V, Otokar Kersovan, 1979.

[9] Sommer RG, Jillson OF, Phyto-photodermatitis (solar dermatitis from plants) Gas Plant and the Wild Parsnip, *New England Journal of Medicine*, 276(26), 1967, 1484-1486.

[10] Moller H, Phototoxicity of *Dictamnus alba*, *Contact Dermatitis*, 4(5), 1978, 264-269.

[11] Ramamurthy V, Sathiyadevi M, Preliminary phytochemical screening of methanol extract of *Indigofera trita* Linn., *Journal of Molecular Histology and Medical Physiol*ogy, **5**(2), 2017, 1-3.

[12] Vimalkumar CS, Hosagaudar VB, Suja SR, Vilash V, Krishnakumar NM, Latha PG, Comparative preliminary phytochemical analysis of ethanolic extracts of leaves of *Olea dioica* Roxb., infected with the rust fungus *Zaghouania oleae* (EJ Butler) Cummins and non-infected plants, *Journal of Pharmacognosy and phytochemistry*, 3(4), 2014, 69-72.

[13] Kokate CK, Khandelwal KR, Pawar AP, Gokhale SB, 2000, *Practical Pharmacognosy*. Nirali prakashan, Pune, pp. 45-46.

[14] Chaturvedi S, Joshi A, Dubey BK, Pharmacognostical, phytochemical and cardioprotective activity of *Tamarinds indica* Linn. Bark, *International Journal of Pharmaceutical Sciences and Research*, **2**(11), 2011, 3019-3027.

[15] Bhandary SK, Kumari SN, Bhat VS, Sharmila KP, Bekal MP, Preliminary phytochemical screening of various extracts of *Punica granatum* peel, whole fruit and seeds, *Journal of Health Sciences*, **2**(4), 2012, 35-38.

[16] Akande IS, Samuel TA, Agbazue U, Olowolagba BL, Comparative proximate analysis of ethanolic and water extracts of *Cymbopogen citratus* (lemon grass) and four tea brands, Journal of Pharmaceutical and Biomedical Sciences, 3, 2012, 29-35.

[17] Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H, Phytochemical screening and extraction: a review, *Internationale pharmaceutica sciencia* 1(1), 2011, 98-106.

[18] Sofowora A, *Medicinal Plants and Traditional medicine in Africa*, John Wiley and Sons Ltd., Chichester, England, 1982, pp. 142-145.

[19] Oguyemi AO, In: Sofowora, A (ed.), *Proceedings of a Conference on African Medicinal Plants*. Ife-Ife: Univ Ife, 1979. pp. 20-22.

[20] Martinez A, Valencia G, 2003, Manual de practicas de Farmacognosia y Fitoquimia Medellin: Universidad de Antiquia: Marcha fotiquimica, 1999, pp. 59-65.

[21] Parekh J, Chanda SV, In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plant, *Turkish Journal of Biology* 31(1): 2007, 53-58.

[22] Gul R, Jan SU, Faridullah S, Sherani S, Jahan N, Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from *Ephedra intermedia* indigenous to Balochistan, *The Scientific World Journal,* 2017, 2017, 1-7.

[23] Ugochukwu SC, Uche A, Ifeanyi, O, Preliminary phytochemical screening of different solvent extracts of stem bark and roots of *Dennetia tripetala* G. Baker, *Asian Journal of Plant Science and Research*, **3**(3), 2013, 10-13.

[24] Harborne, JB *Phytochemical methods*, Chapman and Hall, Ltd. London, 1973, pp. 49-188.

[25] Sofowora A, *Medicinal plants and traditional medicine in Africa,* Spectrum Books Ltd., Ibadan, Nigeria, 1993, pp. 289.

[26] Okwu DE, Josiah C, Evaluation of the chemical composition of two Nigerian medicinal plants, *African Journal of Biotechnology* 5(4), 2006, 357-361.

[27] Edeoga HO, Okwu DE, Mbaebie BO, Phytochemical constituents of some Nigerian medicinal plants, *African Journal of Biotechnology*, **4**(7), 2005, 685-688.

[28] Obadoni BO, Ochuko, PO, Phytochemical studies and comparative efficacy of the crude extracts of some homostatic plants in Edo and Delta States of Nigeria, *Global Journal of Pure and Applied Sciences*, **8**(2), 2001, 203-208.

[29] Abidemi OO, Phytochemicals and spectrophotometric determination of metals in various medicinal plants in Nigeria, *International Journal of Engineering Science Invention* **2**(5),2013, 51-54.

[30] Hagerman A, Harvey-Muller I, Makkar HPS, *Quantification of tannins in tree foliage-a laboratory manual*. FAO/IAEA, Vienna, 2000, pp. 4-7.

[31] Quettier-Deleu C, Gressier B, Vasseur J, Dine T, Brunet C, Luyckx MC, Cayin JC, Bailleul, F, Trotin, F, Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. *Journal of Ethnopharmacology*, 72(1), 2000, 35-42.

[32] Saeed N, Khan MR, Shabir M, Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Trolis leptophylla* L., *BMC Complementary and Alternative Medicine*, 12, 2012, 221

[33] Creelman RA, Mullet JE, Biosynthesis and action of jasmonates in plants, *Annual Review of Plant Physiology and Plant Molecular Biology*, 48(1), 1997, 355-381.

[34] Dourmashkin RR, Dougherty RM, Harris RJ, Electron microscopic observations on Rous sarcoma virus and cell membranes, *Nature,*194, 1962, 1116-1119.

[35] Moses T, Papadopoulou KK, Osbourn A, Metabolic and functional diversity of saponins, biosynthetic intermediates and semi-synthetic derivatives, *Critcal Reviews in Biochemistry and Molecular Biology*, 49(6), 2014, 439-462.

[36] Taiz L, Zeiger E, *Plant Physiology*. 3rd edition, Panima Publishing Corporation, New Delhi - Bangalore, 2003, pp. 283-306.

[37] Gershenzon J, Croteau R, Terpenoids. In: Rosenthal GA, Berenbaum M (eds.), *Herbivores, their Interactions with Secondary Plant Metabolites: Ecological and Evolutionary Processes*. Vol. I. Academic Press, New York, 1992, pp. 165-208.

[38] Langenheim JH, Higher plant terpenoids: a phytocentric overview of their ecological roles, *Journal of Chemical Ecology*, 20(6), 1994, 1223-1280.

[39] Islam AK, Ali MA, Sayeed A, Salam SM, Islam A, Rahman M, Khan GR, Khatun S, An antimircrobial terpenoid from *Caesalpinia pulcherrima* Swartz: its characterization, antimicrobial and cytotoxic activities, *Asian Journal of Plant Sci*ences, 2, 2003, 17-24.

[40] Maimone TJ, Baran PS, Modern synthetic efforts toward biologically active terpenes.

Nature Chemical Biology, 3(7), 2007, 396-407.

[41] Boudet, A, Evolution and current status of research in phenolic compounds. *Phytochemistry,* 68(22), 2007, 2722-2735.

[42] Akhtar M, Malik A, Roles of organic soil amendments and soil organisms in the biological control of plant-parasitic nematodes: a review. *Bioresource Technology,* **74**(1), 2000, 35-47.

[43] Lattanzio V, Lattanzio VMT, Cardinali A, Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects, In: Imperato F (ed.), *Phytochemistry*: *Advances in Research,* Research Signpost, India, 2006, pp. 23-67.

[44] Zengin G, Guler GO, Cakmak YS, Aktumsek A, Antioxidant capacity and fatty acid profile of *Centaurea kotschyi* (Boiss. & Heldr.) Hayek var. Persica (Boiss.) Wagenitz from Turkey, *Grasas y aceites,* 62(1), 2011, 90-95.

[45] Atanassova M, Christova-Bagdassarian V, Determination of tannins content by titrimetric method for comparison of different plant species, *Journal of the University of Chemical Technology and Metallurgy*, 44(4), 2009, 413-415.

[46] Izzi V, Masuelli L, Tresoldi I, Sacchetti P, Modesti A, Galvano F, Bei R, The effects of dietary flavonoids on the regulation of redox inflammatory networks. *Frontiers of Bioscience,* 17, 2012, 2396-2418.

[47] Lepiniec L, Debeaujon I, Routaboul JM, Baudry A, Pourcel L, Nesi N, Caboche, M, Genetics and biochemistry of seed flavonoids, *Annual Review of Plant Biology*, 57, 2006, 405-430.

[48] Winkel-Shirley B, Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology, *Plant Physiol*ogy, 126(2), 2001, 485-493.

[49] Dixon RA, Paiva NL, Stress-induced phenylpropanoid metabolism, *The Plant Cell* **7**(7), 1995, 1085-1097.

[50] Taylor LP, Grotewold E, Flavonoids as developmental regulators, *Current Opinion in*

Plant Biology, **8**(3), 2005, 317-323.

[51] Ryan KG, Swinny EE, Winefield C, Markham KR, Flavonoids and UV photoprotection in Arabidopsis mutants, *Zeitschrift fur Naturforschung C*, **5,** 2001, 745-754.