INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN CHEMISTRY AND PHARMACEUTICAL SCIENCES

(p-ISSN: 2348-5213: e-ISSN: 2348-5221)
www.ijcrcps.com

Research Article

PHYSIOCHEMICAL AND BIOCHEMICAL CHARACTERIZATION OF AN INDIGENOUS MEDICINAL PLANT (*SKIMMIA LAUREOLA)*

SAIRA ARIF[1], RAZIA NADEEM[1*] AND IRAM SHAHZADI[1]

[1]Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad, Pakistan

*Corresponding Author

Abstract

Skimmia laureola is an important medicinal plant that is mostly used for the treatment of many diseases. In the present research work, the plant samples were collected from the mountainous areas of the district Bagh of Azad Jammu and Kashmir State for physiochemical and biochemical analysis. The observed physicochemical parameters of essential oil from *S. laureola* like color, odor, percentage oil yield, refractive index, specific gravity, carbon residues, absolute viscosity, kinematic viscosity and total acid number were light to apple white, pleasant, 0.437±0.021, 1.470±0.0001, 0.637±0.001, 2.821±0.004, 107.92±0.02, 93.03±0.06 and 1.83±0.01 respectively. While among biochemical parameters, the percentage moisture, fat and protein in the leaves of *S. laureola* were found 4.83, 3.50 and 10.71 respectively. The total phenolic contents (13.69 ±0.26 mg/g) were measured through spectrophotometer. The HPLC analysis of the organic extract was also performed which showed the presence of eight antioxidant compounds (chromatotropic acid, gallic acid, caffeic acid, 4-hydroxy-3-methoxybenzoic acid, ferrulic acid, cinamic acid, m-coumeric acid and sinapic acid). Among these compounds, sinapic acid was found with the maximum amount (285.36 µg/ml) followed by chromatotropic acid (115. 64 µg/ml). The observed enzyme activities of antioxidant enzymes, ascorbate peroxidase, peroxidase, catalase, superoxide dismutase and glutathione transferase were 15.94±0.43 U/mg, 56.34±0.21 U/mg, 55.37±0.62 U/mg, 61.09±0.24 and 42.01±0.62 U/mg respectively. The enzyme activity of glutatione transferase was found maximum among activities of other assayed enzymes. On the basis of observed potential biochemical characteristics of *S. laureol*a, this medicinal plant could be a promising source of future research for the natural antioxidants and other bioactive compounds which might be used in pharamaceutical and food industries.

Keywords: *Skimmia laureola*, Physiochemical, Biochemical, Characterization, Bioactive compounds and HPLC.

1.Introduction

The medicinal herbs are the plants whose parts (leaves, seeds, stem roots etc.) extracts have been employed for the treatment of different infections. However, the contribution of plants in our life is more than the animals and the main reason is their (existence ordinary array of diverse classes of biochemical with a variety of biological activities). Plant diversity in reality is an outward manifestation of chemical diversity. Even plants have many chemicals and every one of these is able to decrease the effects of specific disease mechanism. The immense economic potential and the widespread cultural acceptability of plant based products are also the key factors renaissance in plant based medicine development (Iqbal and Rehman. 2004).

Medicinal plants are best source to obtain different drugs. About 80% of people in industrialized countries use traditional medicines, which are derived from medicinal plants (Ahmad *et al.,* 2011). In the modern antibiotic formation the plant is used as raw material and the antibiotic also depends upon the medicinal plants and from many years the antibiotic depends exclusively on leaves, flowers and bark of the plants, but in some cases the roots are also used. In some plants the carbon copies of chemicals is also identified. The world health organization also accepted the importance of the plants in medicines. According to WHO the medicinal plant is that plant in which one or more organs contain any substance which can be used for therapeutic purposes

© 2015, IJCRCPS. All Rights Reserved

or which is useful for the synthesis of drugs (Junaid *et al.,* 2006). The application of various components of several medicinal plants against many diseases has been a trend since past. There is elevating demand to find out the bioactive compounds with antioxidant and antimicrobial activities for treating the infections caused by microbes (Huynh *et al.,* 2001). Biologically active components isolated from natural sources are always considered as great scientific interest regarding research against infections (Perumal and Ignacimuthu, 2000). Medicinal plants and fruits contain significant amount of phenols that may have carcinogenic and antimutagenic activities. Phenols are found in leaves, fruits and as well as in bark of trees. Studies revealed that plants have phenols and may have antioxidant activities (Zhu *et al.,* 1997). Current progress in discovery of the drugs as well as novel chemical diversity have lead the scientists to initiate the efforts for exploring the peptides and protein which is traditional way of medicine in the subcontinent. Various extracts of the medicinal plants are being employed as a source of medicinal agents for treatment of human diseases (Yogesh ***et al.,* 2007).**

Skimmia laureola (*Rutaceae*) is an aromatic gregarious evergreen shrub distributed throughout the temperate Himalayas from Kashmir eastwards at an altitudes of 1800 to 3000 m. The leaves of *Skimmia laureola* are useful against smallpox. The species of *Skimmia* include triterpenoids which are usually lupane type (Barkatullah *et al.,* 2012). Leaves are used as coughs remedy and commercially harvested as flavoring agent in food, and in traditional healing. These are made into garlands and considered sacred culture practices. Smoke from the leaves and twig is considered demon repellent. The smoke of the dry leaves is used for nasal tract clearness. It is also used for cold, fever and headache treatment. A total of 44 species are found in association with *Skimmia laureola* in different localities. Seven species including *Adiantum venustum, Fragaria vesica, Indigofera heterantha, Isodon rugosus, Podophyllum hexandrum, Pteridium aquilinum* and *Taxus baccata* are found to be the constant species (Barkat *et al.,* 2012). Presently, we are reporting the physiochemical and biochemical characterization of the essential oil and organic extracts of *Skimmia laureola* collected from district Bagh of Azad Jammu and Kashmir state.

2.Materials and Methods

2.1 Extraction of essential oil and its Physiochemical characterization

A modified Clevenger type apparatus was used for the extraction of essential oil from the leaves of *Skimmia laureola* through hydro-steam distillation. The leaves were thoroughly washed, cut into small pieces, placed in distillation flask and subjected to hydro-steam distillation. The steam and vaporized oil were condensed into liquid by a vertical condenser and were collected in measuring tube. The essential oil was separated from water and collected in small bottles, dried with anhydrous sodium sulphate and was stored in light resistant vials at 4-6 °C.

The physiochemical characteristics (colour, odour, % yield, refractive index, specific gravity, carbon residue, absolute viscosity, kinematic viscosity and total acid number) of the essential oil extracted from leaves of *Skimmia laureola* was determined (Evans *et al.* 1997; AOAC 2000; Evans, 2002; Juliani *et al.,* 2004; Yadav *et al.* 2005; Essien *et al.,* 2008 and Bamgboye and Adejumo, 2010).

2.2 Biochemical Characterization

2.2.1 Preparation of plant extract

The pretreated and stored sample of *S. laureola* was soaked in one liter of n-hexane and methanol separately with occasional shaking for one week at room temperature (25C°±2C°) in extraction bottle. After 1 week, maximum amount of the solvent was separated from the mixture. Filtrate was filtered twice, first using the ordinary filter paper and then Watt's man No. 4 filter paper. The remaining amount of solvents was then completely evaporated by rotary evaporator at a temperature of 60 °C and a rotation of 80 rpm to obtain the essential extract *S. laureola*.

2.2.2 Determination of moisture, fat and protein

The protein content (mg/mL) was determined through Bradford method, 1976. The percentage of protein of the plant extract was determined through Kjeldahl's method (AOAC, 1990). Percentage of fat and moisture contents of ether extract was calculated as under (AOAC, 1990).

2.2.3 Determination of phenolic compounds by HPLC analysis

The phenolic compounds and antioxidants (Ascorbic acid, coumeric acid, gallic acid, tocopherol) were analyzed through High Pressure Liquid Chromatography (HPLC) technique. The mobile phase comprised of aqueous 0.05% trifluoroacetic acid (TFA) and acetonitrile, reversed phase (C-18) column and Spectral (UV/Visible) detector was used in this method with a gradient programming (Alet *et al.,* 2013).

A mixture of solvent A (H_2O: (0.1%) Triflouroacetic acid-94:6, pH 2.27) and solvent B (Acetonitrile,100 %) was used as mobile phase with different mixing ratio using gradient mode of HPLC. The composition of mobile phase was changed with respect to time during the

© 2015, IJCRCPS. All Rights Reserved

analysis and programming of gradient mode is given in the Table 2. The flow rate was set to 1 mL/min and absorbance was noted at 280nm using UV/Visible detector. The analysis of antioxidants from organic extract of *S. laureola* was performed at room temperature (25 °C) using reversed phase C-18 Octadecyl Silicate (ODS) column having 15cm length, 4.6mm diameter and particle size of 5 micrometer. The chromatograms of sample analysis were compared with available standards of antioxidants for both quantitative and qualitative analysis. Retention time and peak areas of standards were noted and calculated respectively. These calculations were employed for estimation of amounts of different antioxidants present in the organic extract of the *S. laureola.*

2.2.4 Determination of antioxidant activities

For determination of antioxidant enzymes activities, enzyme extraction was performed according to method of Hakiman and Maziah (2009).

2.2.5 Determination of Total Phenolic Contents (TPC)

The amount of total phenolic contents was determined by Folin-Ciocalteau reagent method (Wicks *et al.,* 2006).

3.Results and Discussion

3.1 Physiochemical analysis

Different physiochemical parameters of *S. laureola* were profiled during this research work. These included determination of colour, odour, % yield, refractive index, specific gravity, carbon residue, absolute viscosity, kinematic viscosity and total acid number of the oil extracted from leaves of *S. laureola*. The results of measurement of each physiochemical parameter of the essential oil of *S. laureola* are mentioned in the Table 1.

Table 1: Physiochemical parameters of essential oil from *S. laureola*

S. No.	Physiochemical Parameter	Observation/result
1	Colour	colorless to light apple white
2	Odour	Pleasant
3	Oil yield (%)	0.437±0.021
4	Rrefractive index	1.470±0.0001
5	Specific gravity	0.637±0.001
6	Carbon residue	2.821±0.004
7	Absolute viscosity	107.92±0.02
8	Kinematic viscosity	93.03±0.06
9	Total acid number (TAN)	1.83±0.01.

The color and odor of essential oil of our plant was similar to essential oils extracted from various species of *Skimmia* which have been reported by other researchers (Evans, 1997; Bamgboye and Adejumo, 2010). The essential oil yield of *S. laureola* in the present study was less than the reported yield in Nepal which ranged from 0.93 to 1.12 % (Bhattarai and Karki, 2006). The difference appeared, might be due to various ecological factors. The enhanced value of refractive index of the oil sample showed the richness of long chain fatty acids (Juliani *et al.,* 2004). The value of specific gravity of the extracted oil of *S. laureola* is in accordance with the other reported values of specific gravities of oils extracted from *Skimmia* species (Evans, 2002; Akbar *et al.*, 2009; Minzangi *et al.,* 2011). Similalry, the values of other parameters like carbon residue, absolute viscosity, kinematic viscosity and total acid number of our plant were in correlation with the values found by various researchers working on different species of medicinal plants (Bhattarai and Karki, 2006; Izabela *et al.,* 2010; Osman *et al.,* 2011; Sonata *et al.,* 2011; Mario *et al.,* 2012; Hui *et al.,* 2013; Nuno *et al.,* 2013; Dejan *et al.,* 2014).

3.2 Biochemical analysis

3.2.1 Determination of moisture, fat, protein and TPC

The parameters like moisture, fat, protein and total phenolic contents (TPC) from the leaves and organic extract, respectively of *S. laureola* were measured according to the methods described in the section of materials and methods. The result of these measurements is shown in the Fig 1 and Table 2.

© 2015, IJCRCPS. All Rights Reserved

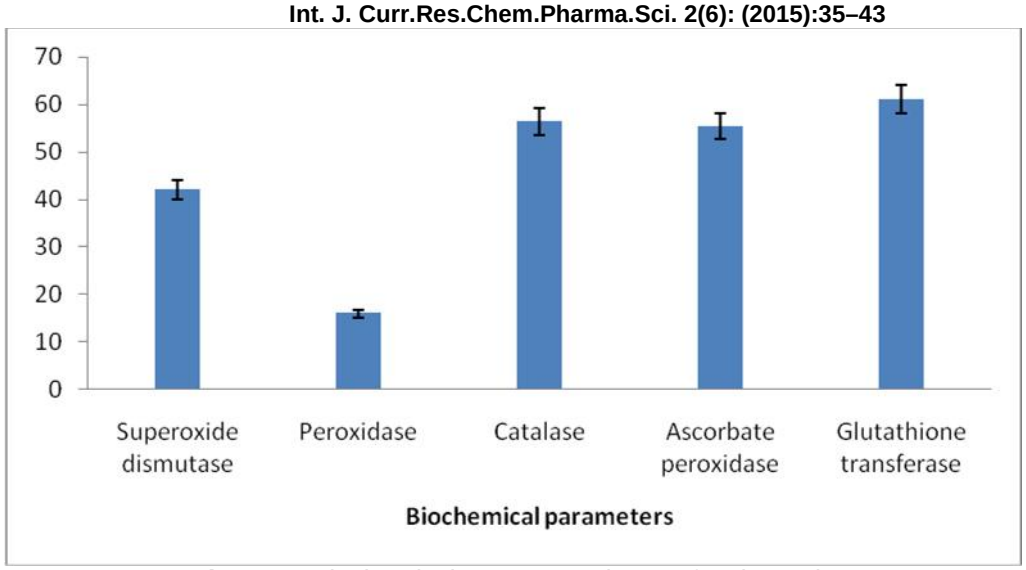

Figure 1: Biochemical parameters leave of *S. laureola*

Table 2: Biochemical parameters leave of *S. laureola*

S. No.	Parameter	Result
1	Moisture	4.83 (%)
2	Fat	3.50 (%)
3	Protein	10.71(%)
4	Total phenolic contents	13.69 ±0.26 mg/g

Our results of the above mentioned biochemical parameters are in accordance with other researchers (Cai *et al.,* 2004; Wong *et al.,* 2006; Feng *et al.,* 2010). The amount of total phenolic contents had been determined through the method of Folin Ciocalteau reagent (Wicks *et al.,* 2006). We expressed the total phenolic contents (TPC) in terms of standard equivalent (mg/g of total extracted compound). We employed Gallic acid being a reference standard, and TPC in the extract of *S. laureola* were found 13.69 ±0.26 mg/g. Phenolic compounds are actually the metabolites of the plants whose characteristics are famous due to the presence of many potential groups of phenols. Few among them are very reactive regarding neutralization of free radicals via chelating metal ions in aqueous solutions or via donation of an electron or hydrogen atom, (Petty and Scully, 2009). In addition to the fact that phenolic compounds have multiple biological characteristics like antimutagenic, antitumor and antibacterial characteristics, and those properties might have a correlation with their antioxidant activities (Shui and Leong, 2002). During our experiments, the observed strong correlations between the results of measuring antioxidant potential and the total phenolic contents exhibited that these phenolic contents had been actually contributing to the antioxidant activities of *S. laureola* and therefore could play a vital role in the beneficial effects from these plants.

3.2.2 HPLC analysis of Phenolic Compounds

Many disorders are raised due to production of free radicals. Therefore, natural antioxidants having pharmacological and food value can be discovered through evaluation of antioxidant capacities of plants. The significance of phenolic components in the medicinal plants as natural antioxidants and their employment as an alternative to the synthetic antioxidants in food additives and flavors has been well known (Branen, 1975; Paramapojn and Gritsanapan, 2009). These observations would facilitate the development of new medicines for the therapeutic applications of human being. However, only limited work has been reported against several species of *Skimmia*.

The chromatograms of sample analysis were compared with standards of antioxidants for both quantitative and qualitative analysis. Retention time and peak areas of standards were noted and calculated respectively. These calculations were employed for estimation of amounts of different antioxidants present in the organic extract of the *S. laureola* (Table 3).

© 2015, IJCRCPS. All Rights Reserved

Table 3: HPLC result of phenolic compounds in organic extract of *Skimmia laureola*

S.#	Name of phenolic compound	Amount (µg/ml)
1	Chlorogenic acid	ND
2	Chromatotropic acid	115.64
3	Gallic acid	8.966
4	Caffeic acid	16.40
5	4-Hydroxy-3-methoxybenzoic acid	196.8
6	Ferrulic acid	149.55
7	Cinammic acid	6.190
8	p-Coumeric acid	ND
9	m-Coumeric acid	16.21
10	Sinapic acid	285.36
11	Syringic acid	ND
12	Vanillic acid	ND

ND= Not detected

Our HPLC analysis showed the presence of eight phenolic compounds (Chromatotropic acid, Gallic acid, Caffeic acid, 4-Hydroxy-3-methoxybenzoic acid, Ferrulic acid, Cinamic acid, m-Coumeric acid and Sinapic acid). As mentioned in Table 5, Sinapic acid was found with the maximum amount (285.36 µg/ml) followed by Chromatotropic acid (115. 64 µg/ml) among all of the phenolic compounds. Our results are comparable with other researchers which have determined the presence of phenolic compounds from various medicinal herbs and plants through chromatographic techniques (Huafa *et al.,* 2004; Maarit and Tuulia, 2007; Nada *et al.,* 2010; Pan *et al.,* 2010; Izabela *et al.,* 2010; Osman *et al.,* 2011; Sonata *et al.,* 2011; Mario *et al.,* 2012; Pasani *et al.,* 2012; Hui *et al.,* 2013; Nuno *et al.,* 2013; Sebastian *et al.,* 2013; Dejan *et al.,* 2014). Huafu *et al.* (2004) observed the content of caffeic acid (0.4 mg/g) in the aromatic herbs which is more as compared to the amount of caffeic aicd in our plant (16.40 µg/ml). Similalry, Hui *et al.* (2013) also detected comparable amounts of chromatotropic acid, rosmarinic acid, chlorogenic acid, caffeic acid and rosmarinic acid through HPLC method. Like our method, Dejan *et al.* (2014) also employed High Pressure Liquid Chromatography (HPLC) technique using reversed phase mode and developed a method for qualitative and quantitative determination of phenolics of forty five plants (including flavonoid aglycones, benzoic acids, cinnamic acids, *C*- and *O*-glycosides, coumarins, and lignans) in plant extracts, through detection of analytes by tandem mass spectrometry. They profiled many phenolic components from organic extracts (80 % methanolic) root, leaf, stem of the *Urtica dioica* L. herbal plant and they also obtained the inflorescence data through HPLC technique.

3.2.3 Determination of Antioxidant enzymes

The results of activities of antioxidant enzymes Superoxide dismutase, Peroxidase, Catalase, Ascorbate peroxidase and Glutathione reducatse are shown in Fig 2 and Table 6.

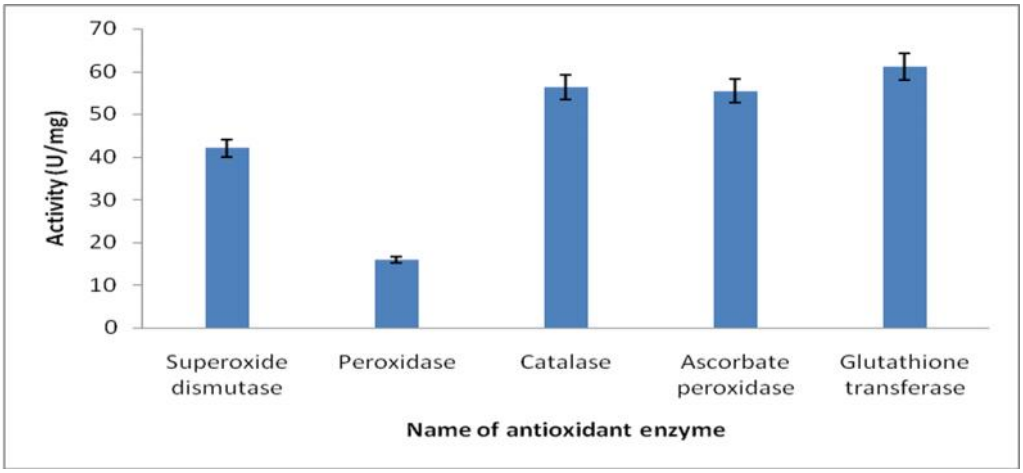

Figure 2: Activities of antioxidant enzymes from organic extract of *S. laureola*

© 2015, IJCRCPS. All Rights Reserved

Table 4: Activities of antioxidant enzymes from organic extract of *S. laureola*

S. No.	Name of antioxidant enzyme	Activity (U/mg)
1	Superoxide dismutase	42.01±0.62
2	Peroxidase	15.94±0.43
3	Catalase	56.34±0.21
4	Ascorbate peroxidase	55.37±0.62
5	Glutathione transferase	61.09±0.24

Our values of antioxidant activities of enzymes are in correlation to the various antioxidant enzymes activities observed in the extracts of other medicinal plants (Reddy *et al.,* 1995; Yamazaki *et al.,* 2003; Ali *et al.,* 2005). The reactive oxygen species (ROS) are formed in the biological systems as a result of normal metabolic activities. Adverse environmental factors including stress and drought lead towards elevated values of ROS that are detrimental to the plants. In order to avoid damage due to production of these excess ROS, plants have adopted many elaborated mechanisms for managing them at sustainable levels. Antioxidant enzymes play a significant role in lowering the levels of ROS and helping avoid oxidative stress. Catalase, superoxide dismutase, ascorbate peroxidase and glutathione reductase also have a significant role in fighting against oxidative stress. The measurement of these enzyme activities through spectrophotometric methods has been a precise way to observe and understand the significant defensive role against oxidative stress (Elavarthi and Martin, 2010). Therefore, in the present research work, we also determined the level of activities of different antioxidant enzymes in the organic extract of the *S. laureola*. Catalase has been a significant antioxidant enzyme that is capable to convert H_2O_2 to water in the peroxysomes (Fridivich, 1989; McCord and Fridovich, 1969). In this organelle, H_2O_2 is produced from photorespiration and beta oxidation of fatty acids (Morita *et al.,* 1994). Enhanced activities of catalase and peroxidase lead to reduction of levels of H_2O_2 in cell as well as these enhance the stability of membranes in addition to CO_2 fixation for the reason that many enzymes of the Calvin cycle found within chloroplasts are much sensitive to H_2O_2 and therefore, an elevated level of H_2O_2 can directly inhibit CO2 fixation (Yamazaki *et al.,* 2003).

The present results suggest that the antioxidant activity of the organic extract of *S. laureola* correlates well with its phenolic contents. In other words the maximum contents of phenolic in the extract of *S. laureola* posses most potent effect on antioxidant activity. Hydroxyl radicals are the most predominant and reactive radicals produced endogenously during aerobic metabolism among the reactive oxygen species (Waling, 1975) which could be produced during hydrogen peroxide and superoxide anion, in metal ions, such as iron or copper and lead to the ageing of human body in addition to various diseases (Siddhuraja and Becker, 2007). Therefore, for successful scavenging of such reactive oxygen species, through the scavenging mechanism, few antioxidant enzymes should cooperate with one another (Ezatollah *et al.,* 2007). Many researchers have reported antioxidant compounds, free radical scavenging properties, TPC and antioxidant properties from organic extracts of different medicinal plants (Ruckmani *et al.,* 2000; Suarez *et al.,* 2002; Ray *et al.,* 2004; Siddhuraju and Becker, 2003; Bajpai *et al.,* 2005; Yogesh *et al.,* 2007). The variation in the profile of phenolics in different medicinal plants may vary due to cultivation conditions, stress or bred lines (Bharali *et al.,* 2003).

4.Conclusion

In the light of observed physical and biochemical characteristics of *S. laureola*, this indigenous medicinal plant might be recommended being a promising source of future research work regarding isolation of various bioactive compounds which are growing and challenging need for food and pharmaceutical industries.

References

Aebi, H. 1984. Catalase in vitro. Methods Enzymol. 105: 121-126.

Ahmad, M., A. S. Shah, R. A. Khan, F. U. Khan, N. A. Khan, M. S. Shah and M. R. Khan. 2011. Antioxidant and antibacterial activity of crude methanolic extract of *Euphorbia prostrate* collected from District Bannu (Pakistan). Afr. J.Pharm. Pharmacol*.,* 5: 1175-1178.

Akbar, E., Z. Yaakob, S. K. Kamarudin, M. Ismail and J. Salimon. 2009. Charact eristic and Composition of *Jatropha Curcas* Oil Seed from Malaysia and its Potential as Biodiesel Feedstock Feedstock. Europ J Sci Res. 29(3): 396-403.

© 2015, IJCRCPS. All Rights Reserved

Alet, Venter., E. Joubert and D. Beer. 2013. Characterisation of Phenolic Compounds in South African Plum Fruits (*Prunus salicina* Lindl.) using HPLC Coupled with Diode-Array, Fluorescence, Mass Spectrometry and On-Line Antioxidant Detection Molecules. 18, 5072-5090.

Ali, M. B., E. J. Hahn and K. Y. Paek. 2005. Effects of light intensities on antioxidant enzymes and malondildehyde content during short-term acclimatization on micropropagated Phalaenopsis plantlet. Environ. Exp. Bot. 54: 109-120.

AOAC (Association of Official Analytical Chemists). 2000. Official methods of analysis. Gaithersburg, MD Washington, USA.

AOAC. 1990. Official Methods of Analysis, 15th ed. Assoc. Offic. Anal. Chem., Arlington, VA.

Bajpai, M., A. Pande, S. K. Tewari and D. Prakash. 2005. Phenolic contents and antioxidant activity of some food and medicinal plants. Int J Food Sci Nutr. Jun;5 6(4):287-91.

Bamgboye, A. I and O. I. Adejumo. 2010. Physicochemical properties of Roselle seed oil. Nutrul and Food Science. 40 (2): 186-192.

Barkat., M. Ibrar, A. Rauf and I. Rahman. 2012. Physicochemical Characterization of Essential and Fixed Oils of *Skimmia laureola* and *Zanthoxylum armatum*. Middle-East J Medicin Plant Res. 1(3): 51-58.

Barkatullah., M. Ibrar, G. Jilani and L. Badshah. 2012. Phytosocioligical and ethnobotanical attributes of *Skimmia laureola*. Int. J. Biosciences. 2 (12): 75-84.

Bharali, R., J. Tabassum and M. R. Azad . 2003. Chemomodulatory effect of Moringa oleifera, Lam, on hepatic carcinogen metabolising enzymes, antioxidant parameters and skin papillomagenesis in mice. Asian Pac J. Cancer Prev. 4(2):131-139.

Bhattarai, N and M. Karki. 2006. Community management of Medicinal plants in nepal: Practices and trends towards Sustainability. Expert workshop on assessing the Sustainable yield in medicinal and Aromatic plant collection. Available on line: http://www.floraweb.de/map-pro/le ctures/ hattarai.pdf

Bradford, M. M. (1976) Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 72: 248–254.

Branen, A. L. 1975. Toxicology and biochemistry of butylated hydroxy anisol and butylated hydroxyl toluene. J Amer Oil Chem Soc. 52: 59-63.

Cai, Y. Z., Q. Luo, M. Sun and H. Corke. 2004. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci. 74: 2157-2184.

Dejan, O., M. Franciskovic, K. Bekvala, E. Svircev, I. Bear, M. Lesjak and N. Mimica-Dukic. 2014. Quantitative determination of plant phenolics in *Urtica dioica*extracts by high-performance liquid chromatography coupled with tandem mass spectrometric detection. Food Chem. 143: 48–53.

Elavarthi, S and B. Martin. 2010. Spectrophotometric assays for antioxidant enzymes in plants. extracts. Am J Chin Med. 36 (6): 1209-1217.

Essien, E. P., J. P. Essien, B. N. Ita and G. A. Ebong. 2008. Physiochemical properties and fungitoxicity of the essential oil of citrus medica L. against ground nut storage fungi. Turk J. Bot. 32: 161-164.

Evans, C. R., N. Miller and G. Paganga. 1997. Antioxidant properties of phenolic compounds. Trends Plant Sci. 4: 152-159.

Evans, W. C., 2002. Pharmacognosy. 15 ed. English L anguage Book, Society BaillereTindall, Oxford.Univ ersity Press.

Ezatollah, E., F. Shekari, F. Shekari, M. Esfandiari. 2007. The effect of salt stress on antioxidant enzymes' activity and lipid peroxidation on the wheat seedling. Not. Bot. Hort. Agrobot. Cluj. 35 (1): 12-17.

Feng, S., G. Ren-You, Y. Zhang, Q. Xiao, L. Kuang and Li. Hua-Bin. 2010. Total Phenolic Contents and Antioxidant Capacities of Selected Chinese Medicinal Plants Int. J. Mol. Sci. 11: 2362-2372.

Fridivich, I., 1989. Superoxide dismutases: An adaptation to a paramagnetic gas. J Biol Chem. 264: 7761-7764.

Habig, W. H., M. J. Pabst and W. B. Jakoby. 1974. Glutathione S-transferases the first enzymatic step in mercapturic acid formation. J Biol Chem. 22: 7130-7139.

Hakiman, M and M. Maziah. 2009. Non enzymatic and enzymatic antioxidant activities in aqueous extract of different *Ficus deltoidea* accessions. Afr. J. Biotechnol. 3(3): 120-131.

Huafu, W., G. J. Provan, K. Helliwell. 2004. Analytical, Nutritional and Clinical Methods; Determination of rosmarinic acid and caffeic acid in aromatic herbs by HPLC. Food Chemistry. 87 (2): 307-311.

Huynh, Q.K., J.R. Borgmeyer C.E. Smith, L.D. Bell and D.M. Shah. 2001. Isolation and characterization of a 30 kDa protein with antifungal activity from leaves of engelmannia pinnatifida. J. Bio. Chem. 316 : 723-727.

Iqbal, C. M. and A. Rehman. 2004. Abstracts international symposium on "Medical plants: linkages beyond National Boundaries 7 (9): 6.*J. Agric. Food Chem.* 1999, *47,* 425-431.

Izabela, K., D. Zabaras, M. Dunstan and P. Aguas. 2010. Antioxidant capacity and phenolic compounds in commercially grown native Australian herbs and spices. Food Chem. 122 (1): 260–266.

Juliani, H. R., J. A. Zygadlo, R. Scrivanti, E. Sota and J. E. Simon. 2004. The essential oil of Anemia

© 2015, IJCRCPS. All Rights Reserved

tomentosa (Savigny) Sw. var. anthriscifolia (Schrad.) Mickel. FlavourFragr. J., 19: 541-543.

Junaid, S. A., A. O. Olabode, F. C. Onwaliri, E. J. Okworiu and S. E. Agina. 2006. The Antimicrobial properties of *Ocimum gratissium* extracts on some selected bacterial gastrointestinal isolates. Afr. J. Biotechnol*.,* 5: 2315-2321.

Maarit, K and H. Tuulia. 2009. On-line coupled dynamic sonication-assisted extraction–liquid chromatography for the determination of phenolic acids in*Lamiaceae* herbs. J Chromat A. 1216 (6, 6): 892–896.

Marino, M., C. Bersani and G. Comi. 2001. Impedance measurements to study the antimicrobial activity of essential oils from *Lamiaceae* and *Compositae*. Int. J. Food Microbiol. 67: 187-195.

McCord, J. M and I. Fridovich. 1969. Superoxide dismutase: An enzymic function for reythrocuprein (Hemocuprein). J Biol Chem. 244: 6049-6055.

Methods Mol Biol. 639: 273-281.

Minzangi, K., A. N. Kaaya, F. Kansiime, J. R. Tabuti and B. Samvura. 2011. Oil content and physicochemical characteristics of some wild oilseed plants from Kivu region Eastern Democratic Republic of Congo. African. J of Biotech. 10(2): 189-195.

Morita, S., M. Tasake, H. Fujisawa, T. Ushimaru and H. Tsuji. 1994, A cDNA clone encoding a rice catalase isozyme. Plant Physiol. 105: 1015-1016.

Nada, B., S. Djilas, M. Jadranind, V. Vajsd, J. Ivanovica, S. Petrovica and I. Zizovica. 2010. Supercritical carbon dioxide extraction of antioxidant fractions from selected Lamiaceae herbs and their antioxidant capacity. Innov Food Sci Emerg Technol. 11 (1): 98–107. Nat Prod Res. 22 (1): 37-47.

Nuno, R., K. Koci, A. V. Coelho, E. Limaa and J. Baptista. 2013. HPLC–UV–ESI-MS analysis of phenolic compounds and antioxidant properties of *Hypericum undulatum* shoot cultures and wild-growing plants. Phytochem. 86: 83–91.

Osman, S., I. Ozturk, G. Ozkan, H. Yetim, L. Ekici, M. T. Yilmaz. 2011. RP-HPLC–DAD analysis of phenolic compounds in pomace extracts from five grape cultivars: Evaluation of their antioxidant, antiradical and antifungal activities in orange and apple juices. Food Chem. 126 (4): 1749–1758.

Pan, S., J. Hong, W. U. Gang, B. Yang, Q. Min-Jian. 2010. Analysis of Flavonoids and Phenolic Acids in *Iris tectorum* by HPLC-DAD-ESI-MSn. Chinese J Natural Medic. 8 (3): 202–207.

Paramapojn, S and W. Gritsanapan. 2009. Free radical scavenging activity determination and quantitative analysis of curcuminoids in Curcuma zedoaria rhizome extract by HPLC method. Current Science. 97(7): 1069-1073.

Pasani, F., V. Verardo, M. F. Caboni, and L. F. D. Antuono. 2012. Determination of glucosinolates and phenolic compounds in rocket salad by HPLC-DAD–MS: evaluation of *Eruca sativa* Mill. and *Diplotaxis tenuifolia* L. genetic resources. Food Chem. 133: 1025-1033.

Perumal, S and S. Ignacimuthu. 2000. Antibacterial activity of some folklore medicinal plants used by tribals in Western Ghats of India. J Ethnopharm. 69 (1): 63–71.

Petty, S and C. Scully. 2007. Oral cancer knowledge and awareness: primary and secondary effects of an information leaflet. Oral Oncol. 43: 408-415.

Ray, K., R. Hazrai and D. Guha . 2003. Central inhibitory effect of Moringa oleifera root extract: possible role of neurotransmitters. Indian J Exp Biol. 41(11):1279-84.

Reddy, K. P., S. M. Subhani, P. Khan and K. B. Kumar. 1995. Effect of light and benzyl adenine on dark treated growing rice (*Oryza sativa*) leaves-changes in peroxidase activity. Plant Cell. Physiol. 26: 987-994.

Ruckmani, K., S.Kavimani, R. Anandan and B. Jaykar. 2000. Effect of Moringa oleifera Lam on paracetamol induced hepatotoxicity. Indian journal of pharmaceutical sciences 601(1):33-35.

Shui, G and L. P. Leong. 2002. Separation and determination of organic acids and phenolic compounds in fruit juices and drinks by high-performance liquid chromatography. J Chromatogr A. 977: 89-96.

Siddhuraja, P and K. Becker. 2007. The antioxidant and free radical scavenging activity of processed cowpea (Vigna unguiculata (L.) seed extracts. Food Chem. 101: 10-19.

Siddhuraja, P and K. Becker. 2007. The antioxidant and free radical scavenging activity of processed cowpea (Vigna unguiculata (L.) seed extracts. Food Chem. 101: 10-19.

Suarez, M. J. M. Entenza C. Doerries, E. Meyer, L. Bourquin , J. Sutherland, I. Marison, P. Moreillo, N. Mermod. 2002. Expression of a plant-derived peptide harboring water cleaning and antimicrobial activities Antimicrob. Agents Chemothe. 59 (5): 513 – 535.

Waling, C. 1975. Fenton's reagent revisited. Acc Chem Res. 8: 125-131.

Wicks, L. K., L. G. Wood and M. L. Garg. 2006. Methodology for the determination of biological antioxidant capacity *in vitro*: a review. J. Sci. Food Agric. 86: 2046-2056.

Wicks, L. K., L. G. Wood and M. L. Garg. 2006. Methodology for the determination of biological antioxidant capacity in vitro: a review. J. Sci. Food Agric. 86: 2046-2056.

Wong, C.C., H. B. Li, K. W. Cheng and F. A. Chen. 2006. A systematic survey of antioxidant activity of

© 2015, IJCRCPS. All Rights Reserved

30 Chinese medicinal plants using the ferric reducing antioxidant power assay. Food Chem. 97: 705-711.

Yadav, S. R., K. Murthy, D. Mishra and B. Baral. 2005. Estimation of petrol and diesel adulteration withkerosene and assessment of usefulness of selectedautomobile fuel quality test parameters. Intern. Jour Env. Sci. and Tech. 1(4): 253-255.

Yamazaki, J., A. Ohashi, Y. Hashimoto, E. Negishi, S. Kumagai, T. Kubo, T. Oikawa, E. Maruta, and Y. Kamimura. 2003. Effects of high light and low temperature during harsh winter on needle photodamage of Abies mariesii growing at the forest limit on Mt. Norikura in Central Japan. Plant Sci. 165: 257-264.

Yogesh S. Biradar, Sheetal Jagatap, K. R. Khandelwal and Smita S. Singhania. 2007. Exploring of Antimicrobial Activity of Triphala Mashi an Ayurvedic Formulation. Oxford Journals.vol:57. pp. 1741-4288.

Zhu, M., H. H. Luk, H. S. Fung and C. T. Luk. 1997. Cytoprotective effects of *Cyperus rotundus* against ethanol induced gastric ulceration in rats. Phytother Res. 11: 392-394.

© 2015, IJCRCPS. All Rights Reserved