Chemical constituents of *Calotropis procera* an indigenous medicinal shrub of Khyber Pakhtunkhwa

Shah Masaud Khan[*1], Ijaz Hussain[1], Abid Farid[4], Noshin Shafqat[7], Alia Gul[8], Sardar Ali[2], Izhar Hussain[2], Sher Aslam Khan[2], Khalid Naveed[3], Sami Ullah Khan[3], Naushad Ali[2], Muhammad Sajid[6], Waseem Ahmad[1], Shujaat Ali[1], Abbas Ali[2], Naveed ul Haq[5], Ashraf Hussain[3]

1. Department of Horticulture, The University of Haripur, Pakistan.
2. Department of Plant Breeding and Genetics, The University of Haripur, Pakistan.
3. Department of Agronomy, The University of Haripur, Pakistan.
4. Department of Entomology, The University of Haripur, Pakistan.
5. Department of Food Science and Technology, The University of Haripur, Pakistan.
6. Department of Agriculture, Hazara University Mansehra.
7. Department of Plant Breeding and Genetics, Hazara University Mansehra.
8. Department of Botany, Hazara University Mansehra.

Corresponding Author: Shah Masaud Khan, Email: Shahmasaudkhan@gmail.com

ABSTRACT

The samples (leaves) were collected from *Calotropis procera* found at their natural habitat of Khanpur Valley in the sub Himalayan Mountains of Pakistan and chemical analysis was carried out at the University of Agriculture Peshawar. Significant effects were observed at different seasons and sites upon various bio-chemical substances. *Calotropis procera* revealed higher significant values for crude proteins (14.58%) at Mang during summer, crude fibers (13.39%) at Dabola during winter, crude fats (11.98%) at Jabri during summer, NFES (50.80%) at Dam during winter, NFEE (203.1%) at Jabri during summer, potassium (500.33mg/100g) at Dam during summer, calcium (274.07mg/100g) at Dabola during winter, magnesium (291.57mg/100g) at Dabola during winter, copper (0.72mg/100g) at Jabri during summer, iron (291.6mg/100g) at Dam during summer manganese (19.27mg/100g) at Dam during summer and zinc (6.03mg/100g) at Mang during winter. It was concluded from the results of the current experiment that medicinal plants under study were rich in bio-chemical substances. *Calotropis procera* showed maximum bio-chemicals in the samples collected during last week of July.

KEY WORDS: Bio-chemical substances, Khanpur Valley, Natural habitat, Summer, winter.

INTRODUCTION

Medicinal plants are staging a comeback and herbal 'renaissance' is happening all over the world (Shah and Krishnamurthy 2013). The herbal medicines today symbolize safety in contrast to the synthetics that are considered as unsafe to human and environment. Plants are the natural resources which contribute major portion to human nourishment and medication (Halberstein, 2005). Historically, plants are harvested or collected from nature by men and women since the birth of life, for their wellbeing (Carvalho and Morales 2010). There are plant species which are used in the preparation of functional food, which serves both the purposes of nutritional as well as medicinal requirements (Chang and Wasser 2012). Such dual purpose species are of immense importance and need comprehensive investigation in the natural flora of ecologically diverse habitats of its existence.

Phyto-medicines are also being used on large scale in Western Europe. Recently the US Government has established the "Office of Alternative Medicine" at the National Institute of Health at Bethesda. Ayurveda, a system of herbal medicine in India, Sri Lanka and South-East Asia has more than 8000 traditional uses while 35,000-70,000 plant species are medicinal (Selvadurai., 2011). China has demonstrated the best use of traditional medicine in providing the health care. China has pharmacologically validated and improved many Phyto pharmaceuticals and eventually integrated them in modern medicines (Pan et al., 2013). Human body needs essential nutrients, bio-chemical compounds (proteins, fats, carbohydrate etc), major minerals (Ca, P, K, Na and Mg) and trace elements (Fe, Zn, Cu, Mn and others), which are essential for general health, growth and reproduction (Prasad and Bisht 2011). We must bear in mind that the consequences of essential trace mineral deficiency may be just as severe as those of a deficiency of a major essential mineral (Ahmed and Chaudhary 2009). Many elements are associated with one another in maintaining our normal growth and health. The medicinal plants may prove to be a useful remedy for many common and complicated ailments and part of essential nutrients required for human nourishment.

Pakistan is very rich in species diversity and its various ecological zones are unique in its ethno botanical and traditional healthcare systems (Shinwari., 2010). Khanpur valley in the sub-Himalayan mountains of Pakistan was selected study the bio-chemical attributes of *Adhatoda vasica* with the aim to achieve the following objectives. To find out the comparative suitability of seasons and sites for the best harvest of bio-chemical attributes of Adhatoda vasica of Khanpur valley. To elaborate the bio-chemical prospects of Adhatoda vasica, for possible use as source of food supplement.

MATERIAL AND METHODS

Impact of different seasons and sites on nutritional/bio-chemical Attributes of Adhatoda vasica of Khanpur valley was conducted during 2012-2013. Mature leaves of Adhatoda vasica were collected from their natural habitat at all the four sites and two seasons. Plant material were collected in summer during last week of July and in winter during last week of December.

Through quadrate transact method, three transacts (replications) were taken and in every transact, the material were collected from different available plants and fresh weights were recorded. The samples were brought to the laboratory of the Agricultural Chemistry, The University of Agriculture Peshawar. The sample was thoroughly cleaned manually and then made into powder by laboratory grinder. The samples in the grinded form were then analyzed for the following bio-chemical attributes, using standard procedures.

RESULTS AND DISCUSSIONS

Proximate Analysis

Moisture (%)

The mean data of moisture % for various seasons and sites is shown in Table-1. The results revealed that the effect of seasons on moisture % was significant while the effect of sites and their interactions was non-significant. Maximum moisture % was observed at summer (92.15%) while minimum at winter (88.87%).

Dry Matter (%)

The mean data of dry matter % for various seasons and sites is shown in Table-1. The results revealed that the effect of seasons on dry matter % was significant while the effect of sites and their interactions was non-significant. Maximum dry matter % was observed at winter (11.13%) while minimum at summer (7.85%).

Ash / Minerals (%)

The mean data of ash / minerals % for various seasons and sites is shown in Table-1. The results revealed that the effect of seasons and sites on Ash% was significant while that of their interactions on % was non-significant. Maximum ash / minerals % was observed at summer (21.25%) while minimum at winter (17.84%). Similarly maximum ash / minerals % was recorded at Jabri site (20.98%) while minimum at Dam site (18.33%).

Crude Proteins (%)

The mean data of crude proteins % for various seasons and sites is shown in Table-1, while their interaction is shown in Figure-1. The results revealed that the effect of seasons, sites and their interactions on crude proteins % was significant. Maximum crude proteins % was observed at summer (14.32%) while minimum at winter (12.47%). Similarly maximum crude proteins % was recorded at Jabri site (13.810%) while minimum at Dam site (12.72%). In case of interactions maximum crude proteins % was recorded at Mang site during summer (14.58%) followed by Jabri site during summer (14.54%) while minimum of it was observed at Dam site during winter (11.19%).

Crude Fibers (%)

The mean data of crude fibers % for various seasons and sites is shown in Table-1, while their interaction is shown in Figure-2. The results revealed that the effect of seasons and their interactions on crude fibers % was significant while that of sites was non-significant. Maximum crude fibers % was observed at winter (11.71%) while minimum at summer (9.06%). In case of interactions maximum crude fibers % was recorded at Dabola site during winter (13.39%) followed by Mang site during winter (11.64%) while minimum of it was observed at Dabola site during summer (8.64%).

Crude Fats (%)

The mean data of crude fats % for various seasons and sites is shown in Table-1, while their interaction is shown in Figure-3. The results revealed that the effect of seasons and interactions on crude fats % was significant while the effect of sites was non-significant. Maximum crude fats % was observed at summer (10.99%) while minimum at winter (9.56%). In case of interactions maximum crude fats % was recorded at Jabri site during summer (11.98%) followed by Dam site during summer (11.12%) while minimum of it was observed at Jabri site during winter (7.13%).

Essential Oils (%)

The mean data of essential oil % for various seasons and sites is shown in Table-1. The results revealed that the effect of sites on essential oil % was significant while the effect of seasons and interactions was non-significant. Maximum essential oil % was recorded at Jabri site (2.03%) while minimum at Dam site (1.36%).

Nitrogen Free Extractable Substances (NFES)

The mean data of NFES % for various seasons and sites is shown in Table-1, while their interaction is shown in Figure-4. The results revealed that the effect of seasons, sites and their interactions on NFES % was significant. Maximum NFES % was observed at winter

(48.42%) while minimum at summer (44.28%). Similarly maximum NFES % was recorded at Dam site (48.44%) while minimum at Dabola site (44.78%). In case of interactions maximum NFES % was recorded at Dam site during winter (50.80%) followed by Jabri site during winter (50.27%) while minimum of it was observed at Jabri site during summer (40.72%).

Net Free Energy Estimation (NFEE)

The mean data of NFEE for various seasons and sites is shown in Table-1, while their interaction is shown in Figure-5. The results revealed that the effect of seasons and their interactions on NFEE was significant while that of sites was non-significant. Maximum NFEE was observed at summer (197.18) while minimum at winter (181.48). In case of interactions maximum NFEE was recorded at Jabri site during summer (203.10) followed by Dam site during summer (199.86) while minimum of it was observed at Jabri site during winter (164.59).

Table-1. Effect of Different Seasons and Sites on Proximate analysis of *Calotropis procera*, indigenous to Khanpur Valley, in sub-Himalayan mountains of Pakistan.

Seasons	**Moisture%**	**Dry Matter %**	**Ash/minerals %**	**Crude Proteins%**	**Crude Fibers %**	**Fats%**	**Essential Oils%**	**NFES %**	**NFEE**
Summer	92.15a	7.85b	21.25a	14.32a	9.06b	10.99a	1.73	44.28b	197.18a

Winter	88.87b	11.13a	17.84b	12.47b	11.71a	9.56b	1.64	48.42a	181.48b
LSD at α 0.05	1.312	1.312	0.847	0.297	0.795	0.527	Ns	1.525	4.300
Sites									
Dam	92.00	8.00	18.33b	12.72b	9.75	10.75	1.36b	48.44a	192.90
Dabola	90.53	9.47	20.71a	13.66a	11.02	9.83	1.47b	44.78b	184.98
Jabri	90.78	9.22	20.98a	13.81a	10.17	9.55	2.03a	45.49b	183.85
Mang	88.72	11.27	18.37b	13.38ab	10.60	10.96	1.87a	46.69ab	195.59
LSD at α 0.05	Ns	ns	1.294	0.692	ns	ns	0.354	2.227	ns
Interactions									
Seasons*Sites	Ns	ns	Ns	*	*	*	Ns	*	*

Means followed by similar letter(s) in column do not differ significantly.

ns = Non Significant.

* = Significant at 5 % level of probability.

It is evident from the results that summer and winter seasons have significantly affected the bio-chemical attributes except essential oils. The bio-chemical compounds like moisture, crude proteins, crude fats, ashes or total minerals and NFEE were higher in summer season than winter season in all the four sites of the study area. In *Calotropis procera*, which is an evergreen species, the plant accumulated maximum bio-chemical compounds in summer to grow at higher rate and to keep nutrients for winter stresses. Summer rains and high water availability to plants facilitated the accumulation of these compounds in leaves during summer. These results were confirmed by many reports (Ara and Sarah 2017). High protein and total minerals found in summer season may be due to availability of high moisture due to heavy rainfall in the valley.

On the other hand dry matter, crude fibers and nitrogen free extracts were found significantly higher during winter than summer in all of the sites. The accumulation of these compounds may be due to low temperature and deficiency of water and stresses (Moura et al., 2010). Furthermore, plants grown in cold areas accumulate oils and sugar during early winter to cope with severe cold and provide support to plant survival and protect it from frost injury (Ruelland et al., 2009). During winter plants faced the low temperature stress as a result of which high accumulation of dry matter and nitrogen free extracts were recorded. High soil EC was also observed during winter which also caused stressed conditions increasing the accumulation of these compounds (Yadav et al., 2011). High NFES might be due to deficiency of nitrogen during winter. Low temperature also reduced absorption of N which further increased accumulation of NFES.

The sites comparison revealed that Dam site produced maximum value for NFES in the leaves of adhatoda. It was observed that the soil of dam site was having higher water holding capacity, as a result of which high moisture were recorded in leaves of Calotropis procera (Frosi et al., 2013). Dabola site gave maximum value for crude fibers and Jabri site showed maximum results for crude proteins, essential oils and ash which might be associated with environmental stresses as both the sites are at higher altitude (Al-Jabri and Baawain 2010). While Mang site produced maximum values for dry matter, crude fats and NFEE which are closely associated with the mineral composition of the soil of the site (Hussain, 2009) and NFE at KG was higher due to high fat and protein contents in plants growing at this site which contain maximum energy. Increase in crude proteins, essential oils and ash might also be related with some environmental stress or anthropogenic stresses as clear cutting and grazing (Zahran., 2010) which are common at the site.

Elementology

Sodium (Na) mg/100g

The mean data of Na for various seasons and sites is shown in Table-2. The results revealed that the effect of seasons and sites on Na was significant while the effect of their interaction was non-significant. Maximum Na was observed at summer (7.16 mg/100g) while minimum at winter (5.41 mg/100g). Similarly maximum Na was recorded at Mang site (8.09 mg/100g) while minimum at Dam site (5.12 mg/100g).

Potassium (K) mg/100g

The mean data of K for various seasons and sites is shown in Table-2, while their interaction is shown in Figure-6. The results revealed that the effect of seasons, sites and their interaction on K was significant. Maximum K was observed at summer (390.79 mg/100g) while minimum at winter (346.92 mg/100g). Similarly maximum K was recorded at Dabola site (447.50 mg/100g) while minimum at Jabri site (276.25 mg/100g). In case of interactions maximum K was observed at Dam site during summer (500.33 mg/100g) followed by Dabola site during summer (454.00 mg/100g) and then followed by Mang site during summer (439.67 mg/100g). While minimum K was observed at Jabri site during summer (169.17 mg/100g)

Calcium (Ca) mg/100g

The mean data of Ca for various seasons and sites is shown in Table-2, while their interaction is shown in Figure-7. The results revealed that the effect of seasons, sites and their interaction on Ca was significant. Maximum Ca was observed at winter (187.00 mg/100g) while minimum at summer (167.67 mg/100g). Similarly maximum Ca was recorded at Dabola site (196.13 mg/100g) while minimum at Dam site (157.38 mg/100g). In case of interactions maximum Ca was observed at Dabola site during winter (274.07 mg/100g) followed by Dam site during summer (198.90 mg/100g). While minimum Ca was observed at Dabola site during summer (118.20 mg/100g)

Phosphorus (P) mg/100g

The mean data of Phosphorus for various seasons and sites is shown in Table-2. The results revealed that the effect of seasons and sites on P was significant while that of their interactions was non-significant. Maximum P was observed at winter (99.49 mg/100g) while minimum at summer (84.75 mg/100g). Similarly maximum P was recorded at Dabola site (100.25 mg/100g) while minimum at Mang site (81.87 mg/100g).

Magnesium (Mg) mg/100g

The mean data of Mg for various seasons and sites is shown in Table-2, while their interaction is shown in Figure-8. The results revealed that the effect of seasons on Mg was non-significant while the effect of sites and of their interaction was significant. Maximum Mg was recorded at Dabola site (244.68 mg/100g) while minimum at Dam site (198.53 mg/100g). In case of interactions maximum Mg was observed at Dabola site during winter (291.57 mg/100g) followed by Mang site during winter (228.67 mg/100g). While minimum Mg was observed at Dam site during winter (193.90 mg/100g)

Copper (Cu) mg/100g

The mean data of Cu for various seasons and sites is shown in Table-2, while their interaction is shown in Figure-9. The results revealed that the effect of seasons, sites and their interaction on Cu was significant. Maximum Cu was observed at winter (0.65 mg/100g) while minimum at summer (0.59 mg/100g). Similarly maximum Cu was recorded at Jabri site (0.67 mg/100g) while minimum at Dabola and Mang sites (0.0.58 mg/100g). In case of interactions maximum Cu was observed at Jabri site during summer (0, 72 mg/100g) followed by Dam site during winter (0.68 mg/100g). While minimum Cu was observed at Mang site during summer (0, 50 mg/100g).

Iron (Fe) mg/100g

The mean data of Fe for various seasons and sites is shown in Table-2, while their interaction is shown in Figure-10. The results revealed that the effect of seasons, sites and their interaction on Cu was significant. Maximum Fe was observed at summer (252.35 mg/100g) while minimum at winter (202.54 mg/100g). Similarly maximum Fe was recorded at Dam site (243.57 mg/100g) while minimum at Mang site (207.13 mg/100g). In case of interactions maximum Fe was observed at Dam site during summer (291.60 mg/100g) followed by Jabri site during summer (249.63 mg/100g). While minimum Fe was observed at Mang site during winter (179.30 mg/100g)

Manganese (Mn) mg/100g

The mean data of Mn for various seasons and sites is shown in Table-2, while their interaction is shown in Figure-11. The results revealed that the effect of seasons and interactions on Mn was significant while the effect of sites was non-significant. Maximum Mn was observed at summer (16.38 mg/100g) while minimum at winter (12.28 mg/100g). In case of interactions maximum Mn was observed at Dam site during summer (19.27 mg/100g) followed by Dabola site during summer (18.97 mg/100g). While minimum Mn was observed at Dam site during winter (10.53 mg/100g).

Zinc (Zn) mg/100g

The mean data of Zn for various seasons and sites is shown in Table-2, while their interaction is shown in Figure-12. The results revealed that the effect of sites and interactions on Mn was significant while the effect of seasons was non-significant. Maximum Zn was recorded at Dabola site (5.55 mg/100g) while minimum at Dam site (4.27 mg/100g). In case of interactions maximum Zn was observed at Mang site during winter (6.03 mg/100g) followed by Dam site during summer (5.97 mg/100g). While minimum Zn was observed at Dam site during winter (2.57 mg/100g).

Table-2 Effect of Different Seasons and Sites on Elemental analysis of *Calotropis procera*, indigenous to Khanpur Valley, in sub-Himalayan mountains of Pakistan.

Seasons	Sodium (Na)	Potassium (K)	Calcium (Ca)	Phosphorus (P)	Magnesium(Mg)	Copper (Cu)	Iron (Fe)	Manganese(Mn)	Zinc (Zn)

Summer	7.16a	390.79a	167.67b	84.75b	215.93a	0.59b	252.35a	16.38a	5.27a
Winter	5.41b	346.92b	187.00a	99.49a	234.25a	0.65a	202.54b	12.28b	4.71a
LSD at α 0.05	0.403	18.237	12.989	4.124	ns	0.044	18.958	0.976	ns
Sites									
Dam	5.12c	432.33a	157.38c	91.50b	198.53b	0.65a	243.57a	14.90	4.27b
Dabola	5.19c	447.50a	196.13a	100.25a	244.68a	0.58b	236.52ab	15.08	5.55a
Jabri	6.75b	276.25c	186.28a	94.87ab	234.73a	0.67a	222.57bc	13.80	4.82ab
Mang	8.09a	319.33b	169.55b	81.87c	222.40ab	0.58b	207.13c	13.53	5.33a
LSD at α 0.05	0.977	32.016	10.457	7.033	30.058	0.047	19.721	ns	0.762
Interactions									
Seasons*Sites	**Ns**	*	*	**ns**	*	*	*	*	*

Means followed by similar letter(s) in column do not differ significantly.

ns = Non Significant.

* = Significant at 5 % level of probability.

The statistical analysis of the data recorded on bio-chemical attributes revealed that bio-chemical elements, Na, K, Fe and Mn were found significantly higher in summer season than in winter season while Mg and Zn concentration in the leaves of *Calotropis procera* was non-significantly higher in summer than in winter. This might be due the finding that during summer, the mineral concentration remain high in most of the species (Kaushik *et al*., 2010) because mineral absorption is associated with high rate of transpiration due to high temperature during summer (Adebooye *et al*., 2010). Another reason for higher concentration of these minerals during summer might be due to the reason that summer is more active period bio-chemically and photo synthetically (Ahmad *et al*., 2013). Similarly Ca, P and Cu were significantly higher in winter than summer.

The sites comparison revealed that Dabola site produced maximum value for K, Ca, P, Mg, Fe, Mn and Zn, while Na at Mang and Cu at Jabri was in maximum concentration in the leaves of *Calotropis procera*. It was observed that the soil of Dabola site was rich in K salts and having higher water holding capacity, which might have caused the accumulation of higher concentrations of K, Ca, P, Mg, Fe, Mn and Zn in leaves of *Calotropis procera* (Al-Yemni et al, 2011). Jabri site showed maximum results for Cu which might be associated with environmental stresses as Jabri is at higher altitude (Begum et al., 2010). While Mang site produced maximum values for Na which is closely associated with the mineral composition of the soil of the site (Gupta and Sinha, 2007). This site to site variation shows that the soil and environmental conditions at sites were different from each other which resulted in the accumulation of nutrients in the plants with different levels of concentrations and that many reports confirm the present findings (Rahim *et al*., 2011). The availability of more bio-chemical elements in the leaves of *Calotropis procera* at Dabola site might be due to stressed conditions like low temperature in winter and soil conditions (Badr et al., 2012).

CONCLUSION

Calotropis procera found greater significant values for crude proteins (14.58%) at Mang during the summer, crude fibres (13.39%) in the winter of Dabola, crude fat (11.98%) in the summer at Jabri, NFES (50.80%) in the winter at Dam, NFEE (2003.1%) in the summer at Jabri and potassium (500.33 mg/100 g) on Dam during the summer, calcium at Dabola during the winter season (274.07 mg/100 g). The results of the current investigation revealed that *Calotropis procera* was rich in bio-chemical compounds. The samples obtained last week in July exhibited maximum bio-chemicals in *Calotropis procera*.

REFERENCES

Adebooye, O. C., et al. (2010). "Inhibitory effects of sub-optimal root zone temperature on leaf bioactive components, photosystem II (PS II) and minerals uptake in Trichosanthes cucumerina L. Cucurbitaceae." Acta physiologiae plantarum **32**(1): 67-73.

Ahmad, I., et al. (2013). "Spatio-temporal variations in some medicinally important biochemical constituents of Peganum harmala (Hermal)." Pak. J. Bot **45**(S1): 601-607.

Ahmed, D. and M. A. Chaudhary (2009). "Medicinal and nutritional aspects of various trace metals determined in Ajuga bracteosa." J Appl Sci Res **5**(7): 864-869.

Al-Yemni, M. N., et al. (2011). "Bioaccumulation of nutrient and heavy metals by Calotropis procera and Citrullus colocynthis and their potential use as contamination indicators." Scientific Research and Essays **6**(4): 966-976.

Al-Jabri, M. and M. Baawain (2010). "Petrochemicals." Water Environment Research **82**(10): 1534-1552.

ARA, M. and S. Sarah (2017). "Population ecology and economic importance of Calotropis procera as an exotic medicinal plant." J. Ecol. Nat. **1**: 1.

Badr, N., et al. (2012). "Phytoremediation: An ecological solution to heavy-metal-polluted soil and evaluation of plant removal ability." World Applied Sciences Journal **16**(9): 1292-1301.

Begum, N., et al. (2010). "Evaluation of insecticidal efficacy of Calotropis procera and Annona squamosa ethanol extracts against Musca domestica." J Biofertil Biopestici **1**(1): 1-6.

Carvalho, A. M. and R. Morales (2010). "Persistence of wild food and wild medicinal plant knowledge in a northeastern region of Portugal." Ethnobotany in the New Europe: People, health and wild plant resources: 147-171.

Chang, S.-T. and S. P. Wasser (2012). "The role of culinary-medicinal mushrooms on human welfare with a pyramid model for human health." International journal of medicinal mushrooms **14**(2).

Frosi, G., et al. (2013). "Ecophysiological performance of Calotropis procera: an exotic and evergreen species in Caatinga, Brazilian semi-arid." Acta physiologiae plantarum **35**(2): 335-344.

Gupta, A. K. and S. Sinha (2007). "Phytoextraction capacity of the plants growing on tannery sludge dumping sites." Bioresource Technology **98**(9): 1788-1794.

Halberstein, R. A. (2005). "Medicinal plants: historical and cross-cultural usage patterns." Annals of epidemiology **15**(9): 686-699.

HUSSAIN, A. (2009). Study Of Seasonal Biomass Productivity And Nutritional Quality Of Major Forage Species In Subtropical Sub Humid Rangelands of District Chakwal, Arid Agriculture University Rawalpindi Pakistan.

Kaushik, R., et al. (2010). "Nutrient composition of cultivated stevia leaves and the influence of polyphenols and plant pigments on sensory and antioxidant properties of leaf extracts." Journal of Food Science and Technology **47**(1): 27-33.

Moura, J. C. M. S., et al. (2010). "Abiotic and biotic stresses and changes in the lignin content and composition in plants." Journal of integrative plant biology **52**(4): 360-376.

Pan, S.-Y., et al. (2013). "New perspectives on how to discover drugs from herbal medicines: CAM's outstanding contribution to modern therapeutics." Evidence-Based Complementary and Alternative Medicine **2013**.

Prasad, I. and G. Bisht (2011). "Evaluation of Nutritive, Antioxidant and Mineral Composition of." Research Journal of Phytochemistry **5**(1): 54-59.

Rahim, S. M. A., et al. (2011). "Effect of calcium, magnesium, sodium and potassium on farm plantations of various agroeclogical zones of Punjab, Pakistan." African Journal of Plant Science **5**(15): 890-899.

Ruelland, E., et al. (2009). "Cold signalling and cold acclimation in plants." Advances in botanical research **49**: 35-150.

Selvadurai, S. (2011). Pharmacognostical, Phytochemical and Pharmacological Studies on Whole Plant of Sida Spinosa Linn, Periyar College of Pharmaceutical Sciences for Girls, Tiruchirappalli, Tamil .

Shah, A. and R. Krishnamurthy (2013). "Swine flu and its herbal remedies." Int J Eng Sci **2**(5): 68-78.

Shinwari, Z. K. (2010). "Medicinal plants research in Pakistan." Journal of medicinal plants research **4**(3): 161-176.

Yadav, S., et al. (2011). "Causes of salinity and plant manifestations to salt stress: a review." Journal of Environmental Biology **32**(5): 667.

Zahran, M. (2010). Climate–vegetation and human welfare in the coastal deserts. Climate-Vegetation, Springer**:** 249-295.