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MADINAT AL-HIKMAH
City of Education, Science and Culture

Shaheed Hakim Mohammed Said (1920-1998), a scion of the renowned South Asian Hamdard family, decided in 1948 to make the newly created Pakistan his home. He settled down in Karachi and by untiring, single minded devotion and commitment, braving all handicaps, created Hamdard Pakistan. He developed it into the leading pharmaceutical complex of Eastern Medicine in the country, run on the latest modern lines and techniques, supplying drugs of high quality and purity, backed with free clinical consultations to help ailing humanity. Hamdard Pakistan, under his leadership, also emerged as the leading philanthropic organization, and also tried to motivate people through dialogue, conferences, and journals like the *Hamdard Medicus*. In addition, he provided help to various institutions and academic bodies.

He was restless to do more, and during one of his *Hajj* pilgrimages, he envisioned the creation of a comprehensive City of Education, Science and Culture: the Madinat al-Hikmah. Work on it was initiated and funded by the Hamdard Foundation Pakistan in 1981, in the picturesque surroundings of Bund Murad Khan, 35 kilometers away from the city centre. Today it is a beehive of activity, with children and youth engaged in academic, technical and sports activities, devoted to promoting learning and culture, and through it, help achieve moral and physical welfare, peace and progress. The Bait al-Hikmah Library, Hamdard Public School, free Hamdard Village School, Centre for Horticulture and the Hamdard University are the major institutions found here. The Hamdard University's mission provides value-based education to all students in its constituent institutions. Its prestigious institutions – some based at the main campus and some in the city and some in Islamabad - include Hamdard Institute of Management Sciences (HIMS), Hamdard College of Medicine and Dentistry (HCMD), Hamdard Al-Majeed College of Eastern Medicine (HACEM), Shifa ul-Mulk Memorial Hospital, Hamdard University Hospital – Naimat Begum Mother & Child Care Unit (part of the Hamdard University Centre of Excellence), Hamdard Institute of Education and Social Sciences (HIESS), Dr Hafiz Mohammad Ilyas Institute of Pharmacology and Herbal Sciences (HMI-IPHS), Hamdard School of Law, Faculty of Pharmacy and Faculty of Engineering Sciences and Technology (FEST). The Madinat al-Hikmah continues to develop and grow.

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The Text For Articles Should Contain: Main headings in caps and bold as:
1. INTRODUCTION, **2. MATERIALS AND METHODS** (2.1 sub-headings [in ***bold italics***] describing animals, chemicals, preparation of extract, experiments and statistical analysis), **3. RESULTS AND DISCUSSION, 4. CONCLUSION** and **ACKNOWLEDGEMENTS** (if needed) before References, **5. REFERENCES.**.
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Estimation of Antioxidant and Cell Growth Inhibition of Some Selected Medicinal Plants from Myanmar

Khin Mar Mya*, Khin Nyein Chan, Chan Myae Nyein, Mya Thida, Shun Lai Ei and Zaw Min Htet

Biotechnology Research Department, Department of Research and Innovation, Ministry of Education, Kyaukse, Mandalay Region, Myanmar.
*Email: khin821@gmail.com.

Abstract

The potential antioxidant activities of ethanolic extracts of twelve medicinal plants samples were screened by determining the total phenolic content and radicals scavenging activity using Folin-Ciocalteau method, and 1,1 Diphenyl-2-picrylhydrazyl (DPPH) assays. Among them, four medicinal plants extracts namely, *Terminalia oliveri*, *Menthalongi folia*, *Garcinia mangostana* and *Curcuma longa* demonstrated potent antioxidant activity with inhibitory concentration IC_{50} value of 2.70± 0.4 µg/ml, 19.78±0.62 µg/ml, 13.76±0.52 µg/ml and 20.29±2.31 µg/ml, respectively. The total phenolic contents and reducing power, also showed high values. Among these four extracts, antioxidant activity of *T. oliveri* was highest in all the three assays. It was also supported by investigating the correlation between DPPH scavenging activity and total phenolic contents. There is strong correlation between two methods with correlation coefficient value of 0.93. Sulphorhodamine B assay was applied in cytotoxicity testing against Rhabdomyo sarcoma cell line (RD) and the results are expressed as 50% growth inhibition (GI_{50}). The *C. longa* has potent growth inhibitory property with GI_{50} value of 7.03 µg/ml followed by *Calotropis gigantean* (9.16 µg/ml) and *Scoparia dulcis* (11.18 µg/ml). Cell growth inhibition, showed no correlation with total phenolic contents.

Thus medicinal and curing properties in these traditionally used plants are most likely to be associated with antioxidant and cell growth inhibitory properties residing in them and needs to be addressed in detail.

Keywords

Antioxidant, Phenolics, Cell Growth Inhibition, Myanmar medicinal plants.

1. INTRODUCTION

Naturally occurring plant phenolics include several groups of compounds that have been associated with health promoting properties. Phenolics may act as antioxidants, thereby reducing the risk of atherosclerosis and coronary heart disease, which may be caused by oxidation of low-density lipoproteins. They may also protect against some forms of cancer (Emmons and Peterson, 2001). Phenolic phytochemicals are known to exhibit several health beneficial activities such as antioxidant, anti-inflammatory, anti-hepatotoxic, antitumor and antimicrobial. Cancer is the 2nd leading cause of death worldwide, and is considered as one of the most

fearsome causes of morbidity and mortality. It is only preceded by cardiovascular, infectious and parasitic diseases. Although, the disease is often been regarded principally as a problem of the developed world, more than half of all cancers occur in the developing countries (Mathers *et al.*, 2001).

Phenolic compounds are ubiquitous and rich in medicinal herbs as well as dietary plants (Fruits, vegetables, spices, cereals, and beverages). Various phenolic compounds possess a diverse range of beneficial biological activities, which contribute to their potent effects inhibiting carcinogenesis. Extensive research has been conducted *in vitro* or/and *in vivo* on antioxidant and anti-cancer activities of phenolic compounds derived from medicinal herbs and dietary plants. Overwhelming clinical evidence has shown that chemoprevention by phenolic phytochemicals is an inexpensive, readily applicable, acceptable, and accessible approach to cancer control and management (Luk *et al.*, 2007). Though these are present in almost all foods of plant origin, fruits, vegetables, but beverages are the major sources of these compounds in the human diet (Hertog *et al.*, 1993).

Myanmar has abundant plant resources and Myanmar people have inherited their traditional medicine to maintain their health and treat various ailments including malaria, diarrhea and fever for over millennia (Kyaw Soe *et al.,* 2004). It is richly endowed with diverse habitat types and natural resources, culture and traditions. According to traditional beliefs in Myanmar, there are 96 diseases which afflict humans and traditional knowledge and medicine is believed to be able to cure all of these diseases by using ingredients such as fresh or dried roots, stems, leaves, buds, and flowers.

In this study the total phenolic, antioxidant, reducing power and cytotoxicity of twelve medicinal plants species collected from Mandalay region and Shan State in Myanmar were evaluated. The antioxidant activities of the ethanolic extracts were tested by three methods: The total phenolic content by Folin-Ciocalteau method; DPPH (2-2-diphenyl-1-picrylhydrazyl) radical scavenging assay and Ferric-Reducing power assay. The cytotoxicity of the extracts were detected by Sulphorhodamine B assay against human rhabdomyo sarcoma cell line.

2. MATERIALS AND METHODS

2.1. *Plant Materials*

The plant materials were collected from Kyaukse district, Mandalay region in Myanmar while *Vitis repens* and *Swertia angusti folia* were collected from southern Shan state. Botanical identifications were conducted in Pharmaceutical Research Department, Department of Research and Innovation, Yangon in Myanmar. The list of the plant samples are presented in Table 1.

2.2. *Chemicals*

Gallic acid monohydrate, 1,1-di-phenyl-2-picrylhydrazyl (DPPH), Ferric cyanide, $FeCl_3$, NaH_2PO4, Na_2HPO4, sodium bicarbonate, Folin-Ciocalteu's reagent, minimum essential eagle medium (MEM), fetal bovine serum (FBS), antibiotics-antimycotics solution, trypsin, tris base and DMSO were purchased from HiMedia Co. Ltd., India. Trichloro acetic acid, sulforhodamine B dye, trysin EDTA was obtained from Sigma-Aldrich Company. All chemicals and solvents used were of analytical grade.

2.3. *Extraction*

All plant samples were cleaned, air dried at room temperature in shade and powdered with the pestle and mortar. The weighed samples were separately extracted with ethanol (95%) using

Table1: Selected Medicinal Plant Species from Myanmar

S.No.	Local Name	Scientific Name	Family	Parts used
1.	Dabindaig-myanan	*Vitis repens*	Vitaceae	Rhizomes
2.	Ma yoe	*Calotropis gigantean*	Apocynaceae	Whole plant
3.	Than	*Terminalia oliveri*	Combretaceae	Bark
4.	Arluu	*Solanum tuberosum*	Solanaceae	Tube
5.	Dantathukha	*Scoparia dulcis*	Scrophulariaceae	Whole plant
6.	Min Gout	*Garciniam angostana*	Hypericaceae	Fruit rind
7.	Ko-yan-gyi	*Crinum asiatium*	Amaryllidaceae	Rhizomes
8.	Nanwin	*Curcuma longa*	Zingiberaceae	Rhizomes
9.	Nga-yant-pa-htu	*Clerodendrum siphonanthus*	Verbenaceae	Leaves
10.	Pyae Sone	*Premna ordorata*	Verbenaceae	Leaves
11.	Pankhar	*Swertia angustifolia*	Gentianaceae	Whole plant
12.	Pusi Nan	*Mentha longifolia*	Lamiaceae	Whole plant

percolation method for one month. After filtration the filtrates were concentrated using rotary evaporator. The concentrated plant extracts were stored at –20°C.

2.4. *Sample Preparation*

The weighed quantities of the extracts were dissolved in DMSO to acquire 40 mg/ml concentration as a stock solution and stored at –20°C for further use.

2.5. *DPPH Scavenging Assay for Antioxidant Activity*

The free radical-scavenging activities of twelve plants extracts were determined using the modified stable DPPH free radical scavenging assay in 96 micro-well plates (Shen *et al.*, 2010). Stock solutions (1 mg/ml in methanol) of the extracts were prepared. Each well was filled with extract in methanol (100 µl) to obtain the final concentrations ranging from 1.95-250 µg/ml. Ascorbic acid (AA) was used as positive control. DPPH solution (0.2 mM, 100 µl) was added to each well. Plates were kept in dark for 30 minutes followed by noting optical density (OD) of each well at 517 nm using SPECTRO star Nanomicroplate reader. Percentage inhibition was calculated using the following formula:

$$\text{Percent Inhibition} = \text{OD (Control)} - \text{OD (Sample)}/\text{OD control} \times 100$$

The values of percentage inhibition of free radical activity was plotted against concentration of crude extract and 50% inhibition (IC_{50}) was obtained graphically. The radical scavenging effect was examined and compared with ascorbic acid which was used as positive control (Tepe *et al.*, 2005).

2.6. *Total Phenolic Content*

The total phenolic content (TPC) of the crude extract was estimated by Folin-Ciocalteu method (Prior *et al.,* 2005, Ainsworth and Gillespie, 2007). Briefly, extract (100 µl) was mixed with 500 µl of Folin-Ciocalteu reagent (10%) . After 5 min, sodium carbonate (2.0 ml 20%) was added, the mixture was shaken and allowed to react for 30 min at room temperature in the dark. The absorbance was measured at 760 nm with Apel PD-303 Spectrophotometer and the concentration of the total phenolic was calculated as mg of gallic acid equivalent by using an equation obtained from gallic acid calibration curve (Ainsworth and Gillespie, 2007). All samples were tested three times.

2.7. *Reductive Potential Test*

The reductive potential of the extracts was measured using a modification of the method described earlier (Oyaizu, 1986). Various concentrations (100, 80, 60, 40, 20 µg/ml) of the plant extracts (250 µl) were mixed with phosphate buffer (250 µl) and potassium ferric cyanide ($K_3Fe(CN)_6$) (1% w/v 250 µl). This mixture was kept at 50ºC in water bath for 20 minutes. After cooling, 250 µl of trichloro acetic acid 10% (TCA) was added and centrifuged at 3000 rpm for 10 min whenever required. The upper layer of solution (600 µl) was mixed with a freshly prepared ferric chloride solution (120 µl) and the absorbance was measured at 700 nm in 96 wells microplate. Control was prepared in similar manner excluding samples. Ascorbic acid at various concentrations, was used as standard. A higher absorbance of the reaction mixture indicated a greater reductive potential power.

2.8. *Cell Culture*

In this experiment, Human Rhabdomyo sarcoma cell line (RD) was used. These cells were grown in MEM (Himedia) medium supplemented with 10% (v/v) fetal bovine serum. All cells are incubated at 37° C with CO_2 (5%), air (95%) and 100% relative humidity. The cell lines were sub-cultured by washing with phosphate buffered saline (PBS) followed by release with trypsin – EDTA solution (Himedia). And then inactivated with washing medium, MEM supplemented with FCS (1%) and antibiotics-antimycotics solution (1%). The tubes containing cell suspension were centrifuged for 10 mins at 1000 rpm and pellet was re-suspended in washing medium (1 ml). The cells are counted with trypan blue (0.4%) in haemocytometer and cell viability was determined.

2.9. *In vitro Cytotoxicity Bioassay*

Sulporhodamine B assay was used for cytotoxic activity screening (Monks *et al.*, 1991; Kasinski *et al.*, 2015). Cells having minimum viability of 80% were inoculated into 96 well micro titer plates in 100 µL at plating densities 7,500-8,000 cells/well. After cell inoculation, the plates were incubated at 37°C with, CO_2 (5%), air (95%) and 100% relative humidity for 24 h prior to addition of tested samples. Aliquots (100 µl) of different sample extract dilutions were added to the appropriate wells containing medium (100 µl), resulting in the required final extract concentrations. Each concentration was tested in triplicate. Doxorubicin was used as positive control. Following sample addition, the plates were incubated for an additional 48 h. Cells were fixed *in situ* by the gentle addition of 50 µl of cold 50% (w/v) TCA (Final concentration, 10% TCA) and incubated for 30 minutes at room temperature. The supernatant was discarded, and the plates were washed five times with tap water and air dried.

Sulforhodamine B solution (100 µl)

at 0.4 % (w/v) in acetic acid (1%) was added to each well, and plates were incubated for 30 minutes at room temperature. After staining, unbound dye was removed by washing three times with acetic acid (1%) and the plates were air dried. Bound stain was subsequently solubilized with tris base (10 mM), and the absorbance was read on a microplate reader at 545 nm. Using the seven absorbance measurements [Time zero, (Tz), control growth, (C), and test growth in the presence of extract at the five concentration levels (Ti)], the percentage growth was calculated for each extract concentrations. The graph was plotted between drug concentrations and percent cell growth to obtain GI_{50} or IC_{50}. All assays were run in triplicate and repeated at least three times (Skehan *et al.*, 1990; Monks *et al.*, 1991; Vichai and Kirtikara, 2006).

3. RESULTS AND DISCUSSION

Twelve Myanmar medicinal plant species were selected and tested for their antioxidant activities by DPPH scavenging assay, reducing power by Fe^{3+} to Fe^{2+} assay and total phenolic contents using Folin-Ciocalteu method. In this screening, four medicinal plant species demonstrated potent antioxidant activities as presented in Table 2.

In DPPH scavenging assay, the radical scavenging activity was determined by IC_{50} values. The results showed that the methanol extract of *T. oliveri* has IC_{50} values of 2.7 µg/ml followed by

Table 2: Scavenging Activity, Total Phenolic Content and Cell Growth Inhibition of Some Selected Medicinal Plants Extracts

Sample No.	Extracts	Scavenging activity (IC_{50}, µg/ml)	Cell growth inhibition (GI_{50}, µg/ml)	Total phenolic contents (mg GAE/g of extract)
1.	*Vitis repens*	99.29±6.81	15.74±1.99	38.65±3.17
2.	*Calotropis gigantean*	259.58±7.55	9.16±0.94	46.96±1.19
3.	*Terminalia oliveri*	2.70± 0.4	61.84±3.86	248.12±3.25
4.	*Solanum tuberosum*	94.94±3.43	53.23±12.29	29.45±2.42
5.	*Scoparia dulcis*	88.20±3.43	11.18±1.08	37.63±2.08
6.	*Garciniam angostana*	13.76±0.52	32.9±1.71	67.72±1.46
7.	*Crinum asiatium*	142.01±12.97	23.34±1.24	20.25±1.66
8.	*Curcuma longa*	20.29±2.31	7.67±0.62	73.05±1.71
9.	*Clerodendrum indicum*	146.69±8.76	77.92±2.14	46.96±1.19
10.	*Premna ordorata*	108.85±8.46	233.18±10.59	45.14±1.01
11.	*Swertia angusti folia*	111.69±5.99	58.24±1.86	39.54±1.94
12.	*Menthal ongifolia*	19.78±0.62	73.04±2.81	67.80±1.87

All the values are Mean±SD of n=3 experiments
Inhibitory concentration causing 50% inhibition (IC_{50})
Human Rhabdomyosarcoma cell growth inhibition by 50% (GI_{50})
Galic acid equivalence (GAE)

G. mangostana (13.76±0.52 µg/ml), *M. folia* with (19.78±0.62 µg/ml) and *C. longa* with 20.29±2.31 µg/ml. *T. oliveri* elicited better scavenging activity than that of ascorbic acid (Fig. 1). It was also confirmed with total phenolic content by Folin-Ciocalteu method. The calibration curve showed linearity for gallic acid in the range of 0-1176 mgL/L, with a correlation coefficient (R^2) of 0.9988 (Fig. 2). The results of total phenolic contents of plant extracts are presented as the gallic acid equivalence (GAE) in Fig. 3. *T. oliveri* showed high content with the value of 248.12±3.25 mg GAE/g of extract. The other three plant extracts had moderate phenolic content between 60-75 mg GAE/g of extract.

Moreover, in detecting the reductive potential of twelve medicinal plant extracts, only four plant extracts demonstrated the potential reducing activity with higher absorbance values. As increase in absorbance value reflects an increase in reducing power of extract. The results are depicted in Fig. 4. *T. oliveri* also appeared as most active with high absorbance value of 1.13 at 100 µg/ml. Its reductive potential was also better than that of ascorbic acid which has absorbance value of 0.91 at 100 µg/ml concentrations. Therefore, the extract of *T. oliveri* is rich in antioxidant compounds. *T. oliveri* (Than) and *Tectona hamiltoniana* (Dahat) are endemic plants of Myanmar and available in central dry zone of Myanmar (Lemmens *et al.,* 1995) and are mainly distributed in Than-dahat forest. The medicinal properties of these two plant species are not well-known. Recently, we reported that *T. hamiltoniana* has potent antioxidant and cytotoxic properties (Chan Myae Nyein *et al.*, 2017; Khin Mar Mya *et al.*, 2012). Many researchers have reported the medicinal properties of other three plant species except *T. oliveri.* Therefore, it is needed to explore active antioxidant compounds and other medicinal properties of *T. oliveri.*

The growth inbhibition and cytotoxicity of twelve plant extracts was also screened by SRB assay against RD cell line. The GI_{50} values are presented in Table 2 and Fig. 5. *C. longa, C. gigantean* and *S. dulcis* exhibited potent growth inhibition with GI_{50} value ranging from 7 to 12 µg/ml. The other four antioxidant plants displayed moderate activities. The antioxidant activity of *T. oliveri* in ethanolic extract has only moderate GI_{50} value of 61.84 µg/ml. Therefore it is pointed out that the antioxidant activity and cell growth inhibition of the plant species are not directly proportional. However, *G. mangostana* and *M. folia* have moderate cell growth inhibitor and antioxidant activities.

The DPPH scavenging activity was expressed as IC_{50} value. The lower the IC_{50} value of extracts the higher in DPPH scavenging activity. Therefore, the correlation of value of total phenolic content value and DPPH scavenging activity ($1/IC_{50}$ of extracts) of twelve ethanolic extracts were analyzed by Pearson's correlation coefficient (R). These were carried out using Microsoft office Excel 2013. The R value (0.926) indicate that the DPPH scavenging activity and total phenolic contents are strongly correlated (Fig. 6). Therefore, it is pointed out that the phenolic compounds residing in the extracts are mainly responsible for antioxidant activity. Moreover, the beneficial effects derived from phenolic compounds have been attributed to their antioxidant activity (Heim *et al.,* 2002). Many researchers also showed that strong correlation exists between total phenolic contents and scavenging activity (Piluzza and Bullitta, 2011; Sibi, 2015). Similarly, the lower the GI_{50} values of extracts reflects higher cell growth inbhibition of cancer cells. Likewise, the correlation between cell growth inhibition ($1/GI_{50}$) and total phenolic contents of twelve

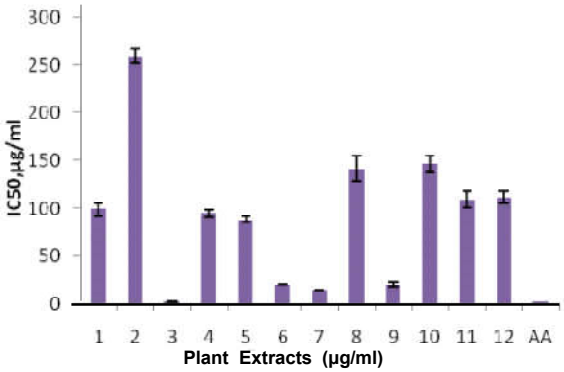

Fig. 1: DPPH Free Radical Scavenging Activity of Selected Myanmar Medicinal Plants Extracts and Ascorbic Acid

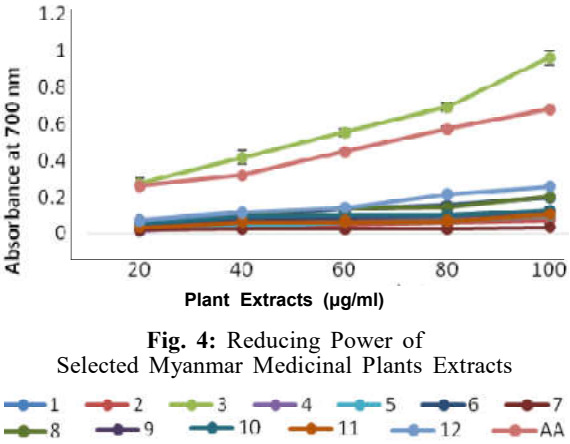

Fig. 4: Reducing Power of Selected Myanmar Medicinal Plants Extracts

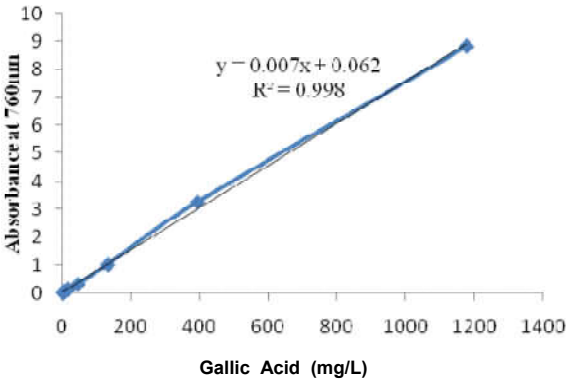

Fig. 2: Standard Calibration Curve of Gallic Acid

Concentrations of gallic acid used: 0, 1.7, 5, 15, 44, 131, 392 and 1176 mg/L

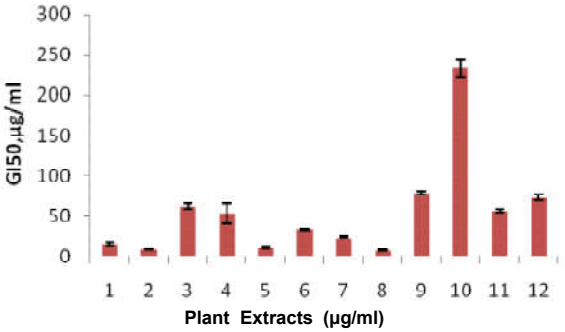

Fig. 5: Cell Growth Inhibition of Selected Myanmar Medicinal Plants Extracts

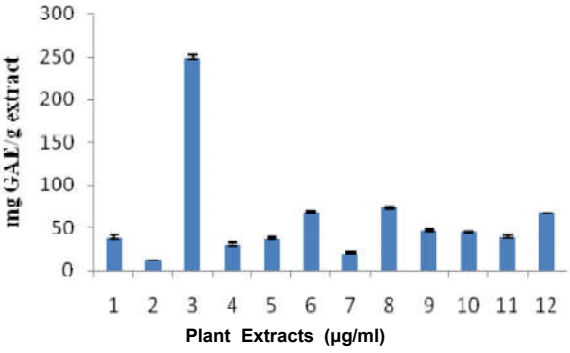

Fig. 3: Total Phenolic Contents of Selected Myanmar Medicinal Plants Extracts

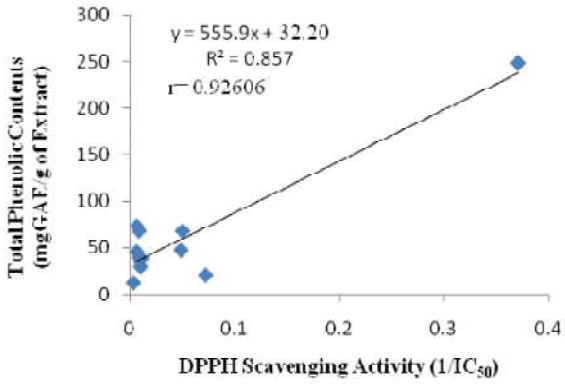

Fig. 6: Correlation Between Total Phenolic Contents and DPPH Scavenging Activity

Values are mean±SD of n=3

plants extracts were also analyzed. On the contrary there was no significant correlation (R= –0.2278) between cytotoxicity and total phenolic contents (Fig. 7). Thus phenolic compounds residing in the extracts are not cytotoxic to Rhabdomyo sarcoma cells. Other types of cytotoxic compounds may be residing in potent cytotoxic plant extracts.

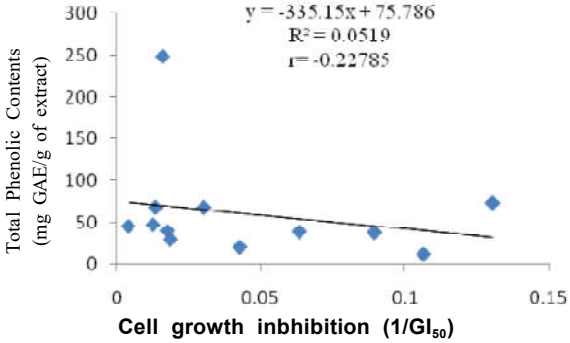

Fig.7: Correlation Between Total Phenolic Contents and cell growth inhibition

4. CONCLUSION

The antioxidant and growth inhibition cytotoxicity of twelve plant samples, two medicinal plant species, *T. oliveri* and *C. longa,* demonstrated potent antioxidant activity and cytotoxicity. In DPPH assay *T. oliveri* displayed highest DPPH scavenging activity with IC_{50} (2.7 µg/ml) comparable to ascorbic acid. It was also confirmed by Folin-Ciocalteu method and reducing power assay. Therefore, *T. oliveri* has emerged as a promising new source of antioxidant compounds. It is an endemic plant of Myanmar and no published research record was documented. It may contain many active constituents and hence appears as excellent candidate for futher exclusive research.

5. ACKNOWLEDGEMENTS

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6. REFERENCES

1. Ainsworth, E.A. and Gillespie, K.M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nat. Protoc.* **2**(4):875.
2. Chan Myae Nyein, Khin Mar Mya, Mya Thida and Khin Nyein Chan. (2017). Detection of Antioxidant and cytotoxic activites of *Tectona hamiltoniana* and *Terminalia chebula. J. Pharm. Sci. & Res*. **9**(10):1750-1754.
3. Emmons, C.L. and Peterson, D.M. (2001). Antioxidant activity and phenolic content of oat as affected by cultivar and location. *Crop. Sci*. **41**(6):1676-1681.
4. Heim, K.E., Tagliaferro, A.R. and Bobilya, D.J. (2002). Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. *J. Nutr. Biochem*. **13**(10):572-584.
5. Hertog, M.G., Feskens, E.J., Kromhout, D., Hollman, P. and Katan, M. (1993). Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *The Lancet*. **342**(8878):1007-1011.
6. Kasinski, A.L., Kelnar, K., Stahlhut, C., Orellana, E., Zhao, J., Shimer, E. *et al.* (2015). A combinatorial micro RNA therapeutics approach to suppressing non-small cell lung cancer. *Oncogene*. **34**(27):3547-3555.
7. Khin Mar Mya, Shyaula, S.L., Marasini, B.P., Dar, A. and Choudhary, M.I. (2012). Anti-cancer activity of tectona hamiltoniana-an endemic plant of Myanmar. *J. Chem. Soc. Pak*. **34**(5):1213-1217.

8. Kyaw Soe, Tin Myo Ngwe, Myo Ngwe Tin. (2004). Medicinal plants of Myanmar: Identification and uses of some 100 commonly used species, Forest Resource Environment Development & Conservation Association.
9. Lemmens, R., Soerianegara, I. and Wong, W. (1995). Plant resources of South-East Asia (PROSEA) 5 (2). Timber trees: Minor commercial timbers. *Backhuys,* Leiden, NL.
10. Luk, J.M., Wang, X., Liu, P., Wong, K.F., Chan, K.L., Tong, Y. *et al.* (2007). Traditional Chinese herbal medicines for treatment of liver fibrosis and cancer: from laboratory discovery to clinical evaluation. *Liver Int.* **27**(7):879-890.
11. Mathers, C.D., Boschi-Pinto, C., Lopez, A.D., Murray, C.J., WHO. (2001). Cancer incidence, mortality and survival by site for 14 regions of the world. WHO.
12. Monks, A., Scudiero, D., Skehan, P., Shoemaker, R., Paull, K., Vistica, D. *et al.* (2001). Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. *JNCI: J. Natl. Cancer Inst*. **83**(11):757-766.
13. Oyaizu, M. (1986). Studies on products of browning reaction. *Jpn. J. Nutr. Diet.* **44**(6):307-315.
14. Piluzza, G. and Bullitta, S. (2011). Correlations between phenolic content and antioxidant properties in twenty-four plant species of traditional ethnoveterinary use in the Mediterranean area. *Pharm. Biol*. **49**(3):240-247.
15. Prior, R.L., Wu, X. and Schaich K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem.* **53**(10): 4290-4302.
16. Shen, C.Z., Jun, H.Y., Choi, S.H., Kim, Y.M., Jung, E.J., Oh, G.S. *et al.* (2010). Evaluation of antioxidant activities and active compounds separated from water soluble extracts of Korean black pine barks. *Bull. Korean Chem. Soc.* **31**(12): 3567-3572.
17. Sibi, G. (2015). Relationship between total phenolics content and antioxidant activities of microalgae under autotrophic, heterotrophic and mixotrophic growth.
18. Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., *et al.* (1990). New colorimetric cytotoxicity assay for anticancer-drug screening. *JNCI: J. Natl. Cancer Inst*. **82**(13):1107-1112.
19. Tepe, B., Daferera, D., Sokmen, A., Sokmen, M., Polissiou, M. (2005). Antimicrobial and antioxidant activities of the essential oil and various extracts of Salvia tomentosa Miller (Lamiaceae). *Food Chem*. **90**(3):333-340.
20. Vichai, V. and Kirtikara, K. (2006). Sulforhodamine B colorimetric assay for cytotoxicity screening. *Natrprotoc*. **1**(3):1112.

Survey and Assessment of Muscoloskeletal Disorders (MSDs) Related to Computer Use in Subset of Karachi Population

Ghazala H. Rizwani[1], Salman Shah[2]*, and M. Rafay Baig[1]

[1]Hafiz Muhammad Ilyas Institute of Pharmacology & Herbal Science,
Main Campus, Hamdard University, Karachi, Pakistan.
[2]Department of Computing, Hamdard Institute of Engineering and Technology,
Faculty of Engineering Sciences and Technology, Main Campus, Hamdard University, Karachi,
Email: salman.shah@hamdard.edu

Abstract

The use of computer and computer related devices like mobile phones especially the smart-phone types, tablets, netbooks, notebooks and laptops, besides the classical desktop versions, has been extensively increased in the past two decades and their use is increasing rapidly with the advent of common day usages, at home or office. Another important phenomenon to note in this regard is the generality and oversimplification of their usage, irrespective of the gender and age groups. This upsurge and intensification in usage of such devices has radically increased the chances of becoming a victim of physical disorders, as common as hand, wrist, neck bone, shoulder and back pains, are commonly referred to as Musculoskeletal Disorders (MSDs).

The aim and objective of this research is two-folds. Firstly, to generate knowledge and realization, by highlighting some common aspects of MSDs. The awareness of the situation, cause and symptoms of these disorders will allow to take appropriate precautions and measures if fell victim of such cases in time.

Secondly, to analyze the performed survey, accomplished in a closed circle of professionals, using well known probabilistic models. The survey results are alarming to the professionals who are continuously using the computer devices, which are explicitly referred to as the Work-Related MSDs (WRMSDs) which itself has grown into a major category of disorders due to their extensiveness, severity and commonality.

Keywords

Computer usage, Musculoskeletal Disorders (MSDs), Work related MSDs, Ergonomics.

1. INTRODUCTION

Musculoskeletal disorders are nowadays very common globally. Many researchers have surveyed and notified that MSDs has become the most communal sign and reason of many types of pains, and discomforts that genuinely lead to disorders and disabilities. Well developed nations like United States of America (Cevik *et al.,* 2003) and United Kingdom (2014) have

fallen victim of MSDs. The numbers of emergency accidental cases are in millions per annum and these figures are on rise directly due to MSDs (Bussières, 2008).

Similar situation has also emerged in Pakistan. The societal practices, general behaviors, common routines, slackness in attitude, lack of interest in getting appropriate knowledge before adapting tools and technologies are the major culprits that have given space to MSDs but health care and monitoring policies from government offices have also influenced the situation negatively. The effects of MSDs can easily be nullified and avoided by improving individuals' lifestyles and enhancing their knowledge base towards their body, mind, health and wellbeing. Thereby, surely minimize the hazards of the MSDs.

1.1. *Uses in Today's Society*

Considering the lifestyle of our society and especially focusing only on the areas where mostly engaged with, four important aspects can easily be highlighted where are mostly indulged in with and where there is high utilization of tools and technologies during the work hours and hence are briefly stated below.

Education

Nowadays use of technology is quite essential for everyday academics. Many educational software are available, even books are now accessible in soft versions. Teachers are using it in order to capitalize and improve their content delivery. Similarly students use such academic versions and software to perform their educational assignments. Also numerous software help and assist both teachers and students in attaining their educational objectives and enhance their learning quite easily and effectively. Their role in swift and rapid research is also undeniable. Due to these reasons the use of computer is very common and regular in routine.

Health Care

As many persons are commonly associated with health care organizations, whether as patients or as staff working there. Advancements in tools and technology has also changed rather enhanced and upgrade this domain of work too. Especially uses of computers have introduced much efficiency and effectiveness in medical record keeping as well as patients' management. Their use has also proved to be helpful and assistive in monitoring critical situations and observing vital signs. Another important feature is the use of implants and various sorts of operative gadgets that has deepened its roots with the passage of time.

Travel

Traveling is also one important and common feature that we are coming across on routine basis. Mostly everyone in our society is traveling daily to and from their work places, may it be on foot or using some vehicle. Use of computers is also getting more and more visible and fitting in this aspect. May it be a case of finding a place, of getting help in getting directions, locate a commuter for your destination or even track your route or in fact your stolen car, we are involving computers and associated specialized devices more often and not just in assistance in case of emergency situation or dispatching roadside notions as they were used to be. Their use in case of localized mobility, and not just long distance travelling, has drastically been increased since past couple of decades than ever before, and very commonly by very common man.

Government

Many of the official government jobs are every now and then getting more and more

computer oriented and their accessibility is not only with the staff but is extended to the public too; many official services are now getting online and computers are being used at offices by the employees or from home by the citizens. Common services like tax filing, license application and tracking, security agencies and services are also generally using computers for information processing and tasks performing on regular basis.

The above discussion is a simple elaboration showing the extent of our usage of computers and an expression highlighting that this usage is getting more and more with the passage of time.

1.2. *Musculoskeletal System*

The Musculoskeletal System includes human body Skeletal system, joints, muscles, ligaments and tendons. Bones and bursae are also considered to be part of this system. This system is quite important for maintaining body movement and support, allowing it to be able to move or pertain it to be stationary at different positions. Not only the mobility aspect but it is crucial in terms of maintaining and managing the mineral storage and reserves for calcium and phosphorus.

1.3. *Musculoskeletal Disorders (MSDs)*

The musculoskeletal system includes human body skeletal system or mechanics which protects body, joints, brain, spinal cord, ribcage and organs (heart, lungs, digestive and reproductive organs along with muscles, ligaments, tendons, bones and bursae are also considered to be part of this system). Besides protection it also offers haemopoiesis, blood cell and platelets formation in bone marrow. This system is quite important for maintaining body movement and support.

Certain illnesses and infections are known for creating negative impact on the musculoskeletal system such as tuberculosis, poliomyelitis, diabetes mellitus, etc. Certain types of trauma and accidents may also lead to injuries to any of the component of the musculoskeletal system. Such effects and impacts are also generally referred to as the Musculoskeletal Disorders (MSDs). MSDs are hard to be detected and treatment in earlier stages and most of the times they are even ignored or not attempted. Most effective diagnostic criterion is the clinical examination itself that promptly and properly identifies the preciseness and severity of the MSDs. The common signs and symptoms that should alarm oneself for MSDs are inflammation, discomforting pain, numbness, noises, weakness and stiffness in joints and lack of mobility. These signs, if remain unnoticed, may lead to serious injuries of muscles, joints, tendons and even of nerves; and thereby, may lead to Cumulative Trauma Disorders (CTDs) which is comparatively more painful.

1.4. *Work Related Musculoskeletal Disorders (WRMSD)*

MSDs are also very common in today's workplaces, may that be agricultural or industrial, outdoors on site or indoors in offices due to overload, overstretching, overuse or overexertion to the Musculoskeletal System. The factors may also be the repetitiveness of work, the posture of body while at work, temperature, pressure and vibration dynamics and the contact stress that is localized to the work space.

The tasks that cause MSD can be performed anywhere including the home or office. However, the physical demands of the office are usually greater than those at home. In fact, most MSD is caused by office-related tasks. Since such disorders are usually caused

by occupational tasks, or work-related MSD or simple WRMSD.

This is not just one medical condition or disorder rather than it is a name for a group of conditions. The severity of these conditions can vary from mild/sporadic to severe/chronic/ debilitating. The most common one is Carpal Tunnel Syndrome (CTS) that is related to Nerve entrapment. Other WRMSD are:

De Quervains' tenosynovitis, Epicondylitis; Synovitis, Muscle strains, Ganglion cyst, Raynaud's phenomenon, Sciatica, Tendonitis, Shoulder impingement, Tennis Elbow, Trigger finger, Trigger points, Lower back pain, Pronator syndrome and Cervical Myofascial pain syndrome (MPS). Most of which are elaborated in Table 1.

The average reader and computer programmer does not need to know precisely what these medical conditions are. But rather needs to know how they are caused? What the symptoms are? And how to prevent or cure such afflictions?

1.5. *Stealth Symptoms of MSDs*

Since the MSDs are within the biological system of musculoskeletal system, so they are very dynamic, slow and silent. Unfortunately so, the body does not alarm its situation and most likely try to respond in adapting to the stressed situation and tolerating the impact. Therefore, the alarming situation should be considered while observing through the neighboring parts of the effected body part.

For instance, in Carpal Tunnel Syndrome (CPT), that effects on hands and produces sore and tingly influence, like they have fallen asleep.

Although here the main cause is not the hand itself but a nerve located at the wrists, but the wrists feel no pain or show no stress and may only mild sore may be felt.

Table 1: Common Symptoms that Lead to Musculoskeletal Disorders (MSDs)

Symptoms / MSDs	Pain	Numbness	Burning	Tingling	Weakness	Stiffness	Swelling	Cramping	Color loss	Reduce grip/ Motion
CTS	✓	✓		✓			✓		✓	✓
CbTS	✓	✓		✓						
TOS		✓			✓					
RS	✓	✓		✓			✓		✓	✓
RCS	✓					✓		✓		✓
DQT	✓					✓	✓		✓	✓
Tendinitis	✓					✓		✓	✓	✓
SNI			✓		✓	✓	✓			
Herniated discs	✓	✓			✓	✓		✓		

Carpal Tunnel Syndrome (CTS), Cubital Tunnel Syndrome (CbTS), Thoracic Outlet Syndrome (TOS), Raynaud's Syndrome (RS), Rotator Cuff Syndrome (RCS), De Quervain's Tenosynovitis (DQT), Sciatic Nerve Impingement (SNI)

Same is the case with 'Muscle knots' or 'trigger points' where the pain or symptom is usually somewhere else, but the trigger points are not there.

Warning Signs

The following are the warning signs that signal an MSD may be present in a person and should not be taken lightly, also highlighted and suffice in Table 1 above:

Pain and Discomfort; Numbness; Tingling or stinging; Burning sensation; Swelling and Stiffness; Cramping or Hampering; Reduced grip in hand or strength in a muscle and Reduced motion range or loss of flexibility. These often produces a subtle change in color at the affected area, like blanching or going pale. Sometimes it also changes tightness of muscle at that region.

Ergo Stressors

Repetition and stress are generally the main features involved in any sort of work. Whatever kind of work we do there are always chances present of repetitions, and overdoing any activity, and thus producing stress on our body and hence sometimes called as stressors.

Yet, not all kinds of stressors cause MSD, and only those that involve longer involvement or is largely repetitive are main sources of MSDs. These stressors are called ergonomic stressors or, for short, ergo stressors. Some examples of such ergo stressors are:

a. Repeated or sustained activity like typing on a keyboard or using mouse.
b. Undue Force applied in a grasp, pinch, twist, push, etc.
c. Low temperatures.
d. Continuous Contact stress like resting your hands on the edge of the desk, or armrests in awkward position
e. Damaging Postures. There are 3 kinds of postures that classify as damaging; one is 'Awkward Postures' that is outside of neutral positions, for example tilting your head back to see through bifocals. Second is 'Extreme Posture' that is joint positions close to the ends of the range of motion and the third is 'Static Posture' like sitting for a longer time.

Carpal Tunnel Syndrome

If you feel pain in your fingers, usually the first three, and your thumb or numbness or tingling effect in the surrounding areas, be attentive for CTS.

CTS is the effect of the median nerve, that feeds the first three fingers and the thumb can become impaired from pressure, caused by irritation and swelling of the flexor tendons in the carpal tunnel in the wrist. Fig. 1 (a) exhibits this CTS effect.

Cubital Tunnel Syndrome

It is resulted by the compression on the ulnar nerve when the elbows are exposed to hard surfaces such as unpadded tables or armrests, and thus compromises pressure near the elbows and tendons of the forearm flexors and muscles as shown in the Fig. 1 (b).

It produces pain in the ring and little fingers or numbness or tingling effect in the surrounding areas.

Thoracic Outlet Syndrome

If you start feeling your arms "falling asleep", then be observant, you may be falling victim of TO Syndrome. It happens so while reaching above shoulder or by carrying heavy objects or having incorrect, improper postures involving a forward head tilt, therein and hence resulting in firmness of the blood vessels running in between the neck and shoulder which may be seen in Fig.1 (c) too. Sometimes you may

also notice a weakened pulse or numbness in the fingers.

The brachial plexus, a Neurovascular bundle, which goes from the collar bone to the top rib, can become impaired and causes these two bones to be positioned close together.

Raynaud's Syndrome

It is also known as Vibration White Finger. The Blood vessels of the hand are damaged or narrowed from repeated exposure to vibration for longer period of time resulting in blanching or whitening of one fingertip.

Excessive use of vibration producing tools, such as hair clippers and jack hammers may cause RS. Pain, numbness and tingling in the fingers during vibration exposure may also be felt and may increase with time. Fig. 1 (d) exhibits this effect.

Rotator Cuff Syndrome

The disorder the produces pain, swelling and stiffness due to the tendons one of the four rotator cuff muscles, namely Subscapularis, Supraspinatus, Infraspinatus, and Teres minor. Supraspinatus acts throughout the range of abduction of the shoulder. Pain on arm movements outwards against resistance that may worsen at night and may also produce stiffness in the shoulder joint. The Fig. 1 (e) shows both the front and the back views of the muscles of the rotator cuff.

De Quervain's Tenosynovitis

De Quervain's Disease is an inflammation of the tendon sheath of the thumb due to forceful repetitive or sustained thumb abduction with ulnar deviation, shown in Fig. 1 (f).

Signs of pain, irritation, and swelling, loss of motion and loss of strength become obvious due to constant rubbing of the synovial sheath that covers the tendon against the tendon.

Tendinitis

Tendinitis is a common CTD due to the inflammation and irritation of the tendon of Shoulder, Elbow, Wrist, Achilles, and knee bones and muscles. Terms like "Tennis elbow" and "Golfer's elbow" are also related to Tendinitis. These different variations are exhibit in Fig. 1 (g). Point tenderness and swelling around these areas are general signs to be cautious for.

Ganglion Cyst

It is a mass or bump anywhere in the hand under the skin but mostly on the back of wrist, quite visible in Fig. 1 (h). Though it is harmless but may produce tingling sensation, muscle weakness, pain and difficulty in joint movement caused by gathering of thick fluid surrounding the tendon sheath.

Sciatic Nerve Impingement

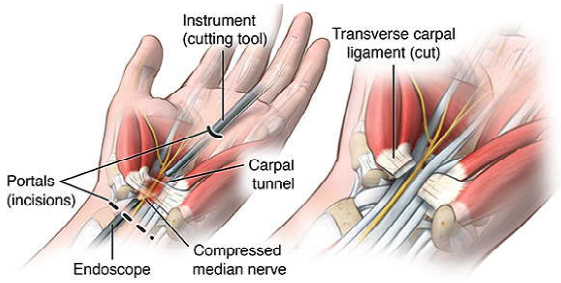
It is also called Sciatica in general. It is common in people who remain seated for prolonged period of time. The sciatic nerve which is the longest nerve in the body runs from the lower back, down the back of leg and into the feet as shown in the Fig. 1 (i).

Any sort of muscular swelling in the buttocks can cause pressure on the sciatic nerve. Consequently pain radiates from the buttock down the leg and can reach as far as the feet and toes.

Herniated Discs

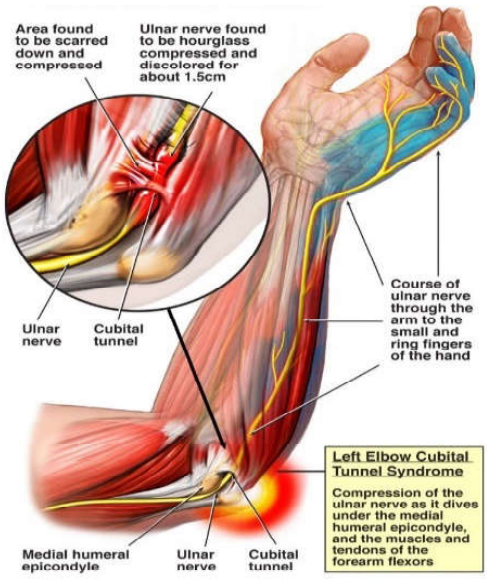
It is one of the most common and misunderstood back problem and can be very painful and damaging.

It causes pressure on the nerve roots leading from the spine leading to back injury and aging due to the pinched spinal nerve obtrudes by the inner portion of the herniated disc, exhibited in Fig. 1 (j).



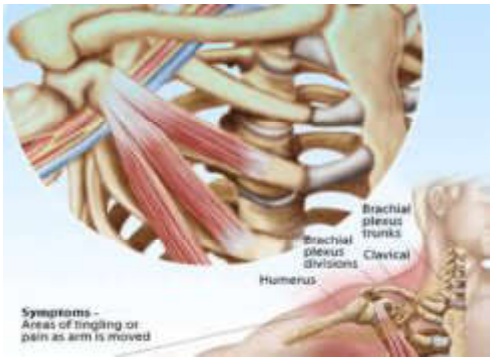
(a)

(Courtesy: https://www.hopkinsmedicine.org)



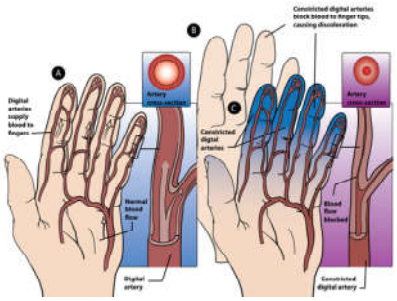
(b)

(Courtesy: https:/ orthoinfo.aaos.org)



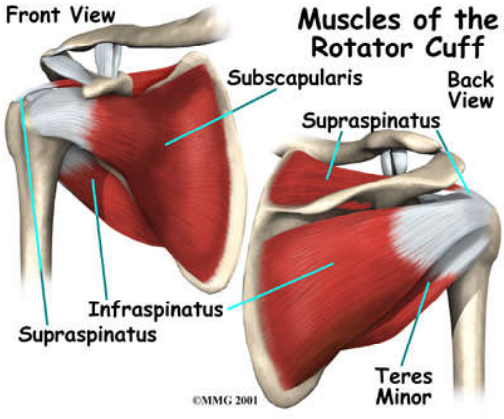
(c)

(Courtesy:https://www.mayoclinic.org)



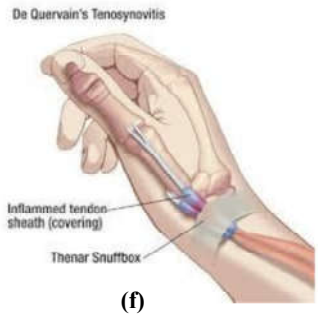
(d)

(Courtesy: https://www.orthobullets.com)



(e)

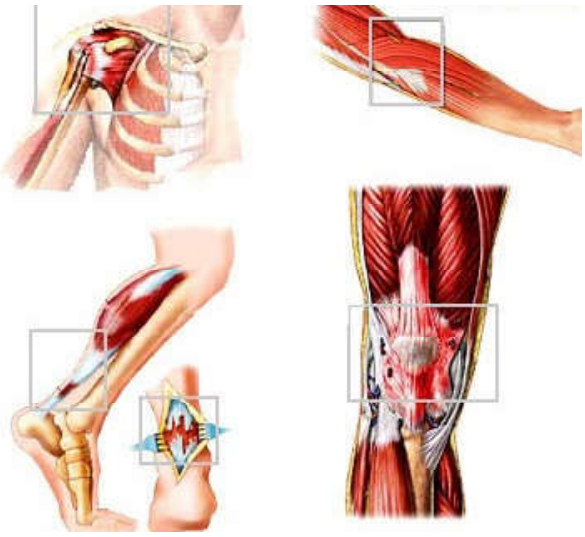
(Courtesy: https://www.eorthopod.com)



(f)

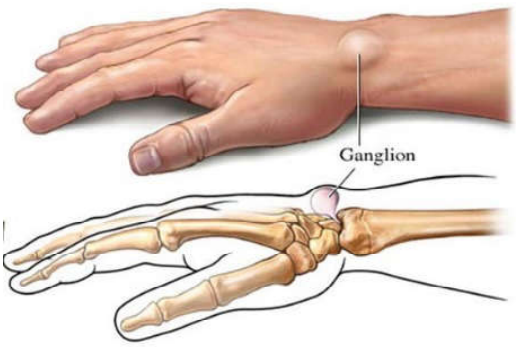
(Courtesy: https://www.orthobullets.com)

Fig. 1 (a) Carpal Tunnel Syndrome; (b) Cubital Tunnel Syndrome; (c) Thoracic Outlet Syndrome; (d) Renaud's Syndrome; Pain on the base of thumb; (e) Rotator Cuff Syndrome; (f) Pain on the base of thumb



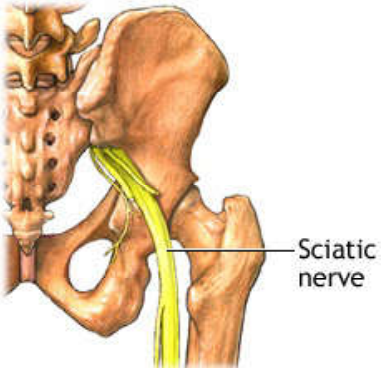
(g)

(Courtesy: https://www.webmd.com)



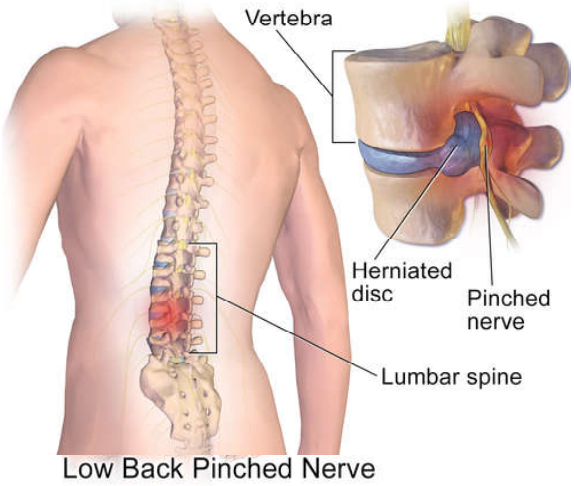
(h)

(Courtesy: https://www.webmd.com)



(i)

(Courtesy: https://www.spine-health.com)



(j)

(Courtesy: https://www.spine-health.com)

Fig. 1 (g) Variations of Tendinitis; Ganglion Cyst; (i) Sciatica; (j) Herniated disc, tensenerve

1.6. *Preventions*

The best way to avoid MSDs, including mouse elbow, is to make sure to take all the steps that can be thought of to eliminate ergo stressors from workstation.

1. **Have workstation set up so that it is workable in neutral positions.** A fairly comprehensive explanation of how to set up workstation that can be found on the web.
2. **Have workstation setup checked by an in-house ergonomist**, after step 1, have an ergonomist come in and check the workstation adjustments.
3. **Get up, stretch, and walk around**

periodically at least once an hour. This is good for vision, cardiovascular health, etc. This is probably the best thing for the protection of the bodies after insuring that the workstation is set up properly.

4. **Scatter non-keyboarding tasks with keyboard.** This provides the body time to repair itself and limits the intensity of the keyboarding.
5. **Do upper-body strength training** two or three times a week, to help the body be more resilient to ergo stressors.
6. **Move the mouse to the left-side of the keyboard,** even if the person is right-handed. It can take as little as a day to become accustomed to the new mouse location. This location has three advantages. First is the right-handed, movement some of the work from the dominate hand (the hardest working hand) to the secondary hand, thus having stress more evenly distributed across both hands. Second, it aligns the center of the alphabetic keyboard with the center of the monitor where it should be. Third putting the mouse close to the body reducing the ergo stress.
7. **Learn how to use the keyboard instead of the mouse** part of the time. There are ways of navigating the desktop from the keyboard rather than using the mouse ("Shortcut Keys"). If you have a 104-key Windows keyboard (the current de facto standard), one can use the WINDOW key for a variety of functions ("Windows Shortcut Keys"). Another alternative is the Context Menu Key. It simulates the "right" mouse click. Then it can use the ALT key (Also in "Shortcut Keys") For example, it can close a Microsoft window with ALT-F4 rather than pointing to and clicking the "X".
8. **Consider obtaining a mouse alternative.** Some alternatives are trackballs, joysticks, touch screens, head pointers, touch pads (cats), a whale (or whale mouse)
9. **Consider purchasing single-prescription computer glasses** (glasses designed specifically for working at a computer). There are many types of glasses available.
10. **Blood flowing** or circulation must be active in condition for proper functioning of body by:

a. Avoiding tight/restrictive clothing,
b. Rotating ankles and contract leg muscles, while sitting,
c. Limiting caffeine drinks,
d. Drinking plenty of water,
e. Avoiding sitting cross-legged,
f. Asking doctor about taking low-strength aspirin, if there is a higher risk of blood clots (for example, some kind of coronary artery disease).

1.7. *Reactive Steps and Treatment – How To Cure MSDs*

If the symptoms of MSD appeared, first inform to the employer then medical advice is very important in this regard. Also taking rest for some time is always helpful. According to the Occupational Safety Health Act (OSHA) a person must maintain record of the incidences of CTD or MSD. Since the 1970 passage of the Occupational Safety Health Act (OSHA) employers are required to maintain a report of all cumulative trauma disorders (CTD) or MSD. Employers are supposed to monitor the incidence of these disorders and intervene when new cases or high-incidence jobs are identified.

The doctor can help to identify the problem and may suggest overcoming such problems

through specific stretches or exercises. The doctor may also prescribe medicine to reduce inflammation and pain intensity. To cure a specific MSD, it is probably best to go way beyond what the doctor orders and use the shotgun approach of doing everything reasonable and imaginable to help. In this regard:

a. Doctor's instructions must be followed
b. Every attempt should be made in this respect as mentioned previously
c. Keep balance in movement and rest
d. Alter routine immediately at home and office to avoid the stressors causing the MSD. For example, if there is mouse elbow, then twisting is bad, avoiding opening bottles and jars without some sort of tool.
e. Do research on specific MSD to know what causes the problem and what can be helpful in curing it.
f. Look into Trigger Point Therapy
g. Consider Massage
h. Stretch

2. MATERIALS AND METHODS

A survey form was developed specifically for this purpose and a simple questionnaire approach was followed as a method for supporting the fact and to verify the objectives.

2.1. *Concept*

Different professionals from various local domains of expertise where use of computer was predominant were selected and those who were using computers only or for most of their job responsibilities were distributed the especially developed questionnaire.

2.2. *Questionnaire*

Professionals answered some series of questions on their computer usage, how often and for what purpose and for how long they use computers at work, and a series of questions relating to their physical experiences and feelings or any complaints or problems or developing abnormal signs in common day routine. Besides getting preliminary data like Agency, Occupation, Designation, and Shift, information rather individual's experiences were also inquired in order to assess their musculoskeletal state and situation. The last series of questions attempted to ascertain whether the pain was mild, adverse and/or consistent. The questionnaire was then evaluated on different aspects related to musculoskeletal disorders. Ranking (strongly agree, agree, disagree and definitely not) and open- ended questions were used.

The principle information that were mainly considered for the statistical assessments as common musculoskeletal symptoms, signs or complaints are elaborated in the Table 2 below. These were implied by few simple questions like "How long do you use computers", "Do you feel pain while working on computers", and "How long your problem sustain" etc.

Table 2: MSDs Symptoms Questioned in Proforma

Questions	Sample Output
Affected area	Full back
Column pain	Lower
Weakness	none
Interval range	1-2 days
Back pain	severe
Leg pain	no
Pain interval	Weekly
Concern, cause	Work overload

The questionnaire was designed to elicit information on the type and intensity of pain felt by the respondents, number of hours the condition remains and the frequency of the signs ensue per week.

All respondent not showing the signs of MSDs or were not feeling any such problems were excluded from the study.

Analysis

The data analyses were carried out using SPSS software (SPSS Inc., Chicago, Illinois, USA). Descriptive statistics for mean (score out of a max. of 4), standard deviation, frequencies, percentages, quartiles and cross tabulations were done.

3. RESULTS AND DISCUSSION

There were 129 respondents, whose responses were considered and their questionnaires were analyzed and assessed and argued that from what they have answered, positive signs of MSDs were prominent and it was statistically proved that they are victim of MSDs and require early attention in order to avoid major loss to their physical activities, health and life.

Statistical computation according to gender, designation and the office environment showed no significant differences and hence results are not shown here.

Working in different time zones did show significant impact for getting MSDs and their results have further been discussed here. Similarly all the said work related MSDs were found to be alarmingly present amongst most of the workers. The responses also highlighted that the impact induced consequently, does retain over a time period and also determines the influence of the level of workload.

Table 3 summarizes that mostly workers are prone to suffering with the MSDs related to whole vertible column extending to neck, and shoulder besides the full back issues. Besides this people are also susceptible to acquire vertible column and arm-to-wrist relatedissues and MSDs.

Table 3: Impact of MSDs

Case Processing Summary	Valid %	Missing %
Upper, lower or full back	37.3	62.7
Neck, shoulder, both	51.0	49.0
Upper, thigh/knee, lower full	5.9	94.1
Numbness, weakness, both, grip	31.4	68.6

Statistical analyses of opinions and feedback of different employees and professionals according to their designation showed no differences (results not shown here). It appeared that professionals who worked for longer hours or were overloaded were affected more with WRMSD.

Table 4 shows different statistical values encompassing different signs and symptoms. Table 5 shows the same statistical values for the duration of the impression and effect of the feelings and magnitudes of the sufferings of work related MSDs. Another aspect that is clear from the statistical values is the wellness of the dataset due to the smaller values of standard deviations and reflects the absence of outliers and closeness of the data values from the mean values.

Table 4: Statistical Values for Different Signs and Symptoms

		Upper, lower or full	Neck, shoulder, both	Upper, thigh/ knee lower, full	Numbness, weakness both, grip
Mean		2.37	2.15	3.33	2.38
Median		2.00	3.00	4.00	2.50
Mode		3	341		
Std. deviation		6984	1.008	1.155	1.360
Sum		45	56	10	38
Percentiles	25	2.00	1.00	2.00	1.00
	50	2.00	3.00	4.00	2.50
	75	3.00	3.00		4.00

Table 5: Duration of Impact of Signs and Symptoms

		Interval Range in days	Continuous WL, extra Wl or late sitting
Mean		843	5.06
Median		1.000	8.00
Mode		1.0	8
Std. deviation		3239	3.029
Sum		43.0	285
Percentiles	25	500	2.00
	50	1.000	8.00
	75	1.000	8.00

It is also quite noticeable people working on a general routine timings from morning to evening i.e., 9 to 5 are having major concerns and disorders related to both neck and shoulder and thus commonly being object of numbness and can clearly be seen from the Figs. 2 and 3.

A couple of more aspects that this study covers; one being the feature of Work load be it continuous, or extra within the job timings or late sitting due within, which is demonstrated by the Fig. 3. The second is the duration of the time period in days and the responses showed that the effect and influence of the hazards and injuries mostly retains for a day or so, shown in Figs. 4, 5 and 6, hence, it all supports our argument that the issues relating to MSDs are on upsurge and intensification.

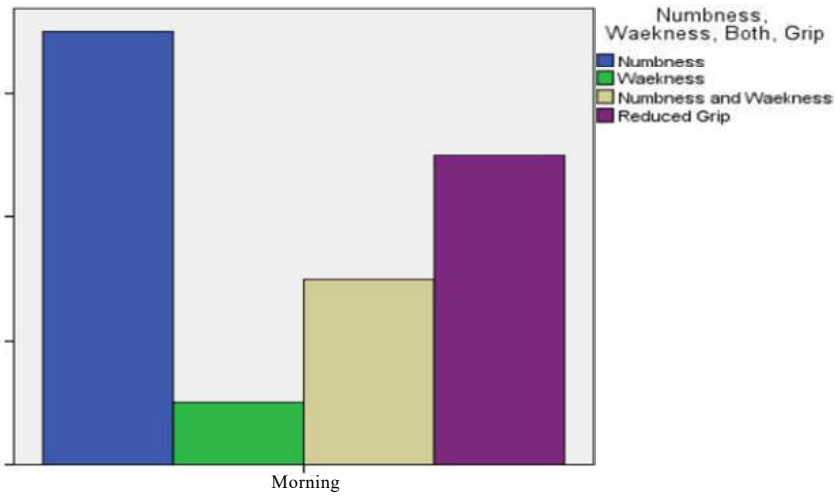


Fig. 2: Major influence of MSDs in Regular shift

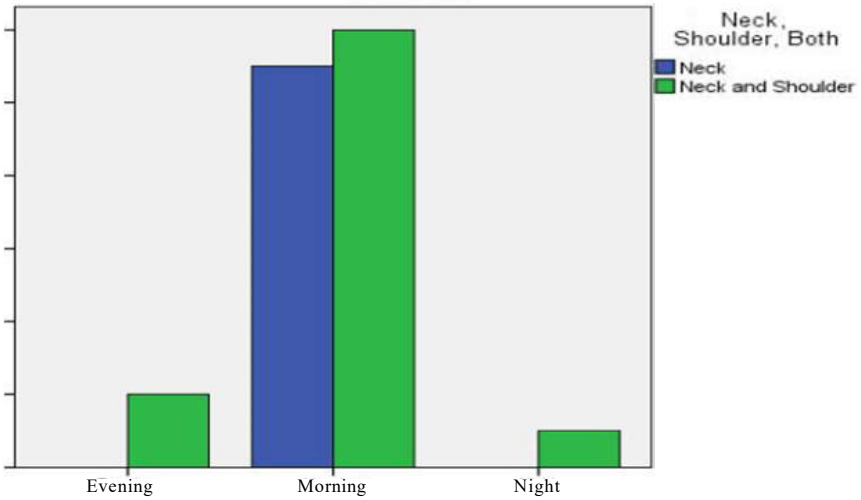


Fig. 3: Major influence of MSDs in different shifts

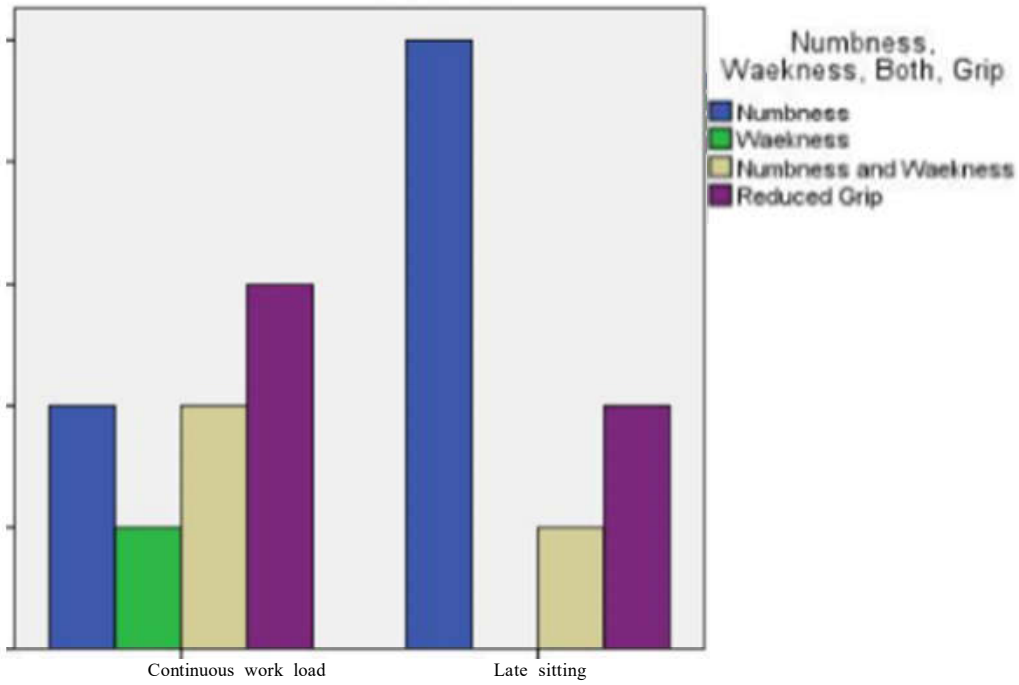


Fig. 4: Major influence of MSDs causing Numbness, Weakness and Reduced Grip

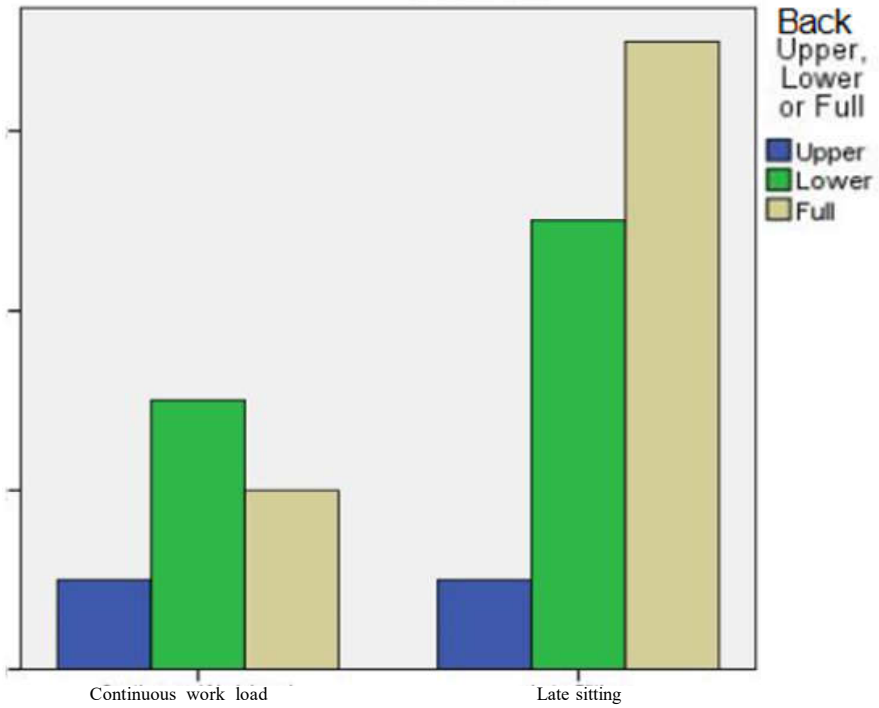


Fig. 5: Major influence of MSDs causing Upper, Lower and Full Back issues

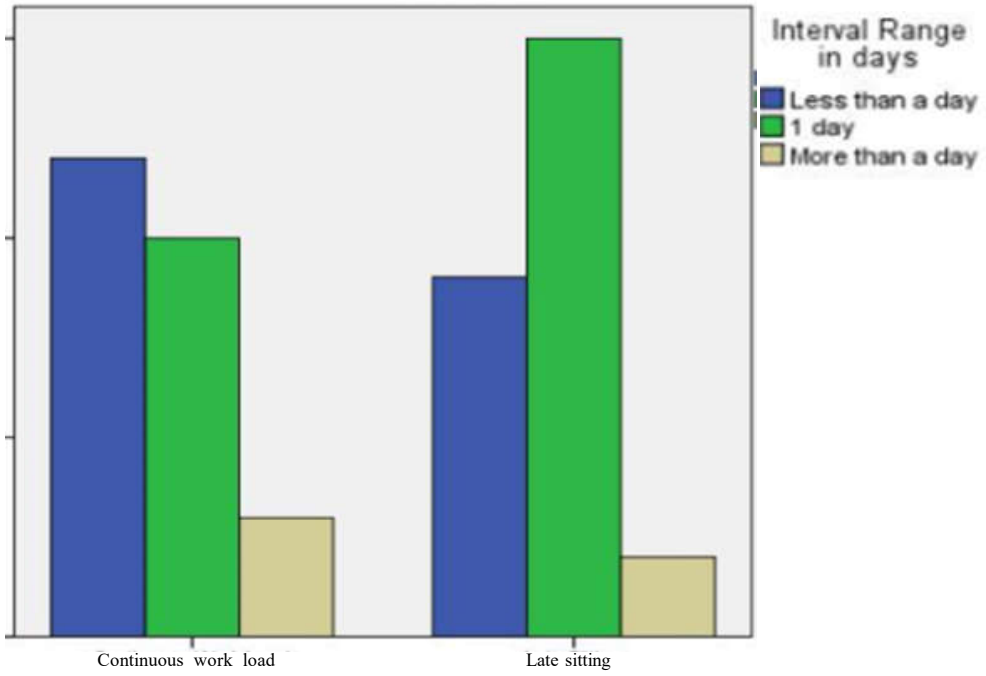


Fig. 6: Major influence of MSDs, impact duration

4. CONCLUSION

The incident of musculoskeletal disorder (MSD) in computer users is on the rise. Work-related musculoskeletal disorders (WRMSD) are a common cause of pain among computer professionals and can lead to aches, stiffness in the joint, pins and needles sensation, tingling and/or a burning sensation, sickness, serious injury and in some cases long-term irreversible disability. General signs, symptoms and causes of WRMSD include

a. Poor and/or static posture for longer period of time
b. Repetitive movements
c. Transducer grip pressure and the use of force
d. Psychosocial factors and less intake of brain strengthening food
e. Workload management issues

Therefore, it behoves all computer users to be aware of MSDs, their causes, symptoms, and measures to avoid it. Moreover, computer users if suffering from different disease conditions must use computer according to respective clinical advice.

It is imperative that no one would like to be injured or disabled by MSDs and therefore, necessary care and precautions must be taken to reduce the risks for developing MSDs, essentially, by applying some common but phenomenal procedures like walking, jogging or some specific sort of intermittent and simple exercises on regular basis along with healthy and nourished diet specially for brain functioning as a tonic along with special massage therapy (oil) on head and feet are also beneficial to prevent the progression of disease condition on one hand and regulation and improvement of efficiency on the other hand.

5. REFERENCES

1. Anderson, Bob. (1990). *Stretching: For Everyday Fitness,* Shelter Publications, Inc., Bolinas, CA.
2. Bussières, A.E., Peterson, C. and Taylor, J.A. (2008). Diagnostic imaging guideline for musculoskeletal complaints in adults – an evidence-based approach – Part 2: Upper extremity disorders. *J. Manipulative Physiol.Ther.* **31**(1):2-32.
3. Cevik, A.A., Gunal, I., Manisali, M. *et al.* (2003). Evaluation of physical findings in acute wrist trauma in the emergency department. *Ulus. Travma. Acil. Cerrahi. Derg*. **9**(4):257.
4. Davies, Clair. (2001). *The Trigger Point Therapy Workbook: Your Self-Treatment Guide for Pain Relief,* New Harbinger Publications, Inc., Oakland, CA.
5. Hedge, Muss and Barrero. (1999). Comparative Study of Two Computer Mouse Designs, Cornell Human Factors Laboratory Technical Report/ RP7992, Ithaca, NY URL:http://erqo.human.comell.eduiPub/HFIabReports/MouseRep.pdf
6. Lidgren, L. (2003). The bone and joint decade and the global economic and healthcare burden of musculoskeletal disease. *J. Rheumatol. Suppl.* p. 67.
7. North Carolina Department of Labor, November 10, 1998, News Release, "NC Department of Labor Proposes New Standard Aimed a Reducing Workplace Ills, Workers' Comp Costs", Raleigh, NC Office of Environment, Health and Safety, University of North Carolina at Chapel Hill, "Office Ergonomics", URL:http://ehs.unc.edu/workplace_safety/ergonomics/office/office2.shtml
8. "Shortcut Keys", URL:http://www.c0mputerh0pe.c0m/sh0rtcut.htm#1
9. Tech Connections, "Mouse Alternatives", URL: http://www.techconnections.orq/resources/guides/Mouse.cfm
10. "Windows Key Shortcut", URL:http://www.seoconsultants.com/windows/tips/windows-kev.htm

Molecular Analysis in Medicinally Important Species *Mentha royleana* and *Mentha arvensis* from Gilgit-Baltistan

Nargis Khatoon and Imtiaz Ahmed Khan*

Department of Biological Sciences, Karakoram International University, Gilgit, Pakistan.
*Email: dr.imtiaz@kiu.edu.pk

Abstract

Gilgit Baltistan (GB) is rich in medicinal plants including species belonging to genus *Mentha* of family Laminaceae. Approximately 30 species of genus *Mentha* are found in GB. Two important species *M. royleana* and *M. arvensis* are locally used to treat asthma, gastro intestinal problems, and as haemostatic. Present research is the first documented attempt to utilize Randomly Amplified Polymorphic DNA (RAPDs) primers to estimate existing genetic diversity in the locally available *M. royleana* and *M. arvensis*. Plant samples were collected from four locations *viz*, Gilgit city, Muhammadabad, Daynore (1500 meter above sea level) and Bagrot (2700 meter above sea level). Bivariate (1-0) data matrix was generated and genetic distances were estimated using Unweighted Pair Group of Arithmetic Mean (UPGMA) procedure. The results revealed five RAPD primers demonstrating complete homozygosity among *M. royleana* and *M. arvensis* (Genetic distance 0%) while three RAPD primers showed maximum genetic distance (100%). Crude leaf protein concentrations estimated using UV spectrophotometry ranged from 13.8-21.54%. Maximum leaf protein content (21.5%) was observed in *M. arvensis* collected from Muhammadabad and *M. royleana* collected from Gilgit city and Bagrot. Minimum leaf protein content (13.8%) was observed in *M. arvensis* collected from Danyore. The SDS-PAGE analysis revealed that high altitude specific protein band in both species collected from Bagrot valley (2700 meters above sea level) suggesting that the alterations in genetic expression is modified by the environmental conditions and are altitude-dependent.

Keywords

Mentha royleana, *Mentha arvensis*, Gilgit Baltistan, Randomly Amplified Polymorphic DNA, Genetic distances, protein concentration, SDS-PAGE

1. INTRODUCTION

Pakistan with a wide variety of climates is reflected by the presence of abundant species of medicinal plants growing widely in the forests, deserts, roadsides and river banks. On the other hand, Pakistan spends around Rs.18 billion per year of foreign exchange on the import of raw materials for pharmaceuticals. In Pakistan 6000 plant species have been reported and among these about 3200 species have medicinal uses in Unani, allopathic, and homeopathic medicines (Haq, 1998). However,

only few have been explored while 90% of country's medicinal herbs requirement is imported.

Mountain areas of Gilgit Baltistan (GB) situated between 710 and 750 East longitude and 320 and 370 North latitude, stretched over an area of 28,000 square miles (Rasool, 1998) are especially rich in medicinal plants. One of medicinally important genus of GB is *Mentha* which is also known as mint genus that is widely distributed across Africa, Asia, Australia, Europe and North America (Christopher and Judith, 1997). Genus *Mentha* is a member of the tribe Mentheae in the subfamily Nepetoideae of family Lamiaceae. Generally, *Mentha* species grow near moist places like pools of water, lakes, rivers, and cool moist spots in partial shade. Because of their fast growth they are also considered as invasive species. *Mentha* species are mostly used as treatment against gastro-intestinal, gallbladder and cough problems. Oil extracted from it is rubbed on skin for aches and pains (Khan *et al.,* 2011). Mint extracts and menthol-related chemicals isolated from *Mentha* species are used in food, drinks, cough medicines, creams and cigarettes (Karta *et al., 2008*). Accurate data regarding area, production and yield of *Mentha* species in Pakistan is not available but it has been estimated that more than 200 tons is sold per year (Haider, 1998). Harvesting of leaves from *Mentha* species can be practiced through out the year. Fresh leaves may be used immediately or stored up to few days in plastic bags in refrigerator. Optionally, leaves can be frozen in ice cube trays. Dried mint leaves should be stored in an airtight container placed in a cool, dark and dry area (Elisabeth, 1992).

Present research is the first documented attempt to utilize relatively recently developed DNA based technology (Polymerase Chain reaction using Randomly Amplified Polymorphic DNA) to estimate existing genetic diversity in the locally available *M. royleana* (Benth.) Hook. f. and *M. arvensis* (L.) (Fig. 1). The objective of the study was to investigate

Fig. 1

Mentha royleana *Mentha arvensis*

(Source: https://en.wikipedia.org/wiki/Mentha)

altitudinal effect on their biochemical characters collected from District Gilgit from low and high altitude alongwith estimation of genetic distances using Randomly Amplified Polymorphic DNA (RAPD) primers.

2. MATERIALS AND METHODS

2.1. *DNA Analysis*

The DNA was extracted on small scale by procedure described earlier (Weining and Langridge, 1991). Leaves 1 g were crushed with pre-warmed DNA extraction buffer (TrisCl) (12.1%), cetyl trimethylammonium bromide (CTAB) (10 %), NaCl (5.2%), EDTA (3.2 %), (pH=8.5). β-marcaptoethanol (100 µL) was added in tubes and placed at 65ºC for 4-6 hrs with occasional vortexing. Tubes were centrifuged at 7000 rpm for 5 minutes. Supernatant was transferred to a fresh tubes. Cold chloroform:isoamylalcohol (24:1) was added and the tubes were inverted vertically (5-10 times) followed by centrifugation at 7000 rpm for 5 minutes. After centrifugation supernatant was transferred into fresh eppendorf tubes. Seventy µL of 3M sodium acetate (pH=4.8-5.2) and isopropanol (700 µL) was added in the supernatant and mixed gently by inverting the tubes for about 10 times. Tubes were centrifuged at 7000 rpm for 5 minutes to acquire DNA pellet. The pellet was washed with ethanol (70%), air dried for 30 minutes and resuspended in double distilled, deionized, autoclaved water (40 µL). DNA samples were stored at –20ºC.

The quality and quantity of the DNA was checked on 1% agarose/TBE (Tris-Borate-EDTA) gel. Agarose powder (1 g) was dissolved in tris-borate (TBE) buffer (100 ml). The mixture was boiled in microwave oven. After boiling, ethedium bromide (5 µL) was added in the gel mixture. Gel was casted in a gel tray with comb and after solidifying, gel was placed in gel tank containing TBE buffer. DNA (5 µL) from each sample was mixed with loading dye (5 µL bromophenol blue) and loaded in the wells. Gel was allowed to run at 70 volts for 1.5 hour and observed under UV light using UVitech Gel Documentation System.

Fifteen RAPD primers (Table 1) were used to amplify genomic DNA isolated from *M. royleana* and *M. arvensis*. Component of PCR included genomic DNA used as template, dNTPs (dATP, dCTP, dGTP and dTTP), RAPD Primer, Taq polymerase buffer, Taq DNA polymerase and water with final reaction mixture of 25 µL. Practical formulation of PCR reaction used during present study were same as described by Fadhel and Boussaïd (2004). Thermocycling conditions included denaturation at 94ºC (1 min.), annealing at 34ºC (1 min.) and extension at 72ºC (2 mins.). Forty cycles were used for DNA amplification.

2.2. *Spectrophotometric Determination of Total Protein Concentration*

Crude protein was extracted from leaves of *M. rolyeana* and *M. arvensis* using procedure described earlier (Yeoh and Wong, 1993). Fresh leaves (1 g) were ground using protein extraction buffer (5 ml 10% w/v NaCl, 70% w/v ethanol, 0.1 M NaOH). The mixture was left at room temperature for 4-6 hrs with occasional vortexing. Samples were centrifuged at 7000 rpm for 20 minutes and supernatant was transferred to a fresh tube. Samples were stored at 4ºC till further use.

Protein content was estimated by UV spectrophotometer using Quartz cuvettes. The protein concentration was estimated assuming that a 1 mg/ml solution of protein will be equal to an absorbance of 1.3 at 320 nm (Grimsley and Pace, 2003).

2.4. *Statistical Analysis*

Genetic distances among all the possible

Table 1: Sequence Information of RAPD Primers Used to Estimate Genetic Diversity in *M. royleana* and *M. arvensis*

S.No.	Name of the primer	Sequence	GC (%)	Molecular weight
1.	GLA-01	CAGGCCCTTC	70	2963.97
2.	GLA-10	GTGATCGCAG	60	3068.02
3.	GLB-05	TGCGCCCTTC	70	2954.97
4.	GLB-06	TGCTCTGCCC	70	2954.97
5.	GLC-04	CCGCATCTAC	60	2947.96
6.	GLC-09	CTCACCGTCC	70	2923.95
7.	GLC-10	TGTCTGGGTG	60	3090.04
8.	GLD-03	GTCGCCGTCA	70	3003.99
9.	GLD-06	ACCTGAACGG	60	3037
10.	GLD-09	CTCTGGAGAC	60	3028
11.	GLG-01	CTACGGAGGA	60	3077.02
12.	GLG-07	GAACCTGCGG	70	3053.01
13.	GLH-01	GGTCGGAGAA	60	3117.04
14.	GLI-01	ACCTGGACAC	60	2996.98
15.	GLK-01	CATTCGAGCC	60	2987.98

Adenine (A), Cytosine (C), Guanine (G) and Thiamine (T)
Randomly amplified polymorphine DNA (RAPD)

comparisons were estimated using following formula (Nei and Li, 1979):

Genetic diversity=1-dxy/dx+dy-dxy

Number of common DNA fragments in two samples (dxy), number of total DNA fragments in sample 1 (dx), number of total DNA fragments in sample 2 (dy).

3. RESULTS AND DISCUSSION

Total genomic DNA of good quality (Free of RNA contamination) of two *Mentha* species were successfully extracted. The quality and quantity of DNA was checked on 1% agarose/ TBE gel. Total of 15 RAPD primers were used for the amplification of *M. royleana* and *M. arvensis* genomic DNA. An example of PCR amplification of genomic DNA isolated using RAPD primer GLI-01 is presented in Fig. 2. DNA fragments were scored as present (1) or absent (0). Bivariate 1-0 data matrix was constructed and used for estimation of genetic diversity using Unweighted Pair Group of Arethematic Mean (UPGMA) procedure (Nei and Li, 1979). Genetic distances estimated using 15 RAPD primers ranged from 0 to 100% with an average value of 42% (Table 2). Five RAPD primers *viz*; GLA-10, GLB-05, GLB-06, GLD-06, and GLH-01 showed complete homozygosity between *M. royleana* and *M. arvensis* (GD=0%). Three RAPD primers GLC-09, GLC-10 and GLG-07 showed maximum genetic distance (GD=100%)

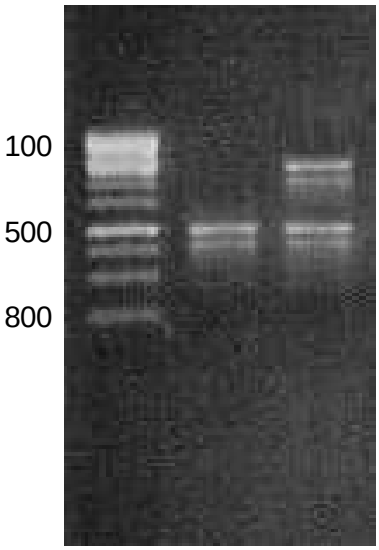


Fig. 2: DNA amplification of *M. royleana* (Lane 1) and *M. arvensis* (Lane 2) using decamer RAPD primer GLI 01. Molecular size marker (M, 100 base pair ladder). Size of DNA fragments (in bp) is presented on the leftside.

among *M. royleana* and *M. arvensis*. Four RAPD primers GLA-07, GLD-09, GLI-01 and GLK-01 showed 50% genetic diversity among the two species. While two RAPD primers (GLD-03, GLG-01) showed 25% genetic distance and one RAPD primer GLC-04 showed 84 % genetic distance among the two species.

Giving due importance to medicinal and commercial value of *Mentha* species, number of studies have been conducted globally using recently developed DNA based technology. Unfortunately, no such work has been reported from Gilgit Baltistan. Present research is therefore the first documented attempt to utilize Randomly Amplified Polymorphic DNA to estimate existing Genetic Diversity in the locally available *Mentha* sps. Genetic diversity of up to 100% reported in present study is in agreement with a previous report (Kazemi and Hanifeh, 2012) of 93% genetic distance in Iranian populations of *Mentha* using (RAPD) primers. Genetic diversity in *Mentha* species

Table 2. Estimates of Genetic Distances among *M. royleana* and *M. arvensis* Using RAPD Primers

S.No.	Code of the DNA primer	G.D. estimate
1.	GLA-10	0.00
2.	GLB-05	0.00
3.	GLB-06	0.00
4.	GLD-06	0.00
5.	GLH-01	0.00
6.	GLD-03	0.25
7.	GLG-01	0.25
8.	GLA-07	0.50
9.	GLD-09	0.50
10.	GLI-01	0.50
11.	GLK-01	0.50
12.	GLC-04	0.84
13.	GLC-09	1.00
14.	GLC-10	1.00
15.	GLG-07	1.00
Average		0.42

Genetic Distance (GD), Randomly Amplified Polymorphic DNA (RAPD)

using Simple Sequence Repeat (SSR) primers also reported amplification of 2 to 4 alleles with an average of 2.33 alleles amplified per SSR (Kumar *et al.,* 2015).

Crude protein percentages in leaves of *M. royleana* and *M. arvensis* collected from 4 locations *viz;* Muhammadabad, Danyore, Jutial Gilgit (considered as low altitude, 1500 meter, above sea level) and Bagrot valley (considered as high altitude, 2700 meter above sea level) ranged from 13.8-21.54% (Table 3). Maximum leaf protein content (21.5%) was observed in *M. arvensis* collected from Muhammadabad and *M. royleana* collected from Gilgit and Bagarot. Minimum protein content (13.8%) was observed in *M. arvensis* collected from

Table 3: Average Protein Content in *M. royleana* and *M. arvensis* Collected from 4 Locations

S.No.	Species	Location	Altitude (meter above sea level)	Leaf protein content (%)
1.	*M. royleana*	Muhmmadabad	1500	20
2.		Danyore	1500	20.77
3.		Jutial Gilgit	1500	21.5
4.		Bagroat	2700	21.54
5.	*M. arvensis*	Muhmmadabad	1500	21.54
6.		Danyore	1500	13.84
7.		Jutial Gilgit	1500	20.7
8.		Bagroat	2700	20

Danyore. Highly significant differences were observed for leaf protein content among two *Mentha* species collected from 4 locations. Crude protein content in *M. royleana* and *M. arvensis* collected from high and low altitude were significantly different t-value=54.2, *p* value=0.0.

Protein quality in 8 samples of genus *Mentha* was studied using SDS-PAGE analysis Fig. 3. The protein bands in *M. royleana* were of lower molecular weight as compared to those observed in *M. arvensis*. In both species (from all locations) 1-2 protein bands were observed. It was also noted that both species collected from high altitude (Bagrot, 2700 meter) had an additional protein band indicating that some fractions of water soluble protein were produced at higher altitude only (Indicated by arrows in Fig. 3). This protein band needs to be analyzed in detail for better understanding of genome structure/protein expression and composition of the two medicinally important species from Gilgit Baltistan.

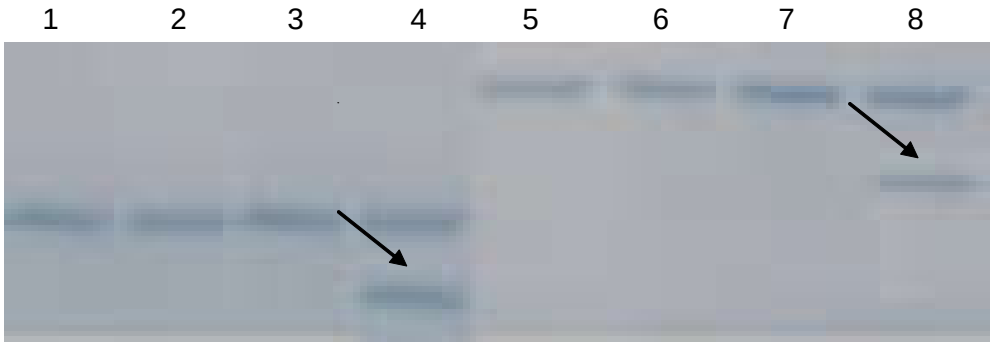


Fig. 3: SDS-PAGE analysis in samples of genus *Mentha*

M. Roliana Muhammadabad (1), Jutial (Gilgt) (2), Daynore (3) Bagrot (4)
M. arvensis Muhammadabad (5), jutial(Gilgt) (6), Daynore (7), Bagrot (8)
The arrow (↘) identifies different electrophoretic bands

4. CONCLUSION

In the present study high range of genetic distance (0-100%) were observed indicating that substantial genetic diversity is present in two species of *Mentha* commonly grown in Gilgit. This existing genetic diversity can be used for the improvement of local genotypes to increase the yield of desired phytochemicals. The protocols optimized during present study may be used at relatively larger scale studies for better understanding of genome structure in medicinal plant species. It is recommended that some of the DNA fragments amplified during present research especially those amplified using RAPD primers GLC-09 should be sequenced which may be used as species specific marker sequence to develop specific PCR primers.

Leaf protein ranging from 13.8-21.54% demonstrated highly significant differences between two *Mentha* species. One additional protein band revealed by SDS-PAGE in both the species collected from higher altitude (Bagrot) should be analyzed in detail for better understanding of genome structure/protein composition of the two medicinally important species from Gilgit Baltistan.

Conflict of Interest

The authors declare there is no conflict of interest concerning publication of the manuscript.

5. REFERENCES

1. Christopher, B. and Judith Z. (1997). The American Horticultural Society: A-Z. *Encyclopedia of Garden Plants.* New York, NY, USA, DK Publishing. p. 668.
2. Elisabeth, O. (1992). *The Encyclopedia of Herbs: Spices & Flavorings.* London, Dorling Kindersley. pp. 36-37.
3. Fadhel, N.B. and Boussaïd, M. (2004). Genetic diversity in wild Tunisian populations of *Mentha pulegium* L. (Lamiaceae). *Genetic Resources and Crop Evolution*. **3**:(309-321).
4. Grimsley, G.R. and Pace, C.K. (2003). *Current Protocols in Protein Science*. 3.1.1-3.1.9 Copyright© 2003 by John Wiley & Sons, Inc. http://onlinelibrary.wiley.com/book/10.1002/0471140864/homepage/Archive.html
5. Haider, Z.S. (1998). Existing indigenous medicinal plant resources of Pakistan and their prospects for utilization. *Proceedings of the meeting held at the Plant Genetic Resource Institute,* Pakistan Agricultural Research Council, Islamabad. pp. 55-64.
6. Haq, N. (1998). *In vitro* production of bioactive compounds from medicinal and aromatic plants. *Proceedings of the meeting held at the Plant Genetic Resources. Institute. Pakistan Agricultural Research Council, Islamabad*. 2-3 December, 1998. (Eds.): R. Anwar, N. Haq and S. Masood. pp. 69-92.
7. *Karta , K. et al.* (2008). The way of ayurvedic herbs. The most complete guide to natural healing and health with traditional ayurvedic herbalism. *Twin Lakes,* Wis.: Lotus. p. 313.
8. Kazemi. M. and Hanifeh, S.H. (2012). Assessment of genetic diversity of mints Iranian wild "*Mentha aquatic*" populations using RAPD marker. *Journal of Agricultural Technology.* **8**(1):327-336.
9. Khan, B. *et al.* (2011). Medicinal uses of plants by the inhabitants of khunjerab national park, Gilgit, Pakistan. *Pak. J. Bot*. **43**(5):2301-2310.
10. Kumar, B. *et al.,* (2015). Identification of EST-SSRs and molecular diversity analysis in *Mentha piperita. The Crop Journal.* **3**(4):335-342.
11. Nei, M. and Li, W.H. (1979). Mathematical model for studying genetic variation in terms of restriction endonuclease. *Proceedings of the National Academy of Science USA*. **76**(10):5269-5273.
12. Rasool, G. (1998). *Medicinal Plants of the Northern Areas of Pakistan: Saving the Plants that Save Us.* Gilgit, Pakistan.
13. Weining, S. and Langridge, P. (1991). Identification and mapping of polymorphism in cereals base on polymerase chain reaction. *Theor. Appl. Genet*. **82**: 209-216.
14. Yeoh, H. and Wong, P.F.M. (1993). Nutriritaive value of leaf protein concentrates from some tropical plants. *Journal of Singapore Academy of Science*. **20**:14-21.

Physicochemical and Phytochemical Studies of Unani Drug Ushba (*Smilax ornata* Hook.)

Reesha Ahmed[1] , Naeem Ahmad Khan[1] and Mohammad Waseem[2]

[1]Dept. of IlmulAdvia, Ajmal Khan Tibbiya College, A.M.U, Aligarh,
[2]Dept. of Psychiatry, A.I.I.M.S., New Delhi,
India.

*Email: dr.reeshaahmed17@gmail.com

Abstract

All herbal drugs should be standardized to ensure uniformity, purity, safety, efficacy and quality by various parameters viz. physical, chemical and biological observation.

Phytochemical investigations along with biological screening is needed to understand the therapeutic dynamics of medicinal plants that will be useful in developing quality parameters and help in the standardization.

Ushba (*Smilax ornata*) used for arthritis, ascites, fever, cough and many other diseases, was standardized using physicochemical parameters. Successive Extractive Values: Pet. ether (2.12±0.01), di-ethyl ether (0.45±0.02), chloroform (0.33±0.01), acetone (1.62±0.01), alcoholic (2.32±0.01), aqueous (4.14±0.02). Non-Successive Extractive Values: Pet. ether (1.03±0.03), chloroform (2.04±0.02), alcoholic (5.90±0.01), aqueous (3.81±0.02). Solubility: water (3.33±0.02) and alcohol (2.28±0.04). Moisture contents (6.66±0.33), total ash values (5.36±0.08), Bulk density 0.33±0.02 (Poured density) and 0.51±0.01 (Tapped density), pH of 1% (5.58±0.02) and 10% solution (5.13±0.01) and loss on drying (4.26±0.33) (Table 2).

Phytochemical analysis revealed the presence of almost all the phyto-constituents in the test drug sample such as alkaloid, flavonoids, glycoside, carbohydrate, tannin, protein, amino acids, starch and resins.

Keyword

Unani drugs, Standardization, *Smilax ornata,* Phytochemistry.

1. INTRODUCTION

Unani system of medicine is a comprehensive traditional system of medicine with its own theory of health and disease (Husain *et al*., 2017). WHO has emphasized the need to ensure quality control of medicinal plant products using modern techniques by applying suitable parameters and standards (Rasheed *et al.*, 2010). WHO has set specific guidelines for the assessment of the safety, efficacy and quality of herbal medicines (Archana *et al.*, 2011).

This variety is obtained from Ushba (*Smilax ornata*) commonly known as Sarsaparilla belongs to family Liliaceae, is a climbing plant with woody stems on ascending lofty trees and springing from a stout, knotty rhizome. The plant is native of Central America.

Roots were formerly exported via Jamaica; hence the designation 'Jamaica' is added with sarsaparilla. Several varieties of sarsaparilla are important but the one known as *Jamaica sarsaparilla* is the most esteemed in India (Greenish, 1999; Wallis, 1985). There are number of species of sarsaparilla, the most common are S*milax ornata* and S. *regelii*, which contain saponins, sarsaponin and parallin sapogenins, sarsapogenin and smilogenin (Greenish, 1999).

The drug has no odour but slightly bitter in taste (Wallis, 1985) and probably other sarsaparillas is Sarsasaponin, $C_{44}H_{76}O_{20}$. $7H_2O$, a crystalline glucoside obtained by hydrolysis of sarsasapogenin and dextrose (Greenish, 1999; Wallis, 1985). Two isomeric genins are known; smilagenin (a name derived from the genus *Smilax*) and sarsasapogenin. Sarsasapogenin also occurs in species of Y*ucca* (Evans, 2009). Sarsaprilla has been administered as an alternative in syphillis, chronic skin disease and rheumatism, but great diversity of opinion exists as to its therapeutic value (Greenish,1999; Evans, 2009).

Two types of drug are used by the name of Ushba. *Ushba magribi* (*Smilax ornata*), its root is used, found in S.America. Another is *Ushba hindi* (*Hemidesmus indicus*), its root have medicinal uses (Saif Uddin, 2010).

Stem of *U. magribi* is climber herb, is wrinkled and fibrous, has bitter taste, found in America while *U. hindi* (*Hemidesmus indicus*) leaves and stem similar to Chameli. Flower is fragnance while root is black non fragrance (Usman, 2008).

There are many adulterated varieties available in market differ from the actual one making it difficult, to attain uniform therapeutic efficacy. Therefore, the present study was conducted to access phytochemicals, its identity and purity by various parameters.

2. MATERIALS AND METHODS

The drug samples of *Ushba* were obtained from Dawakhana Tibbiya College A.M.U. Aligarh, U.P (India) and was properly identified by botanical literature survey.

Ushba (*Smilax ornata*) Fig. 1 coarse powder was subjected to physicochemical and phytochemical studies as per Unani Pharmacopoeia (Anonymous, 2007) followed by fluorescence study of the extract (Afaq *et al*., 1994). Physicochemical parameters studied were ash values (Total ash, acid insoluble ash and water-soluble ash) and loss on drying at 105°C were determined and estimated in percentage using method as recommended in Unani Pharmacopoeia (UPI, 2007). Moisture content was determined using toluene distillation method. Successive extractive value of powder drug in petroleum ether (60-800C), di-ethyl ether, acetone, chloroform, ethyl alcohol and aqueous; pH of 1% and 10% aqueous solution. Water and alcohol soluble matter and fluorescence analysis of the successive extract was studied under day light as well as ultra violet (Short and long wave length) light. The Preliminary qualitative study of phytochemicals was also conducted (Anonymous, 2007).

3. RESULTS AND DISCUSSION

The organoleptic properties of Ushba (*Smilax ornata*) is described in Table 1. However, the Table 2 describes the physico-chemical properties. The phytochemical analysis revealed presence of alkaloids, tanins, glycosides, starchs and carbohydrates. While amino acids, phenols, proteins, terpenes, steroids, flavonoids and resins were absent. Many reasons has been cited for the need for scientific evaluation and standardization of herbal drugs requirement. The present study is an attempt to ascertain the pharmacopoeial standards for the standardization of drug including its quality, identity, purity and

strength of the powder that has been undertaken as a tool to bring out several features like ash standards, solubility in alcohol and water, successive extractive values, and qualitative screening of physicohemicals, total alkaloids, total flavonoids, phenol, nitrogen, fatty matter, sterol/terpenes, protein and carbohydrates.

Herbal medicines are being used by nearly about 80% of the world's population. Herbal drug technology is used for converting botanicals materials into medicines, where standardization and quality control with proper integration of modern scientific techniques and traditional knowledge is important. After organoleptics, physicohemical standardization is of prime importance in quality control of Unani drugs. As the efficacy of various drugs mainly depends upon its physical and chemical properties therefore, the determination of physicochemical characters for the authenticity of a drug is necessary before studying its medicinal properties. Phytochemical constituents present in the drug vary, not only from plant to plant but also among different samples of same species, depending upon various atmospheric factors, storage and drying conditions. According to the macroscopic/organoleptic evaluation (Table 1) of both the drugs i.e. *Ushba magribi* light-brown stem climber herb with parallel lining having no characteristic odour and bitter taste. While *Ushba hindi* is black wrinkled and fibrous root, slightly acrobitter taste without having prominent fragrance were examined and detected the distinctive features of the genuine drug.

However, the Table 2 describes the physiochemical properties. The phytochemical analysis revealed presence of alkaloids, carbohydrates, glycosides, starch and tannins, while amino acids, flavonoids, phenols, proteins, resins, steroids and terpenes, were absent.

Fluorescence analysis of Ushba powder using variety of acids and reagents (Table 4) revealed brown colour in daylight and black under UV (short) indicating λmax 254 and 366.

The fluorescence analysis of successive and non-successive extracts is described in Tables 5 and 6. Only petroleum ether showed similar pattern in both types of extracts while all the other solvents (acetone, alcohol, chloroform, diethyl ether and distilled water) showed variability.

Table 7 demonstrates that benzene chloroform (4:1) is most suitable mobile phase in TLC resulting in maximum number of TLC sports in playing that Ushba is phytochemically rich and hence responsible for multiple medicinal properties associated with alkaloids and tannins.

Fig. 1: *Smilax ornata* (*Ushba*)

Table 1: Organoleptic Characteristics of Ushba (*Smilax ornata*)

S.No.	Organoleptic	Characteristics
1.	Colour	Blackish Brown
2.	Appearance	Solid
3.	Taste	Bitter
4.	Odour	Agreeable
5.	Texture	Powder

Table 2: Physicochemical Parameters of Ushba (*Smilax ornata*)

S.No.	Physicochemical Parameters	Mean±SEM
1.	**Solubility (%)**	
	Alcohol soluble content	2.28±0.04
	Water soluble content	3.33±0.02
2.	**Successive extractive Values in different Organic Solvents (%)**	
	Petroleum ether	2.12±0.01
	Diethyl ether	0.45±0.02
	Chloroform	0.33±0.01
	Acetone	1.62±0.01
	Alcohol	2.32±0.01
	Aqueous	4.14±0.02
3.	**Non-successive extractive Values in different Organic Solvents (%)**	
	Petroleum ether	1.03±0.03
	Chloroform	2.05±0.02
	Alcohol	5.90±0.01
	Aqueous	3.81±0.02
4.	**Ash Value (%)**	
	Total ash	5.36±0.08
	Acid insoluble ash	2.53±0.08
	Water soluble ash	1.33±0.04
5.	**Moisture content (%)**	6.66±0.33
6.	**Loss of weight on drying at 105ºC (%)**	4.26±0.33
7.	**Bulk density (g/ml)**	
	Poured density	0.33±0.02
	Tapped density	0.51±0.01
8.	**pH values of solution**	
	1%	5.58±0.02
	10%	5.13±0.01

Table 3: Qualitative Analysis of Various Phytochemicals

S.No.	Tests	Inference
1.	Alkaloids	+
2.	Amino acids	–
3.	Proteins	–
4.	Phenols	–
5.	Tannins	+
6.	Sterols/terpenes	–
7.	Glycosides	+
8.	Flavonoids	–
9.	Resins	–
10.	Starch	+
11.	Carbohydrate	+

Present (+) and Absent (–)

Table 4: Fluorescence Analysis of Powder Drug in Different Chemical Reagents

S.No.	Powder drug + different reagents	Daylight	UV (short)	UV (long)
1.	HNO_3 (Conc.)	Brown	Black	Brown
2.	HCl (Conc.)	Brown	Black	Greenish brown
3.	H_2SO_4 (Conc.)	Brown	Black	Black
4.	Iodine solution (2%)	Brown	Black	Greenish brown
5.	Glacial acetic acid + HNO_3	Brown	Black	Brown
6.	Glacial acetic acid	Brown	Black	Dark brown
7.	NaOH (10%)	Brown	Black	Green
8.	HNO_3 (Dil)	Brown	Black	Greenish black
9.	H_2SO_4 (Dil)	Brown	Black	Black
10.	HCl (Dil)	Brown	Black	Brown
11.	Dragendroff's reagent	Dark brown	Black	Light brown
12.	Wagner's reagent	Brown	Black	Brown
13.	Benedict's reagent	Brown	Black	Brown
14.	Fehling reagent	Brown	Black	Brown
15.	KOH (10%) methanolic	Brown	Black	Dark brown
16.	$CuSO_4$ (5%)	Brown	Black	Brown
17.	Ninhydrin (2%) in acetone	Brown	Black	Light Brown
18.	Picric acid	Brown	Black	Brown
19.	Lead acetate (5%)	Brown	Black	Brown

Dilution (DIL)

Table 5: Fluorescence Analysis of the Successive Extracts

S.No.	Extract	Daylight	UV (short)	UV (long)
1.	Petroleum ether	Yellow	Green	Brick red
2.	Diethyl ether	Yellow	Green	Brick red
3.	Chloroform	Yellowish brown	Light green	Blackish brown
4.	Acetone	Brown	Dark green	Dark brown
5.	Alcohol	Brownish Yellow	Green	Black
6.	Distilled Water	Black	Greenish black	Black

Table 6: Fluorescence Analysis of the Non-Successive Extracts

S.No.	Extract	Daylight	UV (short)	UV (long)
1.	Petroleum ether	Yellow	Green	Brick red
2.	Chloroform	Dark Yellow	Dark green	Dark red
3.	Alcohol	Redish orange	Green	Redish Black
4.	Distilled Water	Redish brown	Black	Brownish black

Table 7: Thin Layer Chromatography Profile in Different Solvent Systems

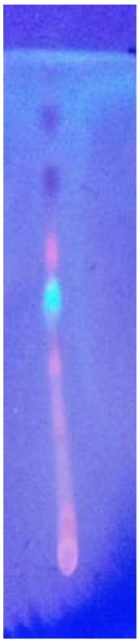
	Mobile Phase and No. of spots		R_f Value and colour of spots
Petroleum ether extract			
U V Short	Benzene: Chloroform (4:1)	5	0.08 (Dark Purple), 0.36 (Dark Purple), 0.53(Light Orange), 0.68 (Dark Purple), 0.85 (Dark Purple)
U V Long		6	0.05 (Light Orange), 0.33 (Light Orange), 0.45 (White), 0.52 (Pink), 0.68 (Purple), 0.83 (Purple)
Iodine Chamber		2	0.42(Orange), 0.91(Orange).
Alcoholic extract			
U V Short	Chloroform: Methanol (18:1)	2	0.31 (Purple), 0.82 (Purple),
U V Long		4	0.49 (Red), 0.76 (Light Blue), 0.94 (White), 0.96 (Red)
Iodine Chamber		2	0.70 (Brown), 0.96 (Light brown)
Acetone extract			
U V Short	n-Buatanol: Acetic acid: Water	1	1.13 (Blue)
U V Long		1	1.13 (Light Orange)
Iodine Chamber		1	1.13 (Dark Yellow)

Relative front value (R_f)

Fig. 2: TLC Profile of different extracts

a)

UV Short



UV Long

b)

UV Short

UV Long

Iodine Chamber

c)

UV Short

U V Long

Iodine Chamber

a) Petroleum ether, b) Alcoholic and c) Acetone

4. CONCLUSION

Herbal drug research currently demands proper identification to differentiate the crude drug material on the basis of their histological sequence of the cell shape and size in their cellular structure. Establishing standards is an integral part of the crude drug utilization as raw material for therapeutic, cosmetic and technical product manufacturing. Moreover, his elementary research on various morphology of plants provoke possibility for establishing the correlation between the Phytochemistry, Pharmacology and Phytotherapy of such excellent natural herbs in light of previous and further advance research findings.

5. REFERENCES

1. Afaq, S.H. and Tajuddin, Siddiqui, M.M.H. (1994). *Standardization of Herbal Drugs,* Publication Division, AMU (Aligarh).
2. Anonymous. (2007). *Unani Pharmacopoeia of India,* Part-I, Vol. II. GOI, Ministry of Health and Family Education, Department of AYUSH, New Delhi. 57 pp.
3. Archana, A. and Bela, Anubha Khale. (2011). Standardization of herbal grugs: An overview. *International Research Journal of Pharmacy,* **2**(12): 56-60.
4. Evans, W.C. (2009). *Trease and Evans Pharmacognosy,* 16th Edn., WB Saunders Elsevier Ltd., London, UK.
5. Greenish, H.G. (1999). *Materia Medica,* 3rd Edn., Scientific Publishers (India), Jodhpur.

6. Husain, G.M., Ahmed, S.S., Azhar, M. *et al.* (2017). Comparative toxicity study on classical and modified version of Jawarish Jalinoos (A traditional Unani formulation) in rats. *Integr. Med. Res.* **6**:66-78.
7. Rasheed, N.M.A. and Gupta, V.C. (2010). Standardization of a compound Unani herbal formulation "Qurs-e-Luk" with modern techniques. *Pharmacognosy Research.* **2**(4):237-241.
8. Saif Uddin, H.S. (2010). *Unani Advia Mufrida,* Qoomi council baraye Farooq Urdu Zubaan, New Delhi. pp. 202-203.
9. Usman, M.I. (2008). *Tanqeeh-ul-Mufridaat,* Ibn Sina Tibbiya College, Azamgarh. p. 172.
10. Wallis, T.E. (1985). *Text Book of Pharmacognosy,* 5th Edn., CBS Publishers and Distributors, Shahdara, Delhi.

In vitro Antibacterial Activity of *Lawsonia inermis* L. Against Pathogens

N. Chandrakala, R. Mekala, S. Rajeswari and G. Soundharanayaki

Department of Zoology, Kunthavai Naacchiyaar Govt. Arts College (W) Autonomous, Thanjavur, Tamilnadu, India.

*Email: Nckalavasan@Gmail.com.

Abstract

The study revealed that aqueous extract of *L. inermis* demonstrated maximum zone of inhibition compared to ethanol or methanol extracts against *V. cholerae.*

Keywords

Aquaculture, *L. inermis*, *V. cholerae*, Pathogens.

1. INTRODUCTION

Aquaculture is the farming of aquatic organisms in both coastal and inland areas involving interventions in the rearing process to enhance production, it is probably the fastest growing food producing sector and now accounts for 50% of the world's fish that is used as food. Currently 567 aquatic species are farmed all over the world, representing a wealth of genetic diversity both within and among species.

Asia is the home of aquaculture, a practice which dates back to thousands of years. In the course of its development, the nature of aquaculture has become more intricate, interwining with other food productive sectors under the influence of political, social, economic, technological and cultural factors. Overall, the aquaculture of South Asia contributed in volume 7.9% to Asian and 7.1% to world production.

Bacterial diseases in aquaculture are mainly controlled by antibiotics, however continuous intensive use of antibiotics is undesirable as this may lead to the development of drug resistance and thereby to a reduced efficacy of drugs in public health context. The alkaloids, polysaccharides and antibiotics accumulate in the environment, hence fish posing a potential risk to consumers and to the environment in general. Antibiotics such as oxytetracyline erythromycin and tetracycline are widely used to prevent bacterial diseases in fish and shrimps.

The use of expensive chemo therapeutants and antibiotics for controlling disease has widely been criticized for their negative impacts like accumulation in the fiddle as residues, development of the drug resistance immune agents are widely used for controlling impaired immune function and status is the promising area in the field of aquaculture. Herbal medicines

are also known to exhibit anti microbial activity facilitating growth and maturation of cultured species inspite intensive farming without posing any environmental hazardness. Administration of herbal extracts or their products of various concentration route enhance the innate and adaptive immune response of different freshwater marine fish and shell fish against bacterial viral and parasitic diseases.

Medicinal plants are part and parcel of human society to combat diseases from the dawn of civilization. According to the World Health Organization, Ayurveda, Siddha and Unani besides folklore practice (Chopra *et al*., 1958). *Lawsonia inermis* syn. *Lawsonia alba* (Henna) is sole species in the genus in the family Lythraceae. Henna has also shown antidiarrhoeal, diuretic emmanagogue and abortifacient properties and found to be non-toxic. The important uses of henna is acting as a cooling agent being astringent, antifungal and antibacterial herb for the skin and hair with 2-Hydroxyn aptho quione to be the main chemical. The different organisms were evaluated against leaf extracts of *L. inermis* (Kelmanson *et al*., 2002).

Plants have been used to treat humans, animals highlighting immemorial herbal medicines have been known to man for centuries (Goun *et al*., 2003). Henna plant i.e. *lawsonia inermis* (Linn.) is known for healing attributes and is now the subject of intones scientific study. Thus, the present study was conducted to evaluate an ornamental evergreen plant cultivated in the tropics from family Lythraleae. It is traditionally used to develop red or black colouring to hands, feet and hair in some occasions such as weddings and religious festivals. The phytochemical analysis of the plant leaves revealed the presence of anthroquinones as major constituents which are commonly known to posses antimicrobial activity (Misra and Sahu, 1977). In the screening of 30 plants for detecting anthroquinones which is found only in *L. inermis* plant extract. It showed broad spectrum of inhibition of 13 different fungal organisms (ring worm fungi) i.e. *Microsporum gypesum* and *Trichophyton mentagrophyles*.

Suliman *et al*., (2014) performed the *in vitro* activity of *L. inermis* (Henna) on some pathogenic fungi. Sarma (2015) the antibacterial activity of *L. inermis.* Nair and Chanda (2005) studied the antibacterial activity of some selected Indian medicinal plants. Obeide *et al.,* (2012) studied the antimicrobial activity of crud extracts of some plant leaves. Hence an attempt has been made to study the *in vitro* antibacterial activity of *L. inermis.*

2. MATERIALS AND METHODS

The leaves of *Lawsonia inermis* were collected from Valangaiman of Thiruvarur District, (10.7713^{o}N, 79.6370^{o}E) of Tamil Nadu, India. About 1 g of sterilized plant were ground in mortar and pestle with 10 ml aqueous and organic solvent (Ethanol and Methanol mixture). It was filtered and the supernatant was stored for antibacterial screening. The bacterial culture such as *E. coli* whereas, *Staphylococcus aureus* were isolated from clinical sample (Chandrakala *et al*., 2003) whereas *V. cholerae* and *V. paraheamolyticus* were isolated from diseased *Pewnaeus monodon* (Fab) subjected to *in vitro* screening (Rao and Satyanarayanan, 1997). The antibacterial activities (Bauer *et al*., 1962) of leaves were tested against the selected pathogenic the nutrients agar and TCBS agar plates were prepared (Himedia Mumbai pH 7±0.1 and 8±0.2). Swab was made with fresh bacterial culture with 10^{-4} cfu/ml (18 hrs old). The discs were impregnated with various extracts and impregnated over the bacterial and lawn incubated for 24 hrs, The plates were observed and the zone of inhibition was

measured. Distilled water and ciprofloxacin were used as negative and positive control respectively.

3. RESULTS AND DISCUSSION

The study of the antibacterial activity of *Lawsonia inermis* revealed the animal pathogen such as *E. coli* showed the zone of inhibition of 14±1 mm. The aqueous extract showed the zone of inhibition of 13±1 mm and the ethanol and methanol extracts showed the maximum zone of inhibition of 12±1 mm and 13±1 mm diameter respectively. Among the animal pathogen ciprofloxacin revealed 32±1 mm zone and 30±1 mm zone in *V. cholerae* and *V. parahaemolyticus.* The aqueous extract demonstrated the zone of inhibition of 37±1 mm for *V. cholerae* and 32±1 mm for *V. parahaemolyticus*. The Ethanol and methanol extract of *L. inermis* showed 32±1 mm and 26±1 mm zone of inhibitance in *V. cholerae* and 26±1 mm and 22±1 mm in *V. parahaemolyticus. Staphylococcus aureus* showed the zone of inhibition of about 17±1 and 15±1 against methanol and ethanol extract. Among the three organisms studied *V. cholerae* produced maximum zone of inhibition in the aqueous extract of *L. inermis* (Table 1).

Arun *et al.,* (2010) analysed the antibacterial activity of flavonoid contents of *Lawsonia inermis* and reported among the five microbes tested with maximum zone of inhibition in *Proteus* sp. followed by *Staphylococus aureus.* Medicinal plants are being used by large proportion of Indian population the reasons include true improvement of disease conduction and no harmful side effects. Plants secondary metabolites have proved to be an excellent reservoir of structurally diverse chemical compounds. Several researches have reported a wide range of pharmacological activities of *Lawsonia inermis* different *in vitro* and *in vivo* test models where henna leaves, flowers, seeds, stem bark and roots exihibited antimicrobial, anti-cancer and anti-inflammatory properties (Chaudary *et al*., 2010).

Phytochemical screening of different plants of *L. inermis* showed that large variety of compounds have been isolated including hapthoquinone derivatives phenolic compounds

Table 1: Antibacterial Activity of *Lawsonia inermis* (Henna)

S.No.	Bacteria	Zone of inhibition (mm in diameter)				
		Water	Ciprofloxacin	Aqueous	Ethanol	Methanol
1.	*E. coli*	—	14±1	13±1	12±1	13±1
2.	*Stathylococus aureus*	—	19±1	27±1	15±1	17±1
3.	*Vibrio cholerae*	—	32±1	37±1	32±1	26±1
4.	*Vibrio parahaemolyticus*	—	30±1	32±1	26±1	22±1

Value are mean ± standard deviation.

terpounds, sterols, tannins, xanthones, coumarin, fatty acids, amino acids and other volatile constituents. Suleiman and Mohamed (2014) reported that henna plants possesses good antimicrobial activity against tested fungi the obtained results demonstrated antifungal activity of both extract the cup-agar diffusion method reveated antifungal activity of the extract yeasts and mound by area of inhibition of growth revealed antifungal activity against dermatophytes.

The results of the antibacterial screening of different solvent extract of *L. inermis* leaf revealed significant antibacterial activity against all tested bacterial strains (Mastanaiah *et al*., 2011). The order of potency of the three extracts are in the following order methanol>chloroform>hexane. The methanol extract exhibited better anti-bacterial activity against *Bacillus subtilis*, *Streptococccus salivarius*, Micrococcus, *Klebsiella pneumonia*, *Streptococcus aureus* as compared to standard antibiotic. Henna contains *Lawsonne* in about 0.5 to 1.5% of its ingredients. *Lawsonne* (2-hydroxynaptho quinine) is the principal constituent responsible for the dyeing properties of the plant however; henna also contains mannite, tannic acid mucilage and gallic acid. These substances are present in henna in the form of a mixture. Anti-bacterial activity may be due to three hydroxyls that have the capability to combine with the carbohydrates and proteins in the bacterial cell wall that may get attached to enzyme sites rendering them inactive. The two other types of extracts and extraction process, when compared with methanol extract, the hexane and chloroform extracts precipitate less quantity of active constituents during extraction of these extracts are also very inhibitory concentration of these extract act against *Streptococcus aureus* but only at high concentrations. It is very effective against *Bacillus subtilis*, *Streptococcus salivarius* when compared to other organisms. The chloroform extract is more effective when compared to hexane extract and it shows maximum effect against *S. salivarius, Staphylococcus epidermis*. The methanol extract is very effective against all organisms when compared to other extracts. Thus the present study on the antibacterial activity of *Lowsomia inermis* showed maximum zone of inhibition in *V.cholera*. The study also showed that the plant possesses appreciable antibacterial activity of animal and human pathogen.

4. REFERENCES

1. Arun, P., Purushotham, K.G., Johnsy Jayarani, J. and Vasanthakumari. (2010). *In vitro* Antibacterial activity and flavonoid contents of *Lawsonia inermis* (Henna). *International Journal of Pharm. Tech. Research.* **2**(2):1178-1181.
2. Chaudhary, G., Goyal, S. and Poonia, P. (2010). *Lawsonia inermis* Linn. aus A phyto-pharmacological. *International Journal of Pharaceutical Science and Drugs Research*. **2**: 91-98.
3. Chopra, R.N., Chopra, I.C., Handa, K.L and Kapur, L.D. (1958). Chopra indigenous drugs of India *in vitro* antibacterial activity and flavonoid contents of *Lawsonia inermis* (Henna). *International Journal of Pharm. Tech. Research.* **2:**1179-1181.
4. Goun, E., Gunningham, G., Chu., D., Nguyen, C. and Miles, D. (2003). Antibacterial and antifungal activity. *Journal of Mycology.* **74**(6):592-596.
5. Kelmanson, J.E. Jages, A.K. and Stoden, JV. (2002). Medicinal plants with antibacterial activity. *Journal Ethnopharm.* pp. 241-246.
6. Misra, S.K. and Sahu, K.C. (1977). Screening of some indigenous plants for antifungal activity. *International journal of Microbiology Research.* **9**:269-272.
7. Nair, R. and Chanda, S. (2005). Antibacterial activity of *Punica granatum* in different solvents. *Journal of Pharm.* **67**:239-243.
8. Obeide, M., Shatnawi, M. and Al Alawi. (2012). Antimicrobial activity of crude extracts of some plant leaves. *Research Journal of Microbilogy*. **7**(1):59-67.

9. Sarma. (2015). Antibacterial activity of *Lawsonia inermis*. *The Beats of Natural Sciences.* **2**(1-6).
10. Suleiman and Elbasheir Ahmad Mohamed. (2014). *In vitro* activity of *Lawsonia inermis* (Henna) on some pathogenic fungi. *Journal of Mycology*. **31**(377-382).
11. Chandrakala, N., Sampathkumar, G. and Karpagam, N. (2003). Isolation and screening of pathogenic bacteria from wound sample. *Indian Journal of Applied Microbiology*. **3**(1):5-6.
12. Bauer, A, Kirby, W.M.M. and Sherris, T.C. (1962). Anitibiotics susceptibility testing by a single disc method, *Am. J. Clinpathol*. **45**:493.
13. Rao, D.P.C. and Satyanarayan, T. (1977). Antibacterial activity of some medicinal plant extract. *Indian Drugs Pharm. Ind.* **12**(3):21-22.
14. Mastanaiah, J. and Varaprasad, B. (2011). *In vitro* antibacterial activity of leaf extracts of *Lawsonia inermis*. *International Journal of Pharm. Tech. Research.* **3**(2):1045-1049.

Physicochemical and Preliminary Phytochemical Analysis of Seeds of *Sambucus wightiana* Wall. ex Wight & Arn.

Anjum Parveen*, Nausheen Ghaffar and Shabir Ijaz

Centre for Plant Conservation, University of Karachi, Karachi-75270, Pakistan.
*Email: anjum_tahir@hotmail.com

Abstract

The phytoconstituents contribute for manufacturing many drugs, helpful for treating different diseases. *Sambucus wightiana* belonging to family sambucaceae traditionally used for its purgative, dropsy, diuretic and diaphoretic properties. The aim of the present study was to introduce a new species of Sambucus and investigate the physicochemical analysis as ash values (Acid soluble and water soluble), extractive values and screening of various phytochemicals (Alkaloid, carbohydrate, protein, flavonoid, tannins, saponins and fixed oil) of seeds of *Sambucus wightiana* in four different solvents (Acetone, chloroform, methanol and water). Moisture content of seed was 29.9%, total ash value were 17.4% while water and acid soluble ash were 37%, 27%, respectively. The maximum extractive value was observed in acetone while the minimum for methanol. Phytochemical investigations revealed that alkaloid is absent in all solvents extracts, whereas carbohydrate and protein were detected in methanol and water extract. The phenolic compound, flavonoids and terpenoid obtained in chloroform, methanol and aqueous extracts. Fixed oil was also present in high amounts.

Keywords

Ash value, Physicochemical, Phytochemical, Phytoconstituents, Seed, *Sambucus wightiana.*

1. INTRODUCTION

Plants have been used for thousands of years by people in China, India and many other countries as traditional medicine (Sneader, 2005). According to the World Health Organization, 80% of the world's population uses plant-based remedies as their primary form of health care. Secondary metabolites are chemicals synthesized in most parts of plant including bark, leaves, stems, root, flower, fruits, seeds, etc. which have little need for them (Charles *et al*., 2013).

Plants always have been a source of important phytochemical agents such as alkaloids, carbohydrates, flavonoids, terpenoid, glycoside, a phenolic compound (Edoga *et al*., 2005). These phytochemical constituents of medicinal plants are helpful for healing as well as for curing human diseases (Nostro *et al.,* 2000).

S. wightiana Wall. belonging to family Sambucaceae, widely distributed in Asia and Europe. In Pakistan it is represented by

2 species *Sambucus wightiana* and *Sambucus nigra*. *Sambucus wightiana* grows wild and commonly found in inner Himalayan ranges from 2000 to 7500 m. The plant is 1-1.5 m tall, herb, leaves are compound, leaf shaped like narrowly lanceolate stipules. Flowers are whitish yellow, minute 5 mm in diameter. The fruit is a drupe, globose, 4-5 mm diameter (Nasir, 1978).

Chemical characterization and phytochemical studies of plants have been reported by several workers (Al-Tameme *et al.,* 2015) leading to idenfication of 15 bioactive compounds in the methanolic extract of *Urtica dioica*. *Sambucus wightiana* used for the treatment of skin diseases (Chashoo *et al.,* 2012). Antimicrobial studies of *S. wightiana.* Its leaves, roots and berries have purgative effects. The decoction of root and inner bark also reported as an effective diuretic. The whole plant is medicinally important having anti-inflammatory, diaphoretic, hypotensive, expectorant and diuretic activities (Kumar *et al.,* 2009). Since there is no published scientific information on *S. wightiana* from Kashmir, Pakistan regarding seed morphology, physiochemical and phytochemical studies therefore the present study was design to address it.

2. MATERIALS AND METHOD

2.1. *Collection of Plant Material*

Seeds (150 g) of *S. wightiana* were collected from Kashmir. Herbarium sheet was deposited in the Karachi University Herbarium, Centre for Plant Conservation. Healthy plants were chosen with high quality seedpods and fruits for seed collection.

2.2. *Macroscopic Study*

Morphological studies were carried out from the fresh and dried seeds (Fig. 1) by observing color, shape, odour size and weight under the stereo microscope.

2.3. *Physicochemical Analysis*

S. wightiana seeds (100 g) were ground well into fine powder using electric blender. Physicochemical analysis including moisture content as per standard method of (Gupta, 1984), total ash content, physical state and color, ash values (acid insoluble ash and water soluble ash) as per standard method of (Gupta, 2003 and Indrayan *et al*., 2005) were conducted.

2.4. *Extractive Value*

Powdered sample (20 g) was soaked in different solvents (200 ml) *viz* acetone, chloroform, methanol (BDH Labortary Supplies) and water separately and placed for continuous shaking. After 48 h extracts was filtered with the help of Whatmann No.1 paper and left for evaporation till all the solvent evaporated. Dried material was collected and extractive value was calculated. Sample was weighed with the help of electrical weighing machine (Sartorious TE214S).

2.5. *Phytochemical Analysis*

Powdered seeds samples (20 g) were soaked in different solvents and placed for continuous shaking for 48 h in shaker followed by. It was filteration evaporation. The different extracts obtained were subjected to identification of various phytochemical (Harborne, 1998) as described below:

2.5.1. *Alkaloids*

Extracts (10 m) were dissolved individually in 2N HCl (4 ml) and filtered. After filtration the sample was used to test alkaloids (Evans, 1997).

i) *Wagner's Test*

In 2 ml of filtrate, added 2-3 drops of Wagner's reagent (0.63 g iodine and 1 g

potassium iodide in a little water volume made up to 50 ml with distilled water). Formation of reddish brown precipitation indicated the presence of alkaloids.

ii) *Mayer's Test*

In 1 ml of filtrate few drops of Mayer's reagent (5 g potassium iodide dissolved in 10 ml water and 1.358 gm mercuric chloride dissolved in 60 ml water, both solutions were mixed with water up to 100 ml). Dense white precipitation confirms the presence of alkaloids.

2.5.2. *Carbohydrates*

Extract (0.1 g) was dissolved in water (2.5 ml) and filtered with the help of filter paper. The filtrate was used in the following tests (Ramakrishan *et al.,*1994).

i) *Benedict's Test*

In a test tube 1 ml of solution and 1 ml of Benedict's reagent (17.3 g sodium citrate 10 g sodium carbonate dissolved in 80 ml distilled water) and boiled to make clear. copper sulphate (1.73 g) dissolved in distilled water (10 ml) was added. The mixture was heated on a water bath for 2-3 min. formation of precipitation indicated the presence of carbohydrate.

2.5.3. *Amino Acid and Protein*

Extract (100 mg) was dissolved in distilled water (10 ml). Filtered with the help of filter paper, filtrate was used to test for protein and amino acid.

i) *Biuret Test*

The filtrate (3 ml) was treated with 2 drops of copper sulphate solution and ethanol (2 ml 95%). Followed ny addition of several pellets of potassium hydroxide. Pink coloration in ethanolic layer confirmed the presence of proteins.

ii) *Millon's Test*

To filtrate (2 ml) few drops of Millon's reagent (Mercury 1 g dissolved in fuming nitric acid 9 ml, after completion of reaction added equal volume of distilled water) were added. White precipitation showed the presence of protein.

2.5.4. *Phenolic Compound*

i) *Lead Acetate Test*

Extract (50 mg) was dissolved in distilled water (5 ml) and (3 ml of 10%) lead acetate solution. Formation of precipitation confirmed the presence of phenolic compounds (Evans, 1997).

ii) *Ferric Chloride Test*

Filtrate (2 ml) was treated with ferric chloride solution (2 ml 10%). Dark brownish green color indicated the presence of phenolic compounds.

2.5.5. *Flavonoids*

Aqueous filterate (1 ml) was treated with ammonia (2 ml 10%) and concentrated H_2SO_4 (1 ml). Development of yellow color confirmed the presence of flavonoids (Ramakrishan *et al.,*1994).

2.5.6. *Glycosides*

i) *Borentrager's Test*

Filterate (2 ml) chloroform (3 ml) was mixed and shaken, then ammonia (1 ml of 10% solution) was added. Pink coloration indicated the presence of glycosides.

2.5.7. *Terpenoids*

Extract (0.5 gm) was mixed with 2 ml

chloroform then added H_2SO_4 (3 ml). Appearance of brownish red color indicated the presence of terpenoid (Evans, 1997).

2.5.8. *Saponin*

Extract (0.5 gm) was dissolved in autoclave distilled water (20 ml), then shaken continuously for 15-20 min. Formation soapy layer indicated the occurance of saponins.

2.5.9. *Fixed Oils (Spot Test)*

A small quantity of dried powdered seed samples was pressed between two filter papers. Oil spot on the paper indicated the existence of fixed oils.

3. RESULTS AND DISCUSSION

3.1. *Seed Morphology of Sambucus wightiana Wall. ex Wight & Arn.*

Seeds of *Sambucus wightiana,* yellow in color, weighing (1.3 mg) in elliptic in shape plano convex. Hilum was basal, 0.25 mm with circular outline.

3.2. *Ethnobotanical Uses*

The plant emits a very irritating odour when bruised. The roots, leaves and berries are used for their purgative properties in dropsy and other ailments presumably possesses other properties attributes to the plant. A decoction of the root or inner bark of the dwarf elder is an effective diuretic. Leaves are expectorant, diuretic, diaphoretic, and purgative, root and berries are used in dropsy and act aspurgative (Shinwari, 2006).

3.3. *Physicochemical Analysis*

Physicochemical analysis of the seeds of *Sambucus wightiana* revealed the total ash value as 17.4%, water soluble ash was 37%, acid insoluble as 73%. Moisture content and extracting values in different solvent also evaluated and results shown in Table 1.

Table 1: Physicochemical Analysis of Seeds of *Sambucus wightiana* Wall.

S.No.	Parameters	Inference
1.	Physical state of ash	Fine powder
2.	Colour of ash	Off white
3.	Loss on drying	29.9%
4.	Ash content	17.4%
5.	Water soluble ash	37%
6.	Water insoluble ash	63%
7.	Acid soluble ash	27%
8.	Acid insoluble ash	73%
9.	Acetone soluble extractive value	15%
10.	Chloroform soluble extractive value	11%
11.	Methanol soluble extractive value	8%
12.	Water soluble extractive value	14%

3.4. *Phytochemical Screening of Seeds of Sambucus wightiana Wall. ex Wight & Arn.*

Phytochemical analysis of seed extracts of *Sambucus wightiana* showed that fixed oil and protein were present in acetone, methanol, chloroform and water extracts. While terpenoids, phenolic compound and flavonoid were present in chloroform, methanol and water extracts. Glycoside was not detected in any extracts. Results shown in Table 2.

Table 2: Qualitative Phytochemical Screening of Seeds of *Sambucus wightiana* using Different Reagents

S.No.	Phytochemical test	Acetone	Chloroform	Methanol	Water
1.	Alkaloid				
	Wagner's reagent	–	–	–	–
	Mayer's reagent	–	–	–	–
2.	Carbohydrate				
	Benedict's test	–	–	++	++
3.	Protein and amino acid				
	Biuret test	–	–	+	+
	Millions test	–	–	+	+
4.	Phenolic compounds				
	Lead acetate	–	+	++	++
	Ferric chloride test	–	++	++	++
5.	Flavonoids	–	+	+	+
6.	Glycoside				
	Borntrager's test	–	–	–	–
7.	Terpenoids	–	++	++	++
8.	Saponin				
	Foam test	–	–	–	–
9.	Fixed oil				
	Spot test	++	++	++	++

Present (+) and Absent (–)

Macromorphological studies showed the seeds of *Sambucus wightiana* was 3x1.5 mm, 0.0013 gm, elliptic in shape, yellow, plano convex, hilum 0.25 mm, basal, circular in outline (Figs. 1A and 1B). *Physiochemical analysis of Sambucus wightiana* seeds are listed in Table 1. Ash represents the inorganic part of the plant due to ashing all the organic material present in sample destroyed. The ash was found as fine powders, off white in color. Moisture content of seed was 29.9%, total ash value was 17.4%. 37% ash was soluble in water, 27% ash was soluble in acid. The maximum extractive value 14% was observed in acetone while the minimum 8% was calculated in methanol.

Results of phytochemical analysis of *Sambucus wightiana* seeds of different solvent extracts confirmed the presence of phytochemicals, which considered as major medicinal constituents (Table 2). The medicinal plants showed healing properties due to the presence of various secondary metabolites such as alkaloids, glycosides, flavonoids, phenols, saponins etc. (Nobori *et al*., 1994), although in this research we could not find the glycosidal components specially in aqueous seeds extract.

Chashoo (2012), also analyzed

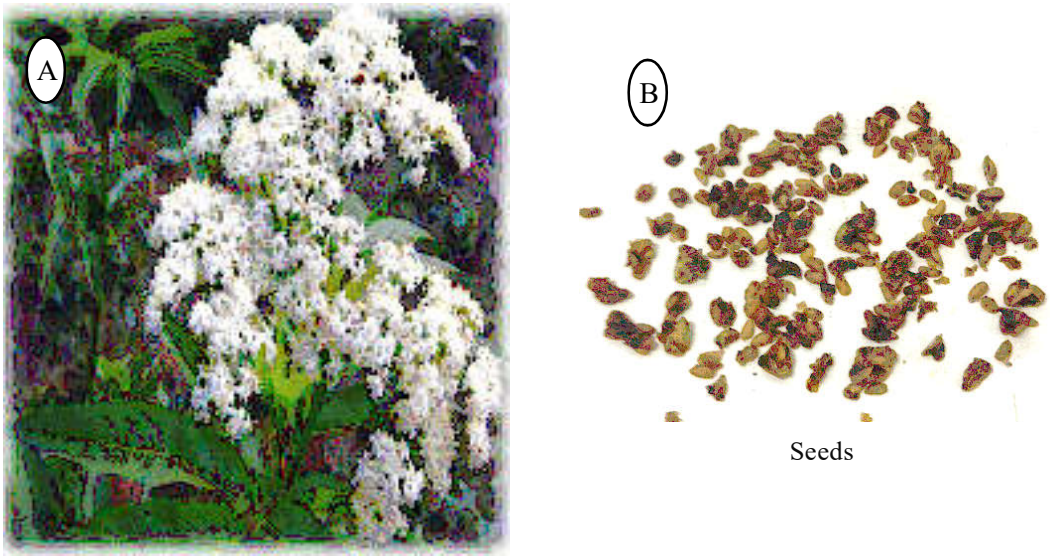


Fig. 1 *Sambucus wightiana*

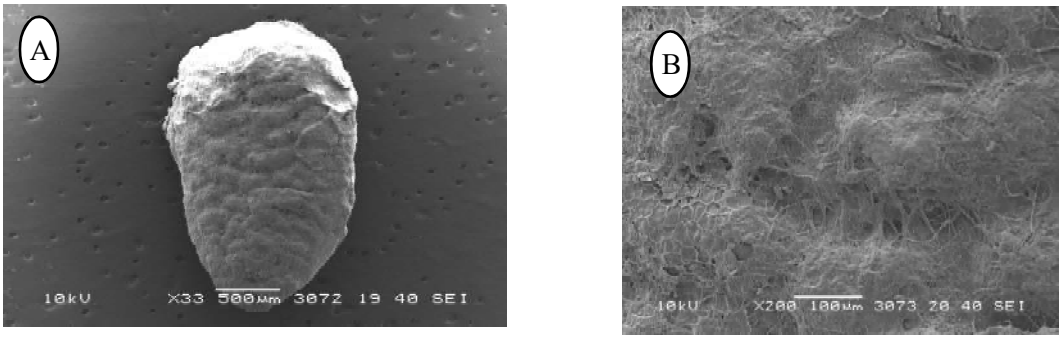


Fig. 2: Scanning Micrograph: *Sambucus wightiana*

A) Entire seed (500 µm) and its B) Surface view [Scale bars: (100 µm)]

phytochemical constituents in root and leaves and antimicrobial activity of *Sambucus wightiana,* and also reported that proteins, carbohydrates, cardiac glycosides were detected in Petroleum ether, chloroform, ethyl acetate, methanol and aqueous extract. Phytochemical investigations revealed alkaloid is absent in all solvents extract, carbohydrate and protein detected in methanol and water extract. Several studies revealed that those plants containing phenolic compounds, showed antioxidant properties (Brown and Evans, 1998; Krings and Berger, 2001). A phenolic compound, flavonoids and terpenoids are strongly present in methanol, chloroform and aqueous extract. Terpenoid has also the property to decrease the blood

sugar level. Flavonoid has anti-allergic and anti-microbial properties (Aiyelaagbe and Osamudiamen, 2009). Fixed oil was present in high amount. Results showed the ideal solvent for extraction from seed is methanol and water which give maximum results.

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4. REFERENCES

1. Aiyelaagbe, O.O. and Osamudiamen, P.M. (2009). Phytochemical screening for active compounds in *Mangifera indica. Asian Journal of Plant Science and Research.* **2**(1):11-13.
2. Al-Tameme, H.J., Hadi, M.Y. and Hameed, I.H. (2015). Phytochemical analysis of *Urtica dioica* leaves by fourier-transform infrared spectroscopy and gas chromatography-mass spectrometry. *J. Pharm. and Phytotherapy.* **7**(10):238-252.
3. Brown, J.E. and Rice-Evans, C.A. (1998). Luteolin rich artichoke extract protects low density lipoprotein from oxidation *in vitro. Free Radical Res.* **29**:247-255.
4. Charles, S. U., Uche, A.I. and Ifeanyi, O. (2013). 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):10-13
5. Chashoo, I.A., Kumar, D., Bhat, Z.A., Khan, N.A., Kumar, V. and Javaid, A. (2012). Antimicrobial studies of *Sambucus wightiana* Wall. ex. Wight & Arn. *J. Phar.* **5**:2467-2468.
6. Edoga, H.O., Okwu, D.E. and Mbaebie, B.O. (2005). Phytochemicals constituents of some Nigerian medicinal plants. *Afr. J. Biotech*. **4**(7): 685-688.
7. Evans, W.C. (1997). *Trease and Evans Pharmacognosy.* Harcourt Brace and Company. Asia Pvt. Ltd. Singapore.
8. Gupta, S. (1984). *The Ayurvedic System of Medicine Occurring in Charka, Sushruta.* Neeraj Publishing House, New Delhi, India, **II**.
9. Gupta, A.K. (2003). *Quality Standards of Indian Medicinal Plants*. Indian Council of Medical Research, India. **I**.
10. Harbone, J.B. (1998). *Phytochemical Methods*. London, Chapman and Hall, Ltd. 49-188.
11. Indrayan, A.K., Sharma, S., Durgapal, D., Kumar, N. and Kumar, M. (2005). Determination of nutritive value and analysis of mineral elements for some medicinally valued plants from Uttaranchal. *Current Sci.* **89**:1252-1255.
12. Krings, U. and Berger. R.G. (2001). Antioxidant activity of roasted foods. *Food Chem*. **72**:223-229.
13. Kumar, M., Paul, Y. and Anand, V.K. (2009). An ethnobotanical study of medicinal plants used by the locals in Kishtwar, Jammu and Kashmir, India. *Ethno. Leaflets.* **13**:1240-1256.
14. Nasir, E. (1978). Sambucaceae. In: Nasir, E. and Ali, S.I. (Eds.). *Flora of Pakistan*. Department of Botany. University of Karachi. **123**:1-4
15. Nobori, T., Miurak, K., Wu, D.J., Takabayashik, L.A. and Carson. D.A. (1994). Deletion of cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. *Nature*. **46**:753-756.
16. Nostro, A., Germano, M.P. Dangelo, V., Marino, A. and Cannatelli, M.A. (2000). Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Lett App. Micro.* **30**:379-384.
17. Rhamakrishnan, S., Prasanan, K.G. and Rajin, R. (1994). *Text Book of Medicinal Biochemistry.* Orient Longman, New Delhi, India.
18. Shinwari, Z.K., Rehman, M., Watanabe, T. and Yoshikawa, Y. (2006). *A Pictorial Guide to Medicinal Plants of Pakistan*. Book Published by Kohat University of Science & Technalogy, Kohat.
19. Sneader, W. (2005). *Drug Discovery: A History*, Wiley, Chichester, UK.

Aspects Responsible for the Beginning of Smoking Habits Among University Students

Tahseen Ahmed*[1], Ambreen Huma[2], Waqar Ahmed[1], Ayaz Unar[1], Bilawal Shaikh[1] and Irfan Ali Solangi[3]

[1]Department of Pharmacy, SMBBMU Larkana,
[2]Faculty of Pharmacy, Zia ud Din University, Karachi,
[3]Department of Pharmacy, BBSU Lyari, Karachi, Pakistabn.
Email: tahseen.channa89@gmail.com

Abstract

The theme of this research is to get knowledge regarding the frequency of Smoking habits and its prevalence among university students and the aspects that arouse students to smoke cigarette and that might stimulate their smoking habits. A Cross-sectional study was conducted to determine the factors associated with starting of smoking cigarette among university students. A questionnaire was filled by the students of university and data regarding their socio-economic values, demographic data, smoking stimulating factors, smoking status and its risk factors along with appearance of various disorders was addressed.

Total 138 students had participated in this study among them 87 were smokers. The age of study subjects was between 18 to 30 and the prevalence rate of cigarette consumption was 28%. The prevalence rate affected by the study year of the participants mostly senior students consume tobacco at their friends' residency. Coughing issue was observed 23% among smokers whereas problem of Asthma was only 6%. People with higher literacy rate and awareness regarding serious hazardous effect of tobacco were non-smokers. The main theme to initiate this fetal habit was just to get entertainment. The smoking habits among university students was very low as compare to society and community population but this is alarming that our youth is heading towards crisis effecting their health and economy.

1. INTRODUCTION

Health is major threat worldwide; the world is advancement in technologies and techniques in the field of medicine and health but at the same time health become major issue globally and the diseases are spread quickly and people become resistant to majority of medicines (Bricker *et al.,* 2006). Our environment is polluted with dirty air and various dangerous gases, which lead to fetal disease such as cardio vascular disease, lungs disease (Tyas and Pederson, 1998). It is assumed that about 8 million peoples are smokers, which is having bad impact on the health ultimately enhance the economic burden on our society (http://www.cdc.gov/tobacco/overview/tobus_us.htm). A survey elaborates that about 82% of smokers belong to either low

socioeconomic regions of the world or developing countries where the rate of death is more as compared to developed countries[http://www.cdc.gov/tobacco/global/GYTS/factsheets/emro/2003/pakistanlahore_factsheet.htm] may be due to establishment of rehabilitation center to control the smoking habits or the majority of population not consuming tobacco (Warren, *et al.,* 2006). According to report it is estimated that almost 3.7% expenditure rate is increasing in the developing countries on smoking (Anonymous, 1994). Africa and Mediterranean countries consume more expenses on the production of tobacco and the tobacco companies of these regions facilitate the young children and females to initiate smoking eventually enhances the risk factors for hazardous disease such as Cancers, Cardio vascular Disease (CVD), lungs Disease, Hepatitis and Diabetee (WHO, 2015; Rudatsikira *et al.,* 2008). The frequency of tobacco consumption is increasing progressively and chances of promoting communicable and non-communicable diseases amplified thrice time with severity. An organization with the collaboration of WHO works on the Global Youth Tobacco Survey (GYTS) to sort out the factors responsible for the starting of tobacco consumption in young age and its economic burden on their families. A combined, report issued by the WHO, on 132 countries is described in such a way that 8.9% peoples initiate consumption of tobacco in University and College life (WHO, 2015). Students with this habit don't know the benefits and harmful aspects of this hazardous habit. The dominance of this habit is very high in some American states and European countries. As far as Pakistan is concerned, the occurrence of this habit is quite different with respect to gender, 36% males are involved in smoking whereas only 9% females take part in smoking. From overall population of Pakistan, the frequency of smoking is 15% among university and college students, these students don't know the actual cause of starting tobacco consumption. According to their behaviors and social characteristics they realize that smoking habit enhances their personality outlook (Shafquat *et al.,* 2007; Murry *et al.,* 1983). According to research almost 1200 new students started smoking or tobacco consumption on daily basis. This burning issue affects the finance and health of youth. The theme of starting tobacco among youth is not plausible. The reason behind this fetal habit is depends on biological, psychological, genetic, economic and social values (Lee *et al.,* 2012). From all these factors Social and environmental issues are very common because new smokers initiated this habit by replication the action of their relatives, friends and family. Family is major cause to startup smoking if father is smoker than chances of smoking among their children increases 50%. Cigarette consumption among university students affects health and economy of our youth simultaneously of new generations of the globe (Jackson, 1998; Warren *et al.,* 2006).

GYTS (Global Youth Tobacco Survey) is conducted in various regions of Pakistan with majority of students. Even school boys were also included and priority was given to those university and school where this habit was very common. The main theme of our study to educate our youth regarding precarious effects of smoking even government of Pakistan also participated in this program to make the country free from this fetal habit of smoking.

2. MATERIAL AND METHOD

A cross-sectional study was conducted for the period of 07 Months from August 2017 to February 2018 at Shah Abdul Latif University (SALU) Khairpur, Pakistan. A questionnaire

was made comprising of 2 parts with measuring scale. First part of that questionnaire comprises of demographic data while another part elaborates frequency of smoking, reasons for starting smoking, motivational factors, smoking place, status of smoking among family members and friends, hazardous effect of smoking and risk factor associated with smoking and socio-economic value of participants. Fagerstrom scale was adopted to measure the severity of nicotine dependence, Scale evaluate the various ranges of addiction of nicotine. From 3 to 6 points scale evaluated the average addiction whereas from 7 to 10 points the nicotine addiction was on the peak level. Status of smoking was set as dependent variable. Verbal communication was also made with each participant to make them know why this study was conducted and what was the theme of study and privacy of each study subject was esteemed. Stratified Cluster sampling techniques was adopted to get demographic data from students. Each batch was considered as a cluster, from all batches total number of 138 students were selected and data was analyzed.

Statistical Analysis

Whole data was analyzed by latest statistical software SPSS 24.00 and p-value of 0.05 was considered as Significant.

3. RESULTS AND DISCUSSION

From total number of 138 study subjects were included in this study from all 56 (40.5%) were consumed tobacco on daily basis with the

Table 1: General Characteristics Of Smokers Study Subjects

Parameters	Variable	Number and Percentage
Gender	Male	87 (63.04%)
Age wise distribution of study subjects	18- 20	07 (8.04%)
	21- 23	23 (26.43%)
	24- 26	41 (47.12%)
	27- 30	16 (18.39%)
Batch wise distribution of particpants	1st Year	03 (3.44%)
	2nd Year	31 (35.63%)
	3rd Year	34 (39%)
	4th or Final Year	19 (21.83%)
Smoking habits	Yes	87 (63.04%)
	No	51 (36.95%)
Knowledge of harmful effects of smoking	Yes	33 (37.93%)
	No	54 (62.06%)
Repeated class of smoking	Non-smoker	51 (36.95%)
	Regular Smoker	63 (45.65%)
	Occasionally	24 (17.39%)

age from 18 to 30 years. and 78 (56.5%) participants were living with their family, 34 (24.6%) were hostelers whereas 26 (18.8%) participants were living alone. Year of study also affect the status of smoking which vary from year to year and the rate of smoking habit was increased with the seniority base and theme for initiation this fatal habit was to get enjoyment without knowing the side and adverse effects of smoking on the human health.

3.1. *Cigarette Smoking Performance*

It was observed that mostly smoking habits was initiated by the study subjects at various functions or events either in university or at their residency with family or friends. Statistically it was proved that Non-smoker were more aware regarding serious outcomes of smoking cigarette as compared to Smokers as 6% of smokers were having asthmatic problem where as 23% of smokers were severe coughing during this habit.

3.2. *Smoking Status of Participants*

Concerning statistics of smoking, 63 participants were regular smokers whereas 24 participants were occasional smokers. Nicotine dependence was also differed in both types, regular smokers were highly reliant on nicotine whereas sporadical smokers had moderate to low nicotine addiction.

3.3. *Purpose for Smoking*

Various aims and reasons were evaluated among study subjects regarding initiation of smoking cigarette including stress 9 (10.34%), failure in love or examination 29 (33.33%), pleasure and fun 32 (36.78%), personal life problems 04 (4.5%) and copying friends 13 (14.94%).

Fagerstrom Score	**Percentage (%)**
Low scale (0-3)	07 (8)
Moderate (4-6)	17 (19.54)
High (7-10)	63 (72.41)
Place of Smoking	
At home	03 (3.44)
At hostel	17 (19.54)
University canteen	16 (18.39)
Together with smokers	37 (42.52)
During reactive parties	14 (16.09)
Reason of Smoking	
Stress	9 (10.34)
Failure in exam/love	29 (33.33)
Pleasure and fun	32 (36.78)
Personal life problems	04 (4.5)
Copying friends	13 (14.94)

Nowadays, it is clearly sumptuous that cigarette smoking has hazardous effect on human health. Most of the people even knew the impact of smoking on human health they started this habit without any hesitation and the persistence of smoking habits was more common among youth as compared to older or adult person. This study showed the alarming situation to health department as smoking is more prevalent in youngsters that are unaware of its impact on health as well as on the economy of the country. From 138 study subjects 87 were regular smokers with prevalence ratio of 40.19%. The measuring scale for the nicotine dependence elaborates that 72.41% were highly dependent, 19.54% were moderately dependent whereas 8% were having minor dependence level of nicotine. Current research particularizes that regularity of cigarette consumption was

26.9%. The frequency is quite below than the previous researches conducted at various parts of the world such as in Italy the persistence rate was 29.0% and a study was conducted in American society with frequency rate of 23.5%. A Sweden researcher elaborates the smoking frequency of 18% at their society which was quite stimulting factor different from recent study. The main stimulating factor for initiation of smoking among university population were friends. Mostly senior students smoke cigarette because of various reason such as failure in exam, stress, burden of study, fun or entertainment (Lee *et al.,* 2012). Besides these factors another reason for smoking was the family history, if parents were smokers than it optimizes to adopt smoking habit among children. From recent study it was analyzed that smoking practices was enhanced with age factor highlighting that maturity claims highly nicotine dependent situation (Shadid *et al.,* 2015). Current studies mostly emphasized on those participants who had smoked on special events or functions. Company of smokers intiates a chain of smokers among new gatherings which had impose on human health and economy of the society. Various researchers from different areas of the world stated the same thing that reason for new smoker just only their friends not any other factor. Research on American society elaborate that if a person is smoker than his company must startup the same habit if they remain in touch with him (Murry *et al.,* 1983; Scragg *et al.,* 2003). Parental smoking is major threat for initiation of tobacco consumption Bricker *et al*, 2006 concluded in his cohort study which was summarized on 05 thousand families that parental smoking has major influence on the children smoking.

4. CONCLUSION

According to a study conducted in various developing countries that children always adopt the characteristics of their family where they grow. Scragg *et al.,* (2003) concluded that if a person smokes cigarette at their home than it causes the bad impression to their children. If a person living in joint family or as a paying guest where smoking is very common than it seems a risk factor for new smokers. Recent study helps to develop new policies and social practices to eradicate this habit by enforcing the academic areas of the country free from smoking as new students or youth of nation save from perilous impact of smoking. Ultimately this research develops the mainstay for upcoming research and also support to vindicate the desires for superior and more erudite trails on smoking being used by our youth.

5. REFERENCES

1. Bricker, J.B., Peterson, Jr A.V., Leroux, B.G., Andersen, M.R., Rajan, K.B. and Sarason, I.G. (2006). Prospective prediction of children's smoking transitions: Role of parents' and older siblings' smoking. *Addiction.* **101**(1):128-136.
2. Bricker, J.B., Peterson, Jr. A.V., Andersen, M.R., Rajan, K.B., Leroux, B.G. and Sarason, I.G. (2006). Childhood friends who smoke: do they influence adolescents to make smoking transitions? *Addict Behav.* **31**(5):889-900.
3. Centers for Disease Control and Prevention: Tobacco use in the United States. Available from: http://www.cdc.gov/tobacco/overview/tobus_us.htm
4. Global Youth Tobacco Survey (GYTS) Pakistan Fact Sheet: Centers for Disease Control. [http://www.cdc.gov/tobacco/global/GYTS/factsheets/emro/2003/pakistanlahore_factsheet.htm]. Accessed February 28, 2006.
5. Jackson, C. (1998). Cognitive susceptibility to smoking and initiation of smoking during childhood: A longitudinal study. *Prev. Med.* **27**:129-134.
6. Lee, S., Ling, P.M. and Glantz, S.A. (2012). The vector of the tobacco epidemic: tobacco industry practices in low and middle-income countries. *Cancer Causes Control.* **1**:117-129.

7. Murry, M., Swan, A.V., Johnson, M.R., Bewley, B.R. (1983). Some factors associated with increased risk of smoking by children. *J. Child. Psychol. Psychiatry.* **24**:223-232.
8. Rachiotis, G., Muula, A.S., Rudatsikira, E., Siziya, S., Kyrlesi, A., Gourgoulianis, K. *et al.* (2008). Factors associated with adolescent cigarette smoking in Greece: results from a cross sectional study (GYTS Study). *BMC Public Health*. **8**:313.
9. Rudatsikira, E., Dondog, J., Siziya, S. and Muula, A.S. (2008). Prevalence and determinants of adolescent cigarette smoking in Mongolia. *Singapore Med. J.* **49**(1):57-62.
10. Scragg, R., Laugesen, M. and Robinson, E. (2003). Parental smoking and related behaviours influence adolescent tobacco smoking: results from the 2001 New Zealand national survey of 4th form students. *N.Z. Med. J.* **116**(1187):707.
11. Shadid, H.M. and Hossain, S.Z. (2015). Smoking behaviour, knowledge and perceived susceptibility to lung cancer among secondary-school students in Amman, Jordan. *East Mediterr Health J.* **21**(3):185-193.
12. Shafquat, R., Zahid, A.B. and Saeed, A. (2007). Correlates of cigarette smoking among male college students in Karachi, Pakistan, *BMC Public Health.* **7**:312-318.
13. Tyas, S.L. and Pederson, L.L. (1998). Psychosocial factors related to adolescent smoking: A critical review of the literature. *Tob Control.* **7**:409-420.
14. US Department of Health and Human Services, 1994. The Health Benefits of smoking Cessation: A Report of the Surgeon General. U.S. Government Printing Office, Washington, DC, DHHS Publication No. (CDC) 90-8416.
15. Warren, C.W., Jones, N.R., Eriksen, M.P. and Asma, S. (2006). Patterns of global tobacco use in young people and implications for chronic disease disease burden in adults. *Lancet.* **367**(9512):749-753.
16. World Health Organization. (2015). WHO Report on the global tobacco epidemic, Geneva, Switzerland, WHO.

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