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Journal of Applied Environmental and Biological Sciences (JAEBS) is a peer reviewed, open access international scientific journal dedicated for rapid publication of high quality original research articles as well as review articles in the all areas of Applied Environmental and Biological Sciences.

Scope

Journal of Applied Environmental and Biological Sciences (JAEBS) is devoted to the monthly publication of research papers of outstanding significance in the all fields of environmental sciences, environmental engineering, environmental Pollution, green chemistry, environmentally friendly synthetic pathways, alternatively fuels, environmental analytical chemistry, biomolecular tools and tracers, water and soil, environmental [management, economics, humanities], Mathematics, multidisciplinary aspects such as Business Management, Organizational Behavior, all areas of biological sciences, including cell biology, developmental biology, structural biology, microbiology, molecular biology & genetics, biochemistry, biotechnology, biodiversity, ecology, marine biology, plant biology, bioinformatics, toxicology, developmental biology, structural biology, microbiology, molecular biology & genetics, biotechnology, biodiversity and related fields. The journal presents the latest developments in the fields of environmental social marketing, environmental journalism, environmental education, sustainability education, environmental interpretation, and environmental health communication.

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ABSTRACT: Plant growth was reduced significantly by salt stress which is a serious environmental problem. Plants have different metabolites that are working in response of different biotic and abiotic stresses and salicylic acid is one of them that act as a vital compound in plants for response against different environmental stresses and modifications and it has also an important role in declining damages in plants that are caused by different stresses. Present project was designed to explore the same phenomenon of salicylic acid on *Vignaunguiculata* irrigated with different sea-salt concentrations. So, *Vignaunguiculata* seeds were grown in pots containing loamy soil in field conditions. Sea-salt concentration(0, 2.5 dS/m and 5 dS/m) and salicylic acid levels(0, 0.5mM and 1mM) were used in this experiment which is complete randomized design (CRD) and factorial experiment. Present investigation revealed reduction in root length, plant height, total fresh biomass, total dry biomass, relative water content (RWC), photosynthetic pigments(chlorophyll a, b, total chlorophyll) and proteins while increase in total carbohydrates as salt concentration increased. Results regarding presence of ions in different parts of plant showed that sodium ion level showed increase while potassium ion level showed decrease in different plant parts as sea salt concentration increased in irrigation water. Different doses of salicylic acid exhibited improvement in studied parameters under non-stressed and stresses environment.

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Siraj Khan, Gul Jan, Hameeda bibi, Kifayat Ullah, Farzana Gul, Shakir Ullah

Plants Traditional Medication in Arid and Semi-Arid Zone of Tehsil Domel, District Bannu, Khyber Pakhtunkhwa–Pakistan

ABSTRACT: The Indigenous communities of tehsil Domel, District Bannu, Khyber Pakhtunkhwa, Pakistan, mainly depend on ethnomedicinal plants, and the study was conducted with the aim to document therapeutic plants and formulae used by the local people for the treatment of different human infirmities. Ethnobotanical data was obtained by conducting many field trips, questionnaires, open-end and semi structured interviews, inquiries, and group gathering from 2016 to 2017.To evaluate the reliability and richness of herbal knowledge, the data were investigated using quantitative Ethnomedicinal indicex Relative Frequency Citation (RFC), Use Value (UV), respectively. During the survey, 49 species of medicinal plants belonging to 28 botanical families were reported to be used in traditional medicines. Lamiaceae and Solanaceae with 6 species are the dominant families, followed by Moraceae and Fabaceae. With regard to growth form, the main source of herbal medicines was herbs (47%). For remedy preparation, leaf (36%) was the most frequently utilized part. Moreover, 25% of the herbal medicines were prepared in the form of Powder and administered orally. The different plant species used by the local people in large numbers for medicinal purposes indicated that tehsil Domel is wealthy in medicinal flora and allied traditional knowledge. This shows that the indigenous people still rely on therapeutic plants for their healthcare needs, and therapeutic plants are the chief source for plants-based medicines for the inhabitants of the study area. A comprehensive data were found on general and worldwide uses of the proposed study to extract new potential species for further ethnomedicinal and/or ethnopharmacological, Phytochemical, and clinical studies.

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Jiawei Zhang, Bingjie Sun, Wenxue Li, Bo Zhang, Wei Zhu, Min Xia

Inhibition of Patulin-Induced Oxidative Stress in Human Esophageal Epithelial Cells by 3, 3'-Diindolylmethane

ABSTRACT: To understand the effects of 3,3'-diindolylmethane on oxidative stress induced by patulin in HET-1A human esophageal epithelial cells and the underlying mechanism. HET-1A cells were treated with patulin to establish an oxidative stress model. The results of the CCK-8 assay indicated that low dose of DIM shows no obviously toxic effects on the HET-1A cells. Flow cytometric analysis indicated that DIM could inhibit patulin-induced ROS production in the HET-1A cells. The Western Blotting results showed that the protein expression of phospho-p38 MAPK and phospho-SAPK in the HET-1A cells gradually decreased; In addition, phospho-NF-kB expression also decreased significantly. It can be concluded that the underlying mechanism of DIM reduced the patulin-induced oxidative stress in HET-1A cells might be the inhibition of ROS production and regulation oxidative stress-related proteins such as NF-kB and the MAPK family. These results indicated that DIM might be categorized as an effective drug for treating or delaying the injury of esophageal epithelial cells due to oxidative stress.

J. Appl. Environ. Biol. Sci. 2018 8(8): 29-34. [Abstract] [Full-Text PDF] [Full-Text XML]

Juarni Anita and Iwan Sudradjat

Housing Adjustment Phenomena in the Coastal Area of Muara Angke, North Jakarta, Indonesia

ABSTRACT: In the early 1970s, the Government of Jakarta developed Muara Angke, a delta located at the north coast of the city as a new settlement to accommodate housing facilities for the local fishery community members. Muara Angke was an area vulnerable to flooding. During the year 1977 to 2012, ten significant floods had occurred at Muara Angke, causing damages to people's houses and degrading the quality of their lives. Consequently, from time to time the residents had to adjust their houses and surrounding environments to overcome the critical conditions. The objective of this study wasto investigate the housing adjustments made by the residents in response to the threat of flood.This research was carried out using interview and field observation methods. Four housing blocks in Muara Angke, namely block H, L, K and Bermis were selected as the objects of study.120 households were purposefully selected as respondents, to represent each housing block or sub-population proportionately. The research findings show that the flood incidents had forced the residents to 1) raise the elevation of the ground floor, to avoid water intrusion into their houses, and hence to prevent any damage to the exterior as well as the interior of the house. 2) heighten the ceiling clearance, to anticipate the effect of land subsidence for many years to come. 3) build additional floor levels as family refuge area in case a flood incident occurred. Also, depending on their financial ability, the residents increase the level of privacy and improve the physical standard of their living space, by 1) expanding floor area, 2) improving spatial configuration, and 3) adding income generating unit such as rental rooms and small-shop.

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Salicylic Acid Prevents the Deleterious Impact of Salt Stress on *Vigna Unguiculata* L.

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Received: February 16, 2018
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ABSTRACT

Plant growth was reduced significantly by salt stress which is a serious environmental problem. Plants have different metabolites that are working in response of different biotic and abiotic stresses and salicylic acid is one of them that act as a vital compound in plants for response against different environmental stresses and modifications and it has also an important role in declining damages in plants that are caused by different stresses. Present project was designed to explore the same phenomenon of salicylic acid on *Vignaunguiculata* irrigated with different sea-salt concentrations. So, *Vignaunguiculata* seeds were grown in pots containing loamy soil in field conditions. Sea-salt concentration(0, 2.5 dS/m and 5 dS/m) and salicylic acid levels(0, 0.5mM and 1mM) were used in this experiment which is complete randomized design (CRD) and factorial experiment. Present investigation revealed reduction in root length, plant height, total fresh biomass, total dry biomass, relative water content (RWC), photosynthetic pigments(chlorophyll a, b, total chlorophyll) and proteins while increase in total carbohydrates as salt concentration increased. Results regarding presence of ions in different parts of plant showed that sodium ion level showed increase while potassium ion level showed decrease in different plant parts as sea salt concentration increased in irrigation water. Different doses of salicylic acid exhibited improvement in studied parameters under non-stressed and stresses environment.
KEYWORDS: Biomass, Chlorophyll, Protein, Potassium, Salinity, Salicylic acid, Sodium.

INTRODUCTION

Salt stress affects negatively the plant development, growth and productivity and it is known as serious environmental problem which badly affect most of the cultivated area of the world (20%) [1].[2] stated that physiological drought caused by high salinity as high salt concentration cause soil porosity and reduction in water potential. [3]revealed that high salt concentration also affects plants at both whole level and cellular level in terms of its physiology. High concentration of salt had negative impact on plants either by osmotic stress or toxic levels of specific ion [4]. Occurrence of high concentration of sodium and chloride ions in the soil solution cause osmotic stress which leads to decrease in availability of water to roots. During salinity stress condition presence of high salt levels in soil force the plant roots to absorbed high concentrations of sodium and/or chloride ions and then moved towards leaves where they stored at alarming level. Nutrient deficiency especially potassium ion nutrition and ion imbalances can also be occur under salt stress[5].

Different phenolic compounds that produced in plants are now considered as hormone (endogenous growth regulator)and one of them is known as salicylic acid (SA). It has been well documented that this compound has positive role in defense mechanism of plant against different types of stress factors (biotic and abiotic)[6, 7]. This compound act as antioxidant (non-enzymatic) and had vital role in different physiological processes regulation of plants including photosynthesis and known as plant growth regulator[8, 9, 10, 11]. Many researchers studied different plants under different stresses and observed the deleterious effects and then amelioration of these effects after exogenous application of salicylic acid, like [12] studied rice under heavy metal stress while [11] studied wheat under salt stress. When plants grown under different stress conditions and then foliarly applied with salicylic acid it can enhance the salinity and drought stress resistance of these plants [13, 14].

Vignaunguiculata sub sp. *unguiculata* (L.) Walp. (Cowpea) is a member of Fabaceae family and known as coope, black eyed pea, coope, southern peas, yard long pea, lobia, china pea, niebe or frijol worldwide. Height of this plant reaches up to 80cm or more and it is a glabrescent scrambling annual herb. In different regions of the world like

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Africa, Southern Europe, Central and South America and semi-arid tropics covering Asia this plant acts as an important food legume crop. This crop introduced as food crop in Pakistan and had 553 tons production from 257 hectares [15]. Human consumption of this plant based on different parts of this plant such as the immature seeds, leaves and fresh or dry seeds which contain 64% carbohydrates and 23-32% proteins. Additionally, animal feeding was also done in dry season by the pods and dry seeds of this plant[16]. So, this plant became a valuable source of income for grain traders and farmers in many countries of Africa[17, 18]. This plant acts as a fodder and considered as crude protein source[19]. Many researchers perform extensive investigations on salinity stress effects on cowpea (*Vignaunguiculata* L.) and used NaCl as a source of salt stress[20, 21, 22, 23, 24, 25]. Taking into consideration the importance of this plant to farmers and to the economy current experiment was designed to investigate the beneficial effect foliarly applied ascorbic acid in different concentrations on salt stressed cowpea (*Vignaunguiculata* L.)

MATERIAL AND METHODS

Plant biomass and growth parameters:

Cowpea (Vignaunguiculata L.) seeds were taken from Agriculture Research Instutute Tarnab, Peshawar. The experiment consisted of 36 pots divided into three groups. All groups were irrigated with three salt treatments (0, 2.5 dS/m and 5 dS/msea-salt irrigation). First group was foliarly sprayed with distilled water, second group was sprayed with 0.5 mM salicylic acid while third group was foliarly applied with 1.0 mM salicylic acid. All these 36 pots were then arranged in a completely randomized design (CRD) in the Department of Botany, University of Karachi, Karachi. 36 pots had basal outlet for leaching of solution purpose and each pot was filled with 3 kg of sandy loam soil having Hoagland's solution at saturation percentage. Same size seeds were surface sterilized with 0.1% $HgCl_2$ for 1 minute and then washed three times with distilled water and 5 seeds were sown in each pot. Seedlings were irrigated with 150 ml tap water daily. When seedlings were reached at 3-leaf stage, they were thinned to one seedling/pot. At this stage, concentration of sea-salt in the irrigation water is gradually increased until the ideal salinity for each treatment reached. Plants were irrigated with 1.5L of tap water/Sea-salt solution two times in a week. When the required salinity level was achieved then different doses of salicylic acid were applied foliarly on plants. At the end of the experiment, root length, number of leaves, number of branches, fresh biomass, dry biomass, and number of pods per plant were recorded in all harvested plants. Samples of leaves were collected for biochemical analysis and relative water content during the grand growing season.

Relative water content: (RWC)

Determination of relative water content was done by method of Mata and Lamattina [26]. First, fresh weight (FW) of leaves was taken then these leaves were placed separately in distilled water for rehydration for 2 hours and then turgid weight (TW) of leaves was measured. In the last step all leaves were dried by keeping them in preheated (80°C) oven for 48 hours and then dry weight of leaves was measured. Relative water content of different leaves was calculated using following formula.

Relative Water Contents (%) = (Fresh Weight-Dry Weight)/ (Total Weight-Dry Weight) *100

BIOCHEMICAL ANALYSIS:

Photosynthetic pigments:

Chlorophyll concentration (Chl) was determined in fresh leaves following the protocol of[27].

Total Carbohydrate Determination:

Total carbohydrate estimation was performed in plant extracts by the method of[28] using an anthrone reagent.

Total Protein Determination:

Total protein contents were extracted and estimated using method described by[29].

Mineral Analysis of Different Vegetative Parts

Samples of leaves, stems and roots were taken for analysis of different cations (Na+ and K+) during the developmental period. The sample were dried and the weight of ash of 0.5 grams of each dry sample was taken. The ash solution was then prepared in 50 ml of deionized water and then diluted in deionized water for mineral analysis. The PFP1 flame photometer was used to measure the concentration of cations in the sample.

Statistical Analysis of Data and Experimental design

The experiment was a completely randomized design (CRD) having different salicylic acid treatments and three salt treatments with three replicates. Statistical analysis was done using SPSS (Ver. 21) software for analysis of variance (ANOVA), Duncan's multiple comparison using mean ($P < 0.05$).

RESULT AND DISCUSSIONS

Plant growth

Data represented in figures 1-4 exhibited that plants treated with salt exhibited significant (P<0.001) reduction in root length, shoot length, fresh and dry biomass. [30] treated sugar beet plants to different concentrations of salt and observed marked reduction in different growth parameters. Different researchers observed same reduction phenomenon in different plants after treatment with salt, e.g. Cotton [31], Tomato [32, 33], Corn [34], pepper and cucumber [35]. Salicylic acid (SA) known as antioxidant and important plant growth regulator [36]and it had importance in plant to allay the harmful effects that induced by salinity on different crops development and growth[37, 11, 38]. Results of the present investigation also provide the evidence of same earlier observations that foliar supply of salicylic acid showed significant (P<0.0001) improvement in above mentioned growth parameters in non-saline and saline environment. [39]treated soybean plants with salicylic acid and conclude that roots and shoots of that plant showed increase in growth under normal conditions. Wheat plants also showed enhanced growth under drought stress when treated with salicylic acid[8] maize [40] and barley [37] under NaCl stress.

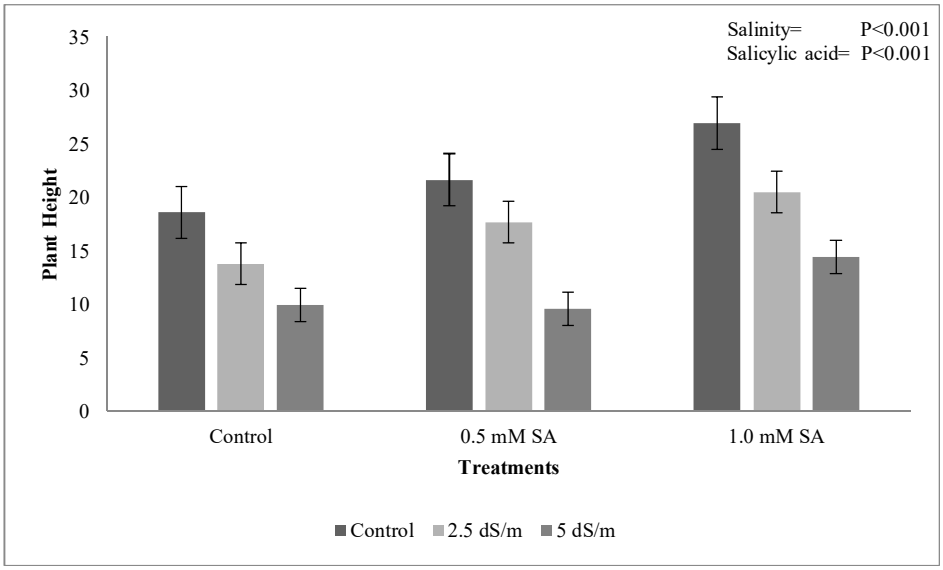

Figure 1. Effect of foliarly applied salicylic acid on plant height (cms) of *Vignaunguiculata* grown under seasalt salinity.

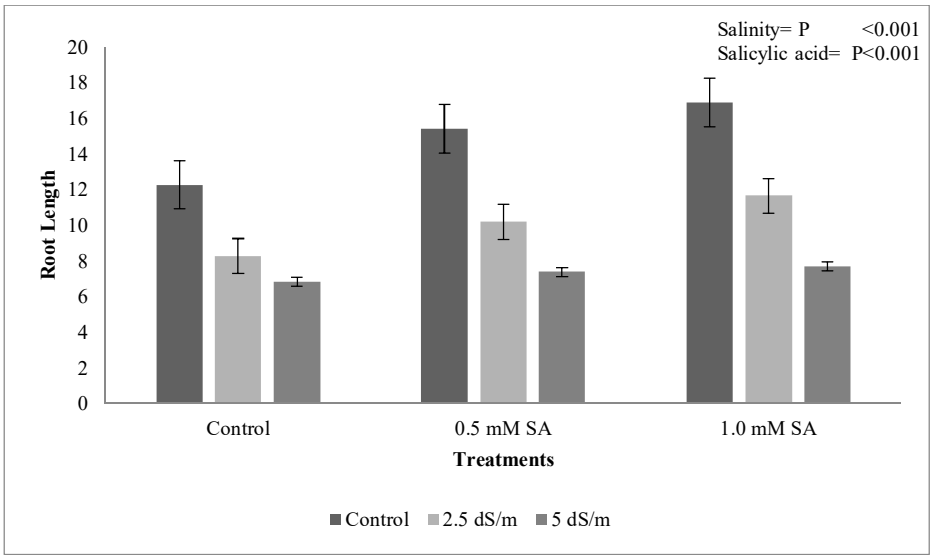

Figure 2. Effect of foliarly applied salicylic acid on root length (cms) of *Vignaunguiculata* grown under seasalt salinity.

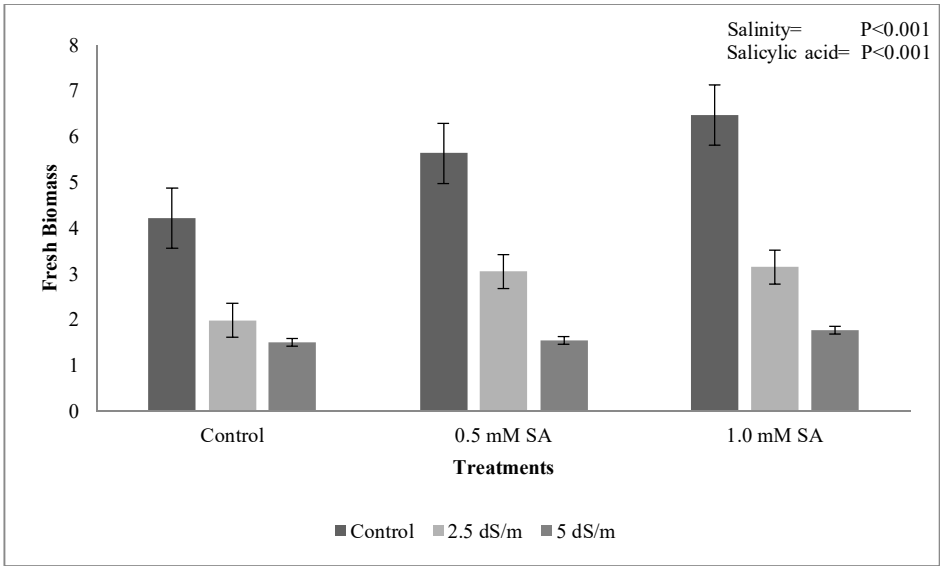

Figure 3. Effect of foliarly applied salicylic acid on fresh biomass (gms) of *Vignaunguiculata* grown under seasalt salinity.

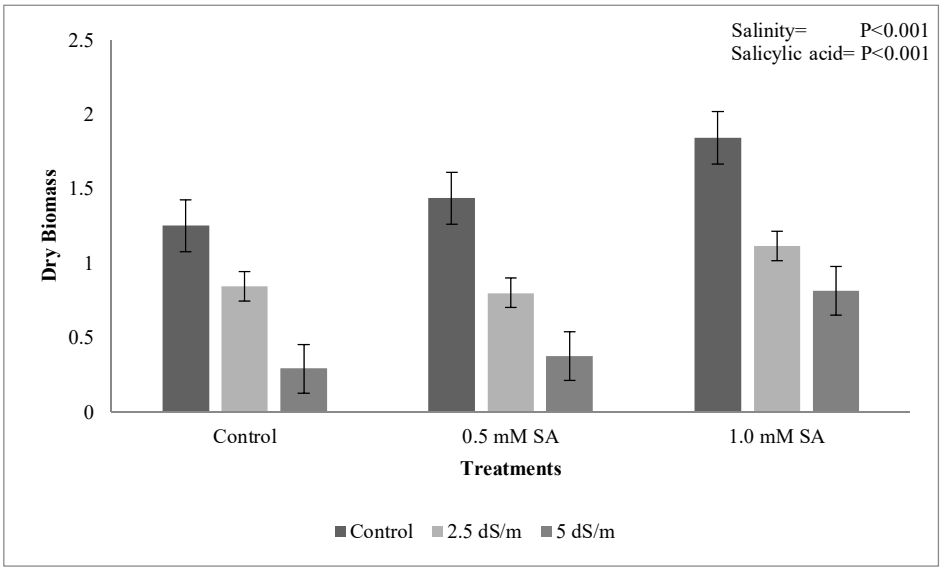

Figure 4. Effect of foliarly applied salicylic acid on dry biomass (gms) of *Vignaunguiculata* grown under seasalt salinity.

Relative water content

Optimal plant growth maintenance is a result of osmoregulation or plant water status maintenance and it is regarded as key physiological process[41]. In present investigation relative water content of cowpea leaves was significantly (P<0.001) decreased at the highest concentration of salinity (Figure 5).[42] studied linblack gram (*Vignamungo*) under salinity stress observed increase in leaf water potential with decrease in osmotic potential. Accumulation of organic and or inorganic solute is the main reason for decrease in osmotic potential. Soluble sugars, amino acids mainly proline, and glycinebetain are different organic solutes that had an important role in adjusting different metabolites osmotically in the cell[43, 44, 45]. Sodium and potassium are inorganic solutes and they are also considered important in osmoregulation but sodium is damaging the cell as compared to potassium and other organic solutes[46, 47]. Increase in salinity in plants results in the more negative values of relative water content, water potential and osmotic potential[48]. Foliar application of salicylic acid showed significant (P<0.0001) improvement in relative water content. Increase in RWC of wheat plants treated with salicylic acid was reported.

Experiments on different crops including tomato [14, 49], barley [37], and cucumber [50] also showed the same phenomenon when grown under salt stress. This fact was also hypothesized that application of salicylic acid on leaves results in reduction of transpiration rate and improvement in leaf diffusive resistance.

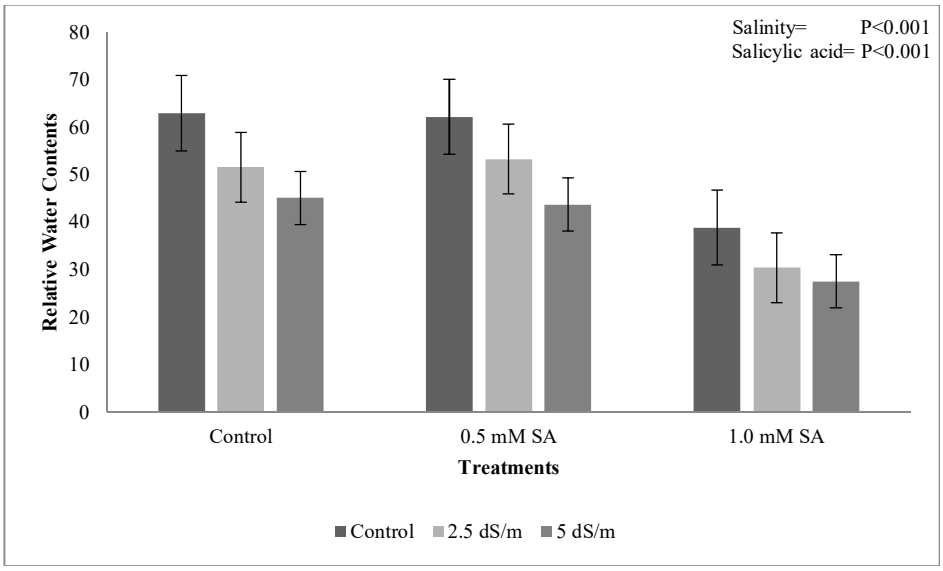

Figure 5. Effect of foliarly applied salicylic acid on relative water content of *Vignaunguiculata* grown under seasalt salinity.

Chlorophyll

In the present study, saline stress cause significant (P<0.001) reduction in the chl *a*, *b*, and total chlorophyll contents (Figures 6-9). [50] for cucumber and [51] for wheat concluded the same results when plants exposed to salt stress. When [52] exposed sorghum plants to different concentrations of salt chlorophyll biosynthesis in leaves showed reduction. [53]also observed same negative effect on chlorophyll content in strawberry plants when treated with different concentrations of salt. [48]also studied the same effect of salt stress on chlorophyll in many crops. When barley plants treated with NaCl leaves carotenoids and chlorophyll a and b showed significant reduction in under stressed plants as compare to normal ones [37]. In another study on bean plants these all parameters showed significant promotion in NaCl treated plants as compared to non-saline ones[54]. According to[55] chlorophyll concentration in salt treated plants depends on concentration and type of salt, biological processes going on in plants and developmental stages of plants. In present investigation foliar application of salicylic acid showed significant (P<0.001) improvement in chlorophyll (a, b and total chlorophyll). Rate of photosynthesis increased in soybean plants which is a result of high pigment contents in the leaves after application of salicylic acid[56]. According to[57] when they worked on maize plants and treated them with salicylic acid carotenoid and chlorophyll content exhibited increase.

Carbohydrates

Reduced water to plant results in increased carbohydrate concentration and lower water potential which helps in maintenance of protein structure and prevent oxidative losses. At molecular level for the activity of sugar responsible genes carbohydrates play key role and as a result plant give different response like expansion of cells and defensive response[58]. Data presented in figure 10 explained that plants subjected to different salinity levels exhibited significant (P<0.001) promotion in total carbohydrates. When *Zea mays* treated with different NaCl concentrations plant exhibited promotion in soluble sugar contents with increase in NaCl concentrations while polysaccharides showed opposite effect in salt treated plants. Maria *et al.,* [59] treated tomato plants to salinity stress and observed increase in soluble sugar content. It is evident that in the presence of salts promotion in soluble carbohydrates in the root region helpful in maintaining balance against osmotic pressure. During salinity stress conditions for escaping from plasmolysis condition plant cell should change macro molecules to micro molecules. Conversion of starch to glucose and decomposition of sucrose to glucose and fructose helps to enhance osmotic pressure of cell[60].

During saline stress condition salicylic acid is responsible for maintaining balance in the sugar level. In present investigation plants treated with salicylic acid exhibited reduction in soluble sugar content as compared to control plants. It is reported that application of salicylic acid to plants stimulates their growth by activating metabolic consumption of total soluble sugar to develop new constituents of cell. It is also evident that total soluble sugars are incorporated into polysaccharides which is accelerated by the application of salicylic acid while polysaccharide hydrolyzing enzyme system inhibited by the treatment of salicylic acid. [61] worked on ray plants and observed that application of salicylic acid reduced soluble sugar levels in plants.[40] stated that enzymatic system that is involved in polysaccharide hydrolysis deranges after application of salicylic acid.

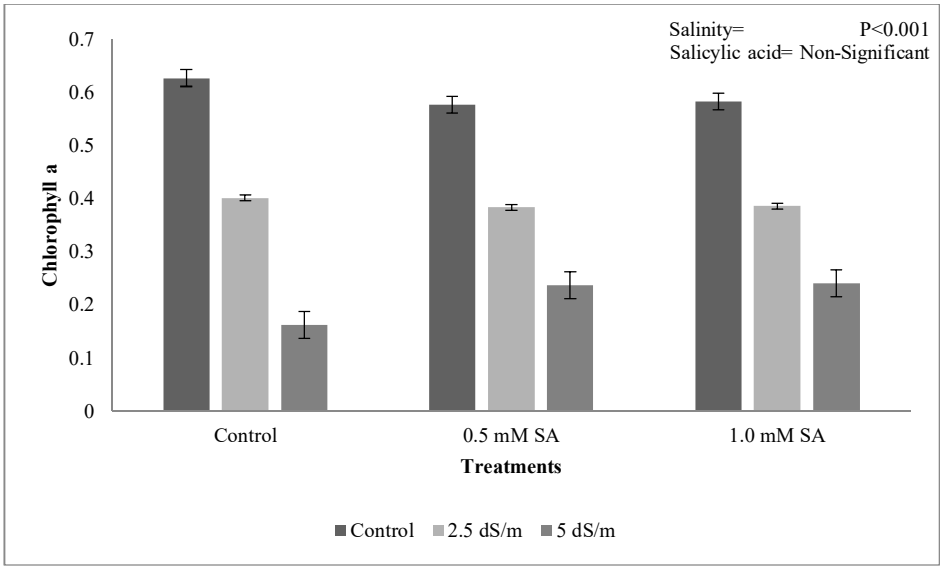

Figure 6. Effect of foliarly applied salicylic acid on chlorophyll a (mg/gmfr.wt.) of *Vignaunguiculata* grown under seasalt salinity.

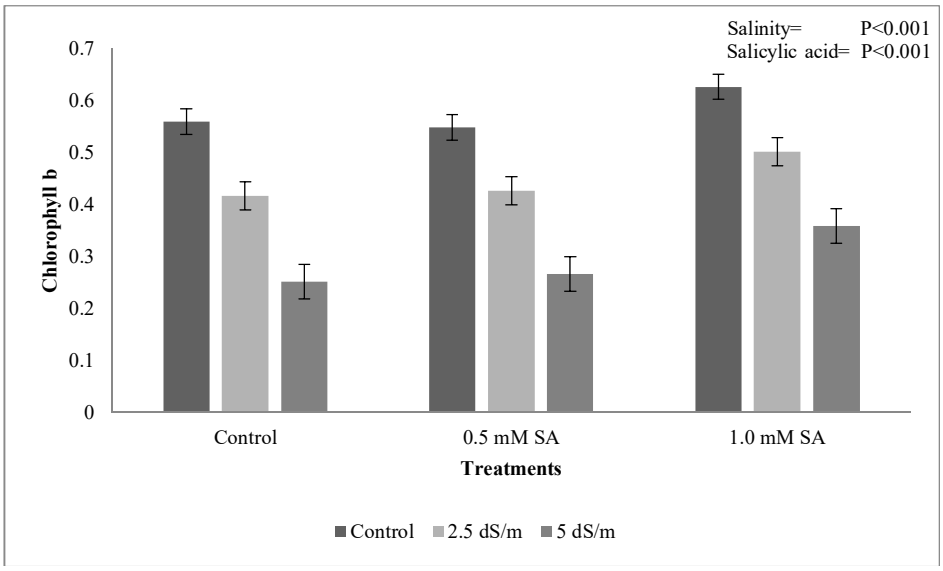

Figure 7. Effect of foliarly applied salicylic acid on chlorophyll b (mg/gmfr.wt) of *Vignaunguiculata* grown under seasalt salinity.

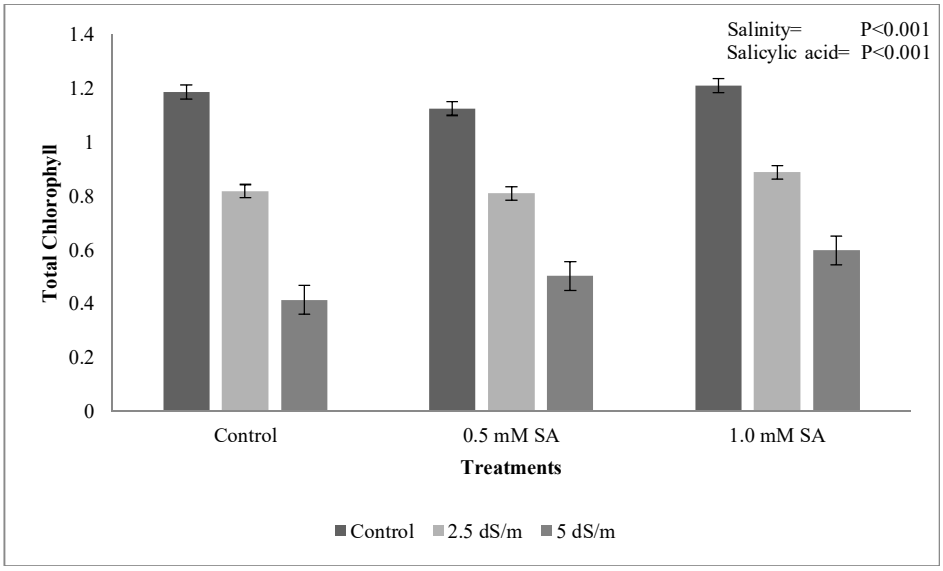

Figure 8. Effect of foliarly applied salicylic acid on total chlorophyll of *Vignaunguiculata* grown under seasalt salinity.

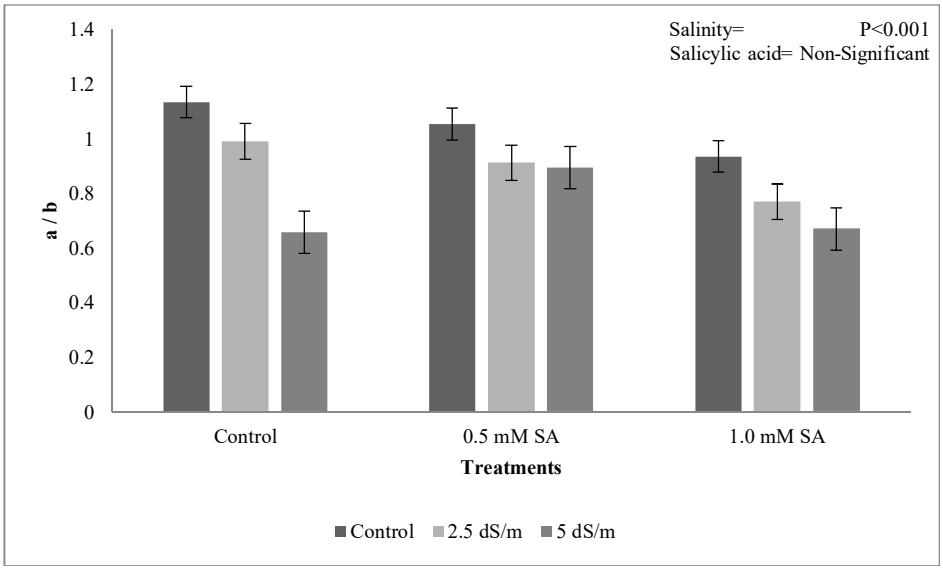

Figure 9. Effect of foliarly applied salicylic acid on chlorophyll a b ratio (a/b) of *Vignaunguiculata* grown under seasalt salinity.

Proteins

Data presented in figure 11 showed significant (P<0.0001) decrease in total proteins when plants subjected to different concentrations of salt. This phenomenon was also reported by [62] and [63] when they treated plants with salinity stress. When [64] treated tomato plants with salt stress they observed reduction in leaf protein level and according to [65] this effect of salt on protein level was created after reduction in the activity of nitrate reductase enzyme. It is evident that when plant treated with salt it created changes in roots and shoots proteins level but in leaf blade there is no effect. [66] also observed reduction in the total protein contents after application of salt on plants. In this study foliar application of salicylic acid exhibited significant (P<0.01) enhancement in protein levels in both non-saline and saline environment. [8] studied plants under water stress they observed reduced soluble protein contents and after salicylic acid application marked a slight increase. A [11] observed that saline media significantly reduce the mean protein in wheat and same plants exhibited significant promotion in total protein contents when treated with salicylic acid.

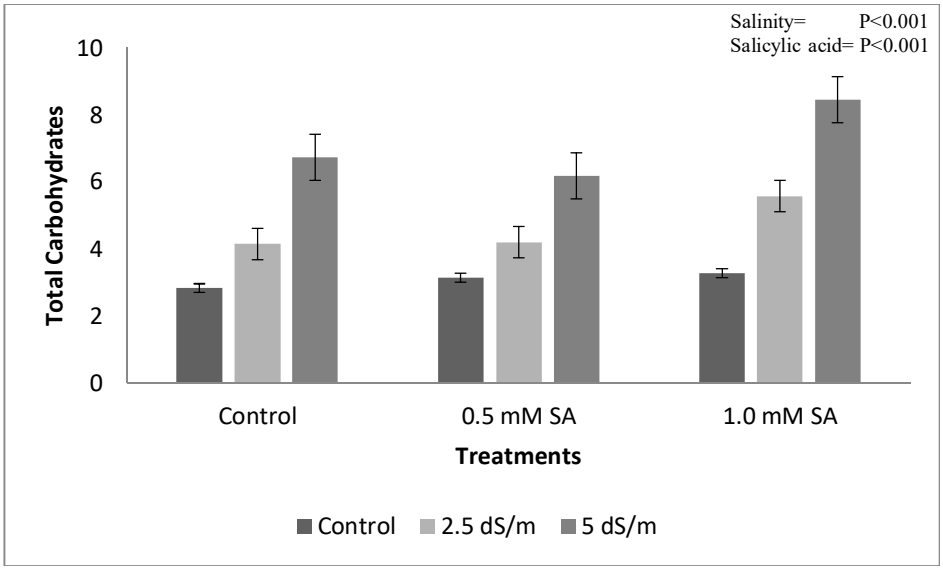

Figure 10. Effect of foliarly applied salicylic acid on total carbohydrates (mg/gmfr.wt) of *Vignaunguiculata* grown under seasalt salinity.

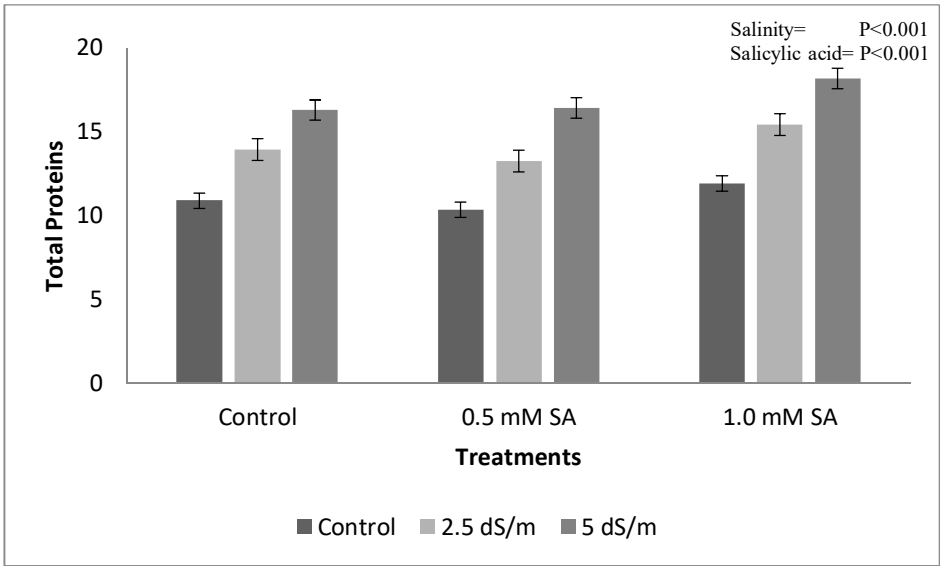

Figure 11. Effect of foliarly applied salicylic acid on total proteins (mg/gmfr.wt) of *Vignaunguiculata* grown under seasalt salinity.

Ions

When plants treated with salt it created a significant (P<0.001) promotion in sodium content and a considerable decrease in potassium concentration which results in a significant promotion in the Na^+/K^+ ratio (Figures 12-14). Salinity treatment in roots increased the level of sodium in the medium which reduced the uptake of potassium by root cells and as a result K+/ Na+ ratio also reduced. After application salt entry and accumulation of high levels of sodium ions in the cell will be toxic for cell and plant.[67] stated that when excess amount of sodium enters the cell it must move out of it or enter the vacuole to prevent cell death or reduced growth. Cytotoxin ions, especially Na+ and Cl- ions in salinity environments enter the vacuole, and are used as an osmotic solution [68].When salicylic acid foliarly applied on plants it exhibited beneficial effect on concentration of sodium and K^+ in different plant parts. [69]observed in tomato plant that accumulated Na+ ions in treated leaf tissue with SA and were placed in salinity and act as inorganic osmolyte.

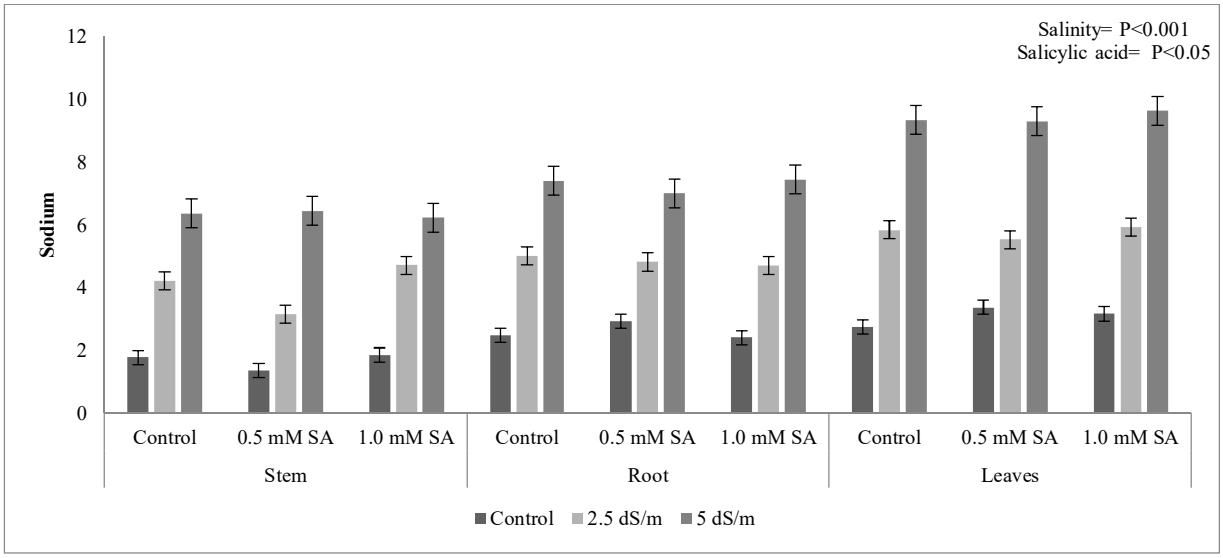

Figure 12. Effect of foliarly applied salicylic acid on Na^+ion concentration of different plant parts (stem, root and leaves) of *Vignaunguiculata* grown under seasalt salinity.

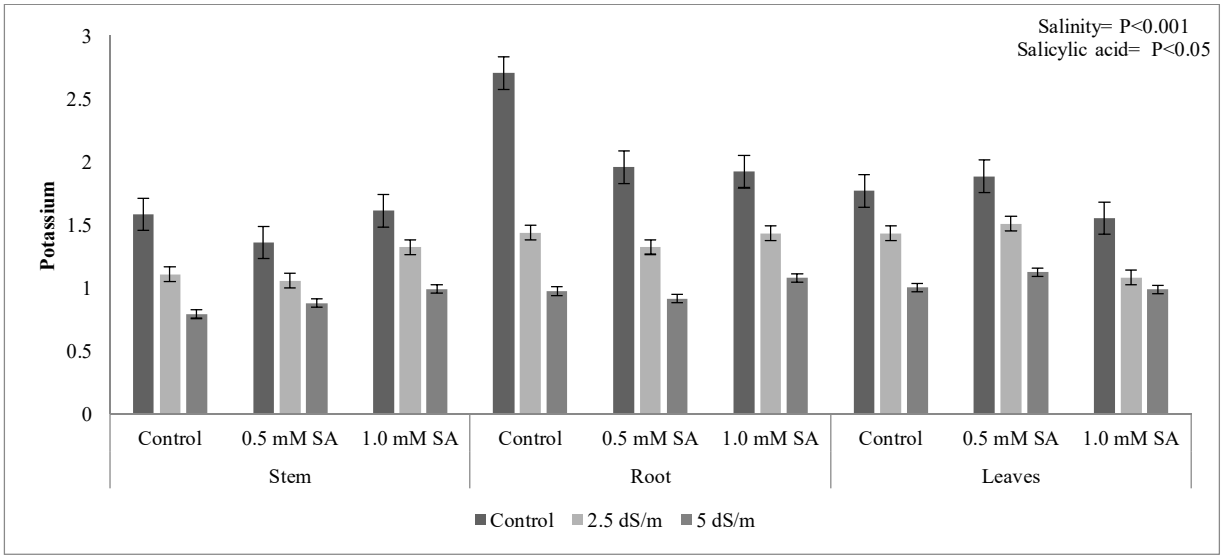

Figure 13. Effect of foliarly applied salicylic acid on K^+ ion concentration of different plant parts (stem, root and leaves) of *Vignaunguiculata* grown under seasalt salinity.

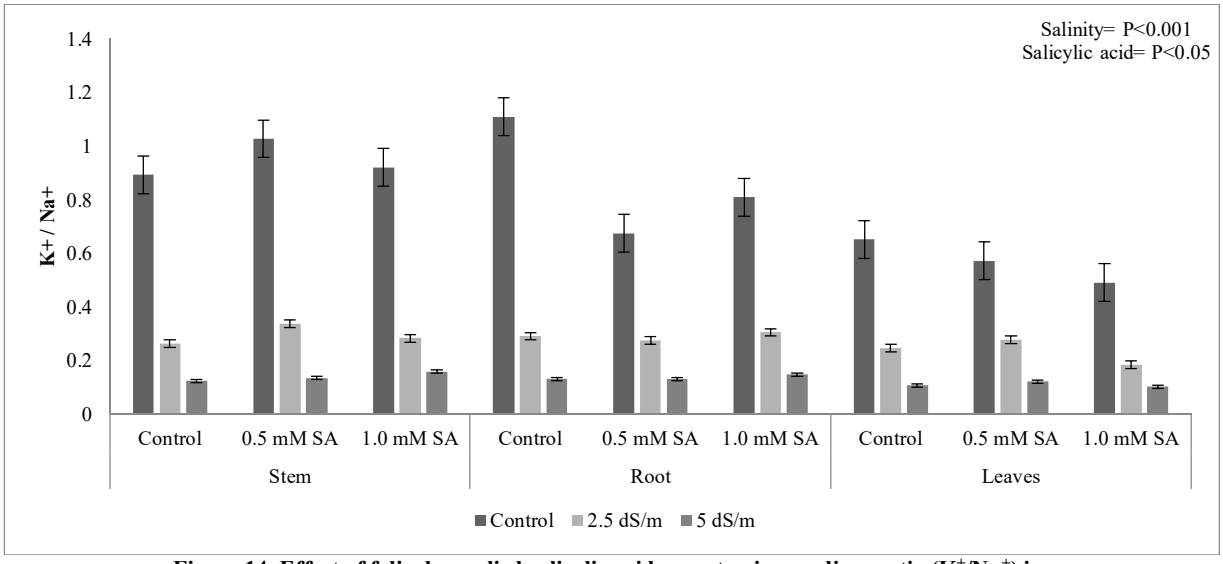

Figure 14. Effect of foliarly applied salicylic acid on potassium sodium ratio (K^+/Na^+) in different plant parts (stem, root and leaves) of *Vignaunguiculata* grown under seasalt salinity.

CONCLUSION

Collective data for vegetative growth, Primary metabolites revealed detrimental effect of salt on plant growth. Sodium also accumulated in different parts of the plant. Application of salicylic acid reduced harmful effect of salt on plant growth and improved growth under normal condition.

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Plants Traditional Medication in Arid and Semi-Arid Zone of Tehsil Domel, District Bannu, Khyber Pakhtunkhwa–Pakistan

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ABSTRACT

The Indigenous communities of tehsil Domel, District Bannu, Khyber Pakhtunkhwa, Pakistan, mainly depend on ethnomedicinal plants, and the study was conducted with the aim to document therapeutic plants and formulae used by the local people for the treatment of different human infirmities. Ethnobotanical data was obtained by conducting many field trips, questionnaires, open-end and semi structured interviews, inquiries, and group gathering from 2016 to 2017.To evaluate the reliability and richness of herbal knowledge, the data were investigated using quantitative Ethnomedicinal indicex Relative Frequency Citation (RFC), Use Value (UV), respectively. During the survey, 49 species of medicinal plants belonging to 28 botanical families were reported to be used in traditional medicines. Lamiaceae and Solanaceae with 6 species are the dominant families, followed by Moraceae and Fabaceae. With regard to growth form, the main source of herbal medicines was herbs (47%). For remedy preparation, leaf (36%) was the most frequently utilized part. Moreover, 25% of the herbal medicines were prepared in the form of Powder and administered orally. The different plant species used by the local people in large numbers for medicinal purposes indicated that tehsil Domel is wealthy in medicinal flora and allied traditional knowledge. This shows that the indigenous people still rely on therapeutic plants for their healthcare needs, and therapeutic plants are the chief source for plants-based medicines for the inhabitants of the study area. A comprehensive data were found on general and worldwide uses of the proposed study to extract new potential species for further ethnomedicinal and/or ethnopharmacological, Phytochemical, and clinical studies.
KEYWORDS: Ethnobotany; Medicinal Plants; Biomedicine; Traditional Medicine; Domel; Pakistan

1. INTRODUCTION

Ethnomedicinal assessment of plants is useful for conservation, plant protection, and developing herbal drugs. Information transfer from generation to generations and closely associated to the people's traditional beliefs (1,2). The local communities all over the world have some knowledge which they have been building up prolonged connections with the natural world which remains vital to their spiritual, physical and social benefits. The medicinal plant knowledge and their uses by local healers and drug development are not only valuable for management of cultural practices and biodiversity but also for community health care. The indigenous knowledge on medicinal plants appears when humans started and learned how to use the traditional knowledge on medicinal plants (3-6). The utilization of ethnomedicinal evaluations of therapeutic plant species is imperative for preservation, assurance and valuable for creating herbal drugs. It is still protected amongst the numerous local groups all over the globe. According to the World Health Organization, around 4000 million individuals in emerging nations trust over the effectiveness of herbal cures and is being utilized in consistency (7-8). Although, modern medicine might be accessible in these nations, herbal medicines have frequently kept up ubiquity as a result of chronicled and social reasons. In Pakistan, more than 6,000 plant species of higher plants exist, among these, 12% of which are utilized as a medicines (9-11). By using these medicinal plants both human and veterinary ailments are treated. In most cases, some plant species are considered particular for a specific illness, but infrequently plants have numerous usages. Pakistan stands among the eight prominent countries, which export medicinal plants, according to the Export Promoting Bureau; there was an export of over 8,500 tones medicinal herbs in 1999, which fetched a petty amount of 6 million US dollars. Mostly, the collectors of medicinal plants are uneducated or ill literate people and they do not know the proper way of collection. Women followed by children and are the principal collectors of medicinal plants. Due to over-collection, several species have been extinct in the Hindu Kush Himalayan regions (12). Traditional medicine system has its roots in the home based medications and this information is moved from one generation to other orally. In spite of the fact that recording of medicinal properties of conventional plant species has contributed as number of contemporary medications (13,14) but still vegetation of some areas in Pakistan is unexplored. These ethnobotanical studies provide an idea of how important medicinal species are within the local sociocultural

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framework and establish a strong association between biological conservation and the conservation of the local cultural heritage (15,16). The synthetic products have already been a harmful effect and the people are unaware about the negative consequences of undefined natural product, while, Pakistan is a developing country with most of the peoples are poor and living in mountainous area have no access to living resources they mostly depends on plants for their daily needs and use them in shelters, wood and medicinal purposes (17-20).

Pakistan is situated at a ltitude of 70.40°-70.30°N and the longitude of 32.48°-33.23°E. It is situated at 340m above the sea level. The proposed area for study Tehsil Domel, District Bannu, Khyber Pakhtunkhwa-Pakistan lies about 605 square kilometers and is surrounded by Lakki Marwat, Karak etc. All these adjacent areas have having dense vegetation, where the traditional uses of medicinal plants as herbal remedies is a common practice among the locals (21-24). Different ethno-medicinal studies were carried out in different regions of the proposed district Bannu Domel but no specific study has done in this area. The most encountered diseases were found in this area was reported diabetes, cough, headache, stomach problem, jaundice, skin diseases and toothache. A few qualitative studies have so far been carried on the ethno-medicinal potential of wild plant resource from the adjoining localities and number of studies on traditional use of plants is increasing day by day in Pakistan, but this is the first independent quantitative ethno-medicinal study in the area to the best of our knowledge.

2. MATERIAL AND METHOD

2.1 Interview

The survey was conducted on a total of 230 individuals from the local population, aged 60 years and above was interviewed. First of all informants were made aware of the importance of the study and knowledge about the medicinal plants were gained from them. Following the ISE (International Society of Ethnobiology) code of ethics the interview was conducted in friendly environment to allow the informants to response the questions naturally. This survey was done using a pre-prepared structured questionnaire, in which the first portion is about personal information: age, educational level and gender, while the second portion collects information regarding medicinal plants vernacular name, life forms, part of plant used, disease treated, mode of utilization and their therapeutic uses. The interview was conducted in native language of the area which is Pashto. Sometimes interviews were taken from the informants using a tape recorder which turned out to be effective and helped recalling the respondent their information resulting in gathering much information. With reference to the particular place, people from local population were interviewed at their homes and the herbalist at their local places.

During the survey, medicinally important plants based upon the local population's information were collected from different locality of the proposed study area. The field work includes collection of plants for the period of 5 months starting from March to July and also collection continued in the period of monsoon. List of plants were arranged alphabetically. Latin name and families were confirmed from online website ''The Plant List'' (http://www.theplantlist.org/), ''The International Plant Name Index'' (http://www.ipni.org/) and Kew naming system "www.mpns.kew.org/mpns-portal/". The identified species were dried, labeled and mounted on herbarium sheets and to deposit to the Herbarium of Abdul Wali Khan University, Mardan, Khyber Pakhtunkhwa, Pakistan

2.1 Study area

Rich floristic and diversified ethnic locality of Tehsil Domel, KPK, Pakistan. The whole area has dense vegetation, and traditional use of medicinal plants as herbal remedies is a common practice among the locales. The people mostly rely on the plants for most of their needs and people have a rich knowledge about different uses of plants for daily needs. But the area has never been explored before for this ethno-medicinal knowledge. This study is significant as this will figured out the traditional medicine and other important uses of plants that are often easily available and affordable to the rural societies in Pakistan (25-30).

3. Data analysis

Compared with several national and global ethno-pharmalogical references (articles, books, Google Scholar) to obtain resemblances, dissimilarities, and new uses of unidentified or well-known medicinal plants.

3.1 Relative Frequency of Citation (RFC)

The indigenous knowledge is evaluated quantitatively using RFC and Frequency of Citation (FC). The RFC was designed to conclude the consent between the informants on the use of medicinal plants in this area. It is calculated by the following formula using equation 1.

$$RFC = FC/N \; (0 < RFC < 1) \quad \text{----------} \quad (Eq.1)$$

Where,

Frequency of Citation; FC, is the number of informants who cited the species; RFC is the relative frequency citation and N is the total number of informants take part in the study.

The value of RFC depends on the citing proportion of informants for that particular species (31,32).

3.2 Use Value (UV)

The formula used for plant calculation is UV, using equation 3 (35).

$$UV = \Sigma U/n \quad \text{----------} \quad (Eq.3)$$

Where,

n = $n$refers to the total number of informants

U = Stands for the number of use reports mentioned by every informant for a certain species

UV= Use value for plants deliver a quantifiable amount for the qualified point of species.

4. RESULTS AND DISCUSSION

4.1 Demographic Characteristics of Informants

In the present investigation, a total 230 informants (Table1) including indigenous people (84.78%) and Traditional Health Practitioner's (THPs) is (15.21%) were interviewed divided into different age groups ranging from 25-35, 35-50, 50-55, 65 and above years, which includes 35 herbalist and 195 people from local population. Among these people, majority of men interviewed (84%) which were 65 and above years old and the remaining (29.13%) were below 65 years, and were the most dominant in local the population. On the basis of education, indigenous knowledge regarding the use of plants is more predominant among illiterate people (41.30%) and this knowledge is decreasing in highly educated class (11.73%).

Table 1: Demographic Data of Informants

S/ No.	Variables	Categories	Number of Persons	Percentage
1	Informant category	Traditional Health Practitioner	35	15.21
		Indigenous People	195	84.78
2	Gender	Female	67	29.13
		Male	163	70.86
3	Age	20-35 years	35	13.91
		35-50 years	52	22.60
		50-65 years	55	23.91
		65-80 years	68	29.56
		80 years & above	20	8.69
4	Educational Background	Illiterate	95	41.30
		5 years education	45	19.56
		8 years education	35	15.21
		10 years education	28	12.17
		12 years education	27	11.73

4.2 Medicinal plant diversity

A total of 49 medicinal plants species belonging to 28 families were documented. The following families i-e Lamiaceae, Solanaceae and Moraceae were of highest numbers of species showing the species presence of 6,6 and 4 species, respectively. During this research, it is clear that most species belongs to herbs, followed by shrubs and trees and 23, 16, 06, respectively. This result is similar to the study reported in Chail valley, District Swat, Pakistan. In Sindh desert of Pakistan, it has reported that the most numerous life forms were herbs and shrubs. However, in Mastung region of Balochistan Province, the most frequent habit was reported herbaceous and shrubby nature of vegetation (41,42).

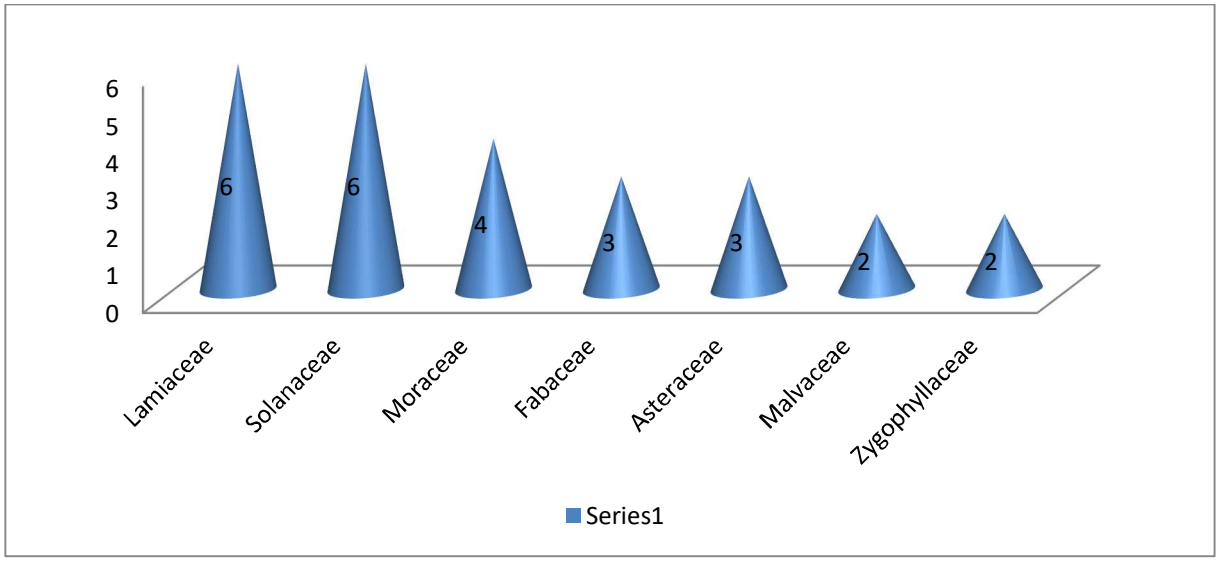

Figure 1: Families of Medicinal Plants

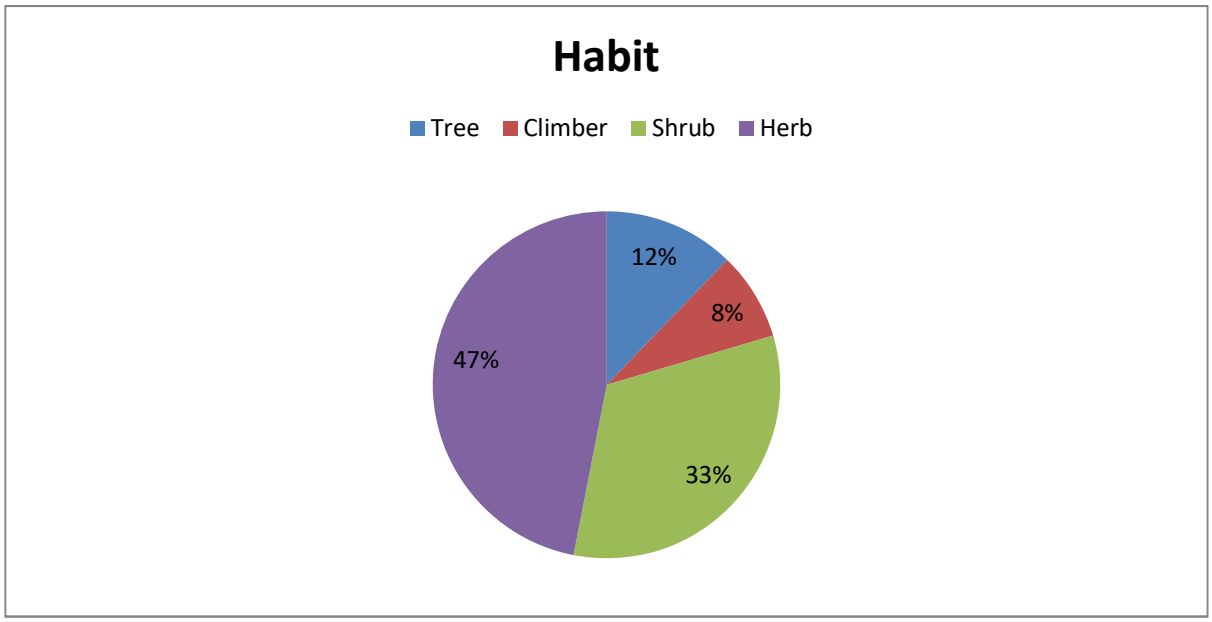

Figure 2: Habitat of Medicinal Plants

4.3 Parts of Medicinal Plants Use

Data on medicinal plant parts used in making herbal medicine are shown in (figure 3). The most used parts of plants are leaves (36%), followed by Fruits (21%) and the Seeds (17%), respectively. The use of leaves in herbal medicine was found commonly in several ethno-medicinal surveys (43, 44). Leaves are more frequently used as compared with other parts because they are more in number, collection is quite easy as compared to roots, tubers and according to conservation point of view, the collection of whole plant cause the plant to be disappeared (45-47).

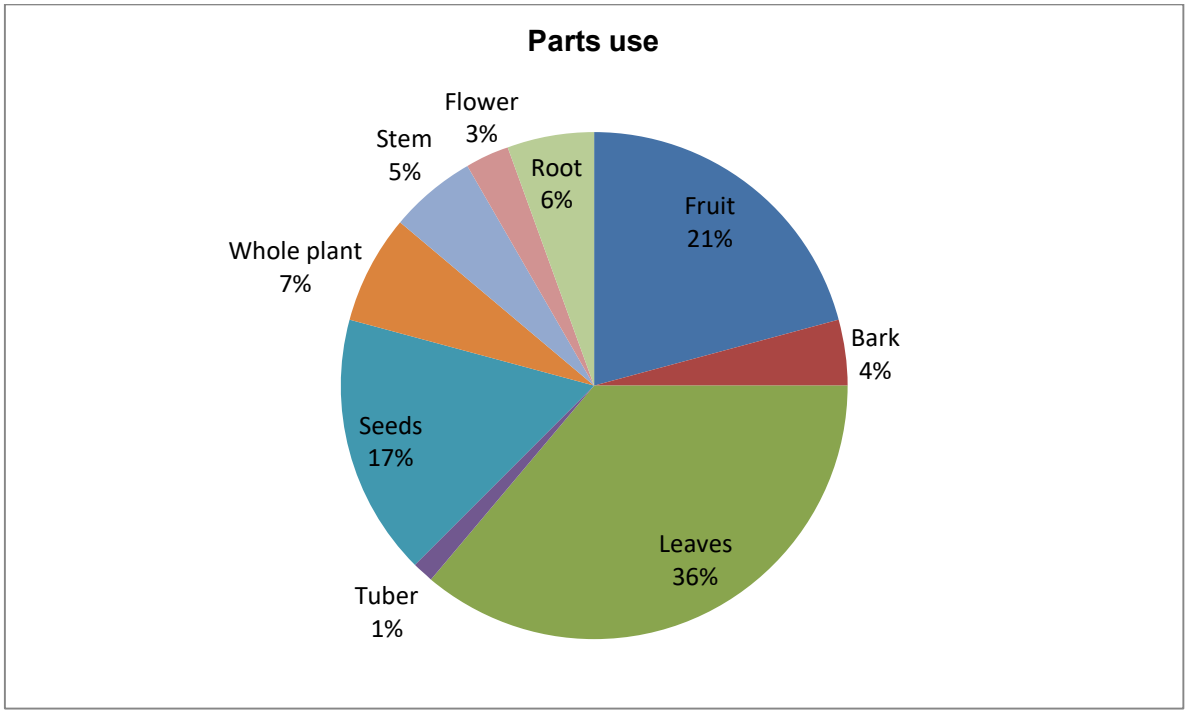

Figure 3: Plant Parts Used

4.4 Method of Preparation

There are two mode of administration of herbal preparations i.e. Internal (decoction, infusion, teas, maceration, powder, juices) and External i-e. paste, oil. The most dominantly used preparation is the powder which is (25%)in number followed by the decoction (23%), and paste (10%), accordingly. Similar results were found in the previous ethno-medicinal studies conducted in different parts of the world. Most of the plants in this study are also used by mixing with honey, milk, water and dates. As these mixture allows easy ingestion of plants that have bitter taste. Some plants are also eaten raw form: *Trachyspermum ammi* L. and*Solanum incanum* L.(48-51)

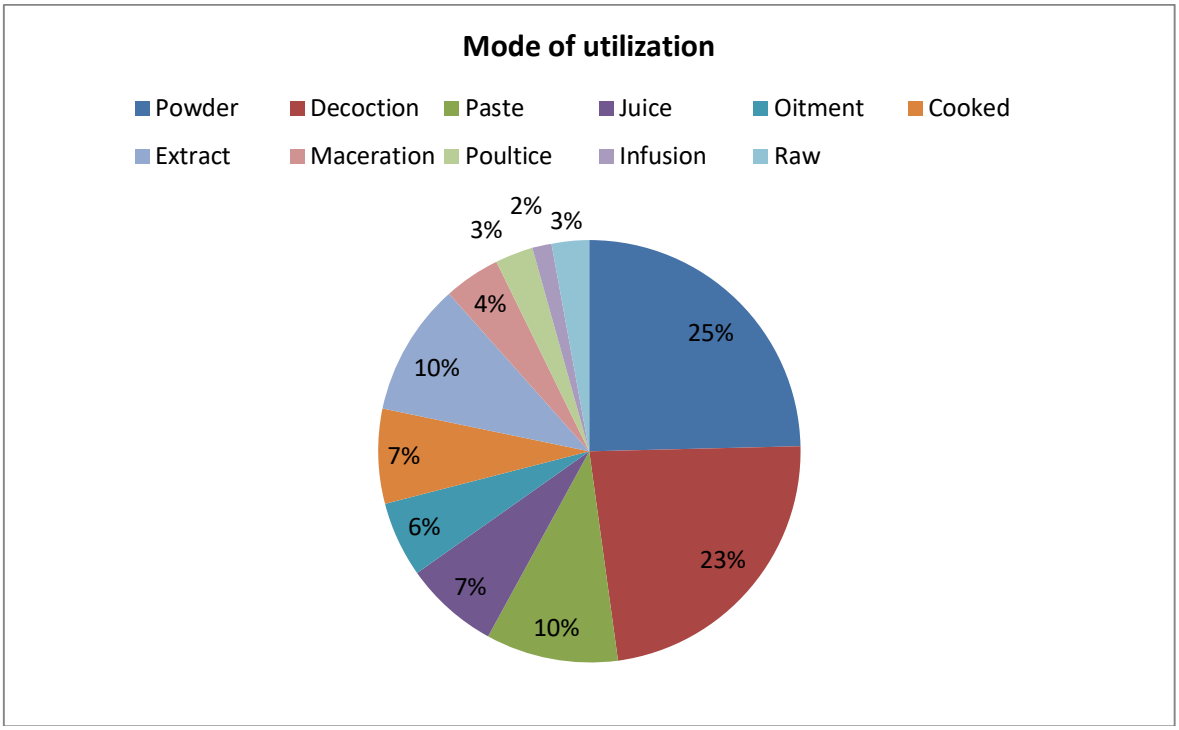

Figure 4: Route of Administration

4.5 Most Frequently Cited Plants and Its Medicinal Uses

In present study, the most cited plant is *Justiciaadhatoda* L. with 80 citations followed by *Morus alba* L. 76 citations, *Rhazyastricta* Decne., 75 citations. The high frequency of citations of therapeutic plants is because these plants are well known and are more frequently used by the local informants, representing a source of consistency. In this study, highest number of citation is of *Justiciaadhatoda* L. which is used to treat Diabetes, cough, asthma, followed by *Morusalba* L. which is used for the treatment of Sexual disorders. We found similar result for *Justiciaadhatoda* L.in other ethno-medicinal surveys as well(52,53). *Rhazyastricta* Decne., is used for treating Blood purification, diabetes, skin disease, disorders, healing wounds and also used as body tonic. High citation is also noted for *Peganum harmala* L. which is used for curing skin disorders, fevers and respiratory disorders, same medicinal uses was reported in other areas of Pakistan (54). This result is similar to other Ethnomedicinal surveys conducted in other regions of Pakistan; Balochistan, Abbottabad, Gilgit, Sindh, Azad (55,56). This resembles well to serious and prolonged diseases being collectively cured by herbal medication.

4.6 New Reports and New Uses

The present study is compared with 20 published research papers, from adjoining areas, country and from all over the world for similar and dissimilar uses. Present study revealed that 20plants were reported for the first time, with novel uses. Remaining 29 plants were already documented in previous literature for various diseases with similar and dissimilar uses. In the proposed work we observed these new medicinal uses for one species in many ailment categories.

5. Quantitative Analyses of Ethno Medicinal Data

5.1 Relative Frequency of Citation (RFC)

The Relative Frequency of Citation **"**RFC" is used to determine the maximum used plant species of the particularly area with reference to informants that have been interviewed for that particular plant species. The range of RFC was plan from 0.34 to 0.01. The highest value of RFC was calculated 0.34 which was observed for *Justiciaadhatoda* L. and its status confirms that this medicinal plant species is frequently found everywhere in the area and the concern people are quite familiar with its medicinal uses, mostly they are in practices for Diabetes, cough, asthma and menses. The most frequently used medicinal plants species in the study area are observed *Justiciaadhatoda* L"0.34", *Melia azedarach* L."0.31", *Morus alba* L."0.32", *and Menthalongifolia* (L.) 0.27 ", respectively.

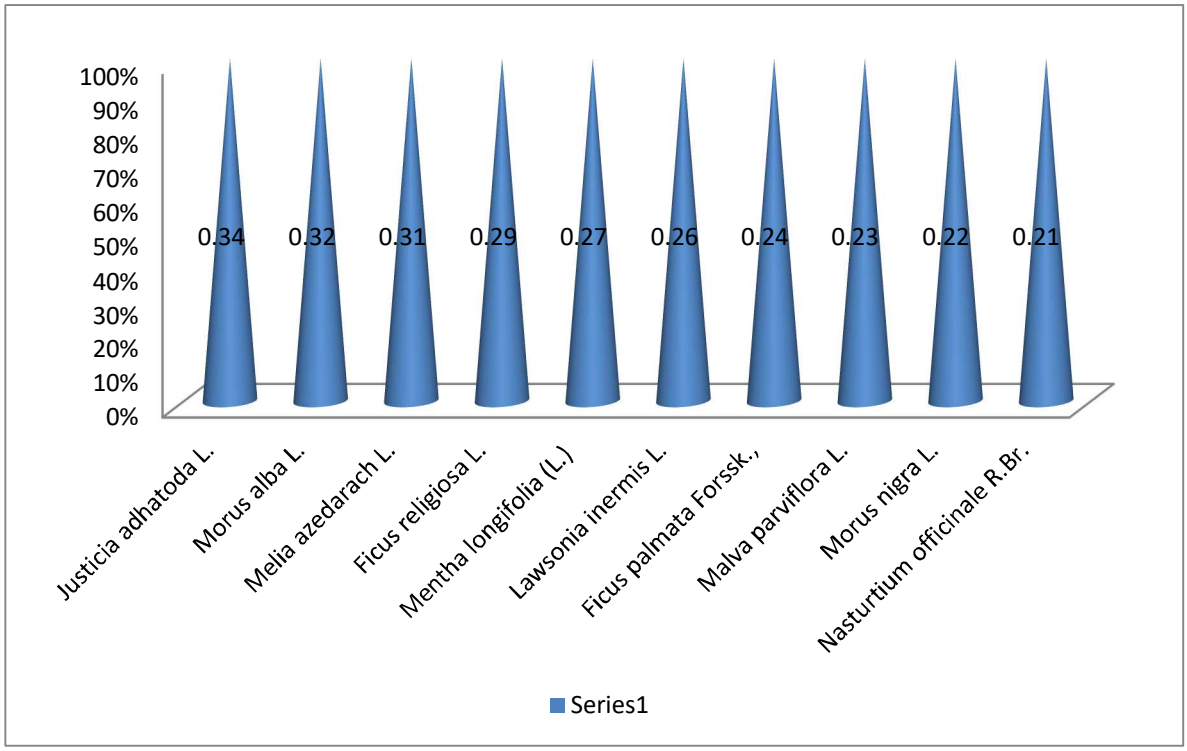

Figure 5: Relative Frequency of Citation (RFC)

5.2Use Value (UV)

The terminology Use Value (UV) is a quantitative method that shows the relative importance of a plant species or plant family among the population. In the present study, the Use Value was calculated and ranges from 0.1 to

0.2 species. The highest Use Value in term of numbering sequence was reported in *Martyniaannua* L. 0.1 followed by *Ricinuscommunis* L0.10, *Trachyspermum ammi* L. 0.11, *Opuntiamonacantha* (Willd.)0.12, and *Tribulusterrestris* L.024, respectively. The high UV values indicate that these particular plant species are the most recommended and well known in used by the local informants, which being indicate the significances of plants. However, the plants species with least UV values were reported the *Morusalba* L. 0.01 and *Ficusreligiosa* L. 0.02, accordingly. The proposed least UV values of the proposed plant species are explained the fact and findings that these particular plant species medicinally uses vary rare in the area due to the medicinal knowledge and concern information by the local community.

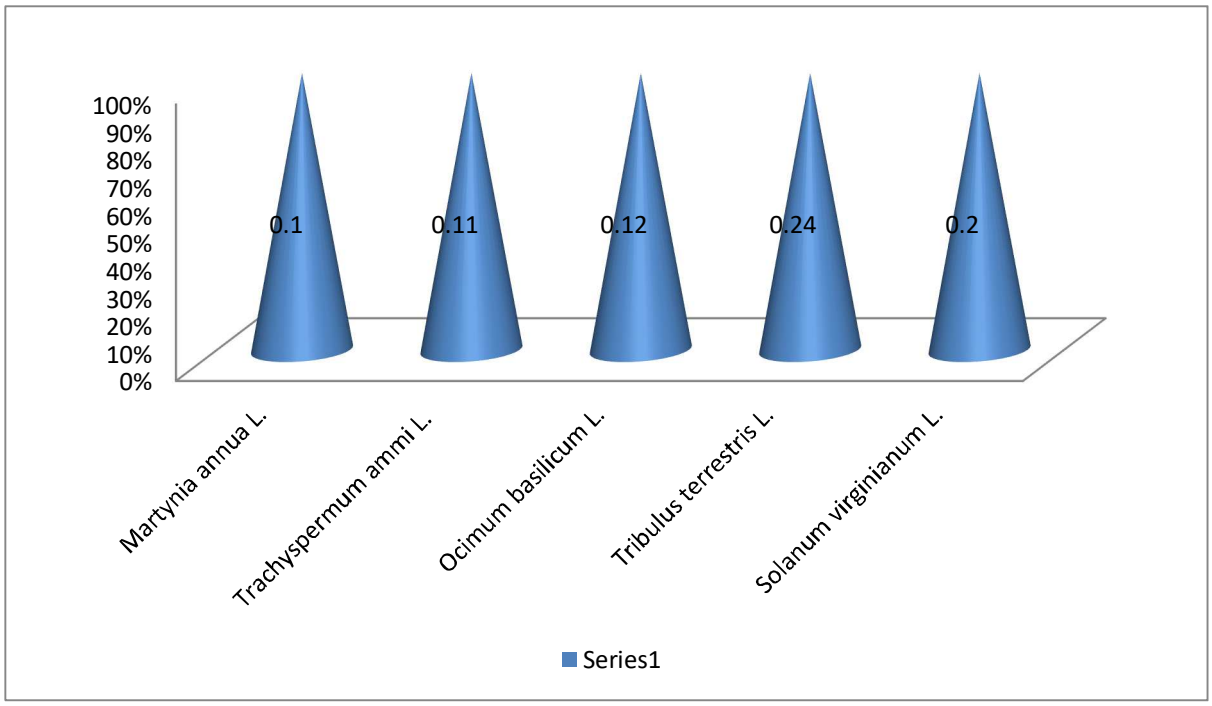

Figure 6: Use Value (UV)

Table 2: Medicinal Uses of Important plants of Domel, KPK, Pakistan with RFC Values, UV, FC and UR Values.

S.NO	Taxon	Family	Local name	Habit	Part used	Mode of utilization	Disease treated	Active Phytochemical constituents	FC*	UR*	UV*	RFC*	Comparative Studies
1	*Ficuspalmata* Forssk.,	Moraceae	Khabara	Tree	Fruit, bark, leaves	Powder	Asthma, diabetes, Constipation, rheumatism	Phenol, tannins (Hegazy et al., 2013)	56	4	0.07	0.24	1● 2 ●3● 4● 5● 6● 7● 8● 9● 10▲ 11 ●12● 13● 14 ●15● 16● 17● 18● 19● 20●
2	*Ficusreligiosa* L.	Moraceae	Pipal	Tree	Leaves	Decoction	constipation, Gonorrhea	Tannins, saponins, flavonoids, steroids, terpenoids and cardiac glycosides (Makhija et al., 2010)	69	2	0.02	0.29	1 ■2● 3■ 4● 5▲ 6● 7 ● 8● 9● 10▲ 11 ●12● 13● 14● 15 ▲16■ 17 ●18 ●19 ■20●
3	*Gloriosasuperba* L.	Colchicaceae	Kiari	Climber	Tuber	Powder	Skin Diseases	Alkaloid, amino acids, carbohydrates and proteins (Ashokkumar et al., 2013)	37	1	0.02	0.16	1● 2● 3 ●4● 5 ●6● 7● 8● 9● 10● 11● 12● 13● 14 ●15● 16● 17● 18● 19● 20●
4	*Guilandinabonduc* L.	Fabaceae	Karajwa	Shrub	Seeds	powder	Fever, kill worms	Homoisoflavonoids, caesalpinianone, and 6-*O*-methylcaesalpinianone (Ata et al., 2009)	44	2	0.04	0.19	1● 2● 3● 4 ●5● 6● 7● 8● 9● 10 ●11● 12● 13 ●14● 15● 16● 17● 18● 19● 20●
5	*Hordeum vulgare* L.	Poaceae	Joa	Herb	Fruits	Powder	Dysentery, typhoid, tuberculosis,	Phenolic compound (Duh et al., 2001)	44	3	0.06	0.19	1● 2 ●3● 4● 5● 6 ●7 ●8● 9● 10▲ 11● 12● 13● 14● 15● 16● 17● 18● 19● 20●
6	*Justiciaadhatoda* L.	Acanthaceae	Bansa	Shrub	Whole plant	Decoction, powder	Diabetes, cough, asthma, menses	Alkaloids, lignin, flavonoids, and terpenoids essential oils, vitamins, fatty acids. (Corrêa and Alcântara, 2012)	80	4	0.05	0.34	1● 2● 3● 4● 5 ●6● 7 ●8● 9 ●10 ●11■ 12● 13● 14● 15 ●16● 17 ●18● 19● 20●
7	*Lawsoniainermis* L.	Lythraceae	Mehndi	Shrub	Whole plant	Decoction, paste	Diuretic, blood purifier	Carbohydrate, proteins, flavonoids, tannins and phenolic compounds. (Chaudhary et al., 2010)	25	2	0.08	0.26	1● 2● 3■ 4● 5● 6 ●7● 8● 9 ●10● 11 ●12● 13● 14 ●15 ●16● 17 ▲18● 19 ●20●
8	*Malva parviflora* L.	Malvaceae	Sonchal	Herb	Leaves, stem	Decoction, cooked	constipation, rickets	Flavonoids, tannins, phenols, saponins, alkaloids, resins. (Farhan et al., 2012)	53	2	0.03	0.23	1● 2● 3● 4● 5▲ 6● 7● 8● 9 ●10▲ 11● 12● 13● 14● 15▲ 16● 17▲ 18● 19● 20●
9	*Martyniaannua* L.	Martyniaceae	Hath Jori	Shrub	Leaves, Fruits	Juice	Inflammation, Epilepsy	Alkaloid, glycosides, tannin, carbohydrates. (Mali et al., 2002)	20	2	0.1	0.09	1● 2 ●3 ●4 ●5● 6 ●7● 8● 9 ●10 ●11● 12● 13● 14● 15● 16 ●17● 18● 19● 20●
10	*Matricariachamomilla* L.	Asteraceae	Baboona	Herb	Flowers	Ointment	Body pain, backbone pain	Terpenoids, flavonoids, Coumarins, and spiroethers. (Tolouee et al., 2010)	61	2	0.03	0.26	1● 2 ●3● 4● 5● 6● 7● 8● 9● 10● 11● 12● 13● 14● 15● 16● 17● 18● 19● 20●

11	*Melia azedarach* L.	Meliaceae	Daraik	Tree	Leaves	Decoction	Leprosy, allergy, litching	Tannins, triterpenes and alkaloids. (Maciel et al., 2006)	72	3	0.04	0.31	1● 2● 3● 4● 5▲ 6 ●7● 8● 9▲ 10 ▲11● 12● 13● 14● 15▲ 16■ 17 ▲18 ●19● 20●
12	*Menthalongifolia* (L.) Huds	Lamiaceae	Podina	Herb	Leaves	Powder, decoction	Dysentery, asthma, jaundice, intestinal worms	*cis*-piperitone epoxide, pulegone and piperitenone oxide. (Gulluce et al., 2007)	64	4	0.06	0.27	1● 2● 3● 4● 5● 6▲ 7● 8● 9● 10▲ 11 ■12● 13● 14● 15● 16 ●17● 18● 19● 20●
13	*Mirabilis jalapa* L.	Nyctaginaceae	Gul Basi	Herb	Leaves, seeds, flower	Powder, ointment, cooked, extract	Wounds, jaundice, deopsy, menses disorders	Phenolic and flavonoid (Hajji et al., 2010)	44	4	0.09	0.19	1● 2● 3 ●4● 5● 6● 7● 8● 9● 10▲ 11● 12● 13● 14● 15● 16 ●17● 18● 19 ●20●
14			Janglikaraila	Climber	Fruit	Juice, powder	Diabetes's, jaundice, irritation, kidney stone, gonorrhea	Triterpenes and steroidal compounds. (Luo et al., 1998)	36	5	0.13	0.15	1● 2● 3● 4 ●5● 6 ●7● 8● 9● 10● 11● 12● 13● 14● 15● 16 ●17● 18● 19● 20●
15	*Morus alba* L.	Moraceae	Chitta toot	Tree	Fruit	Extract	Sexual tonic	Flavonol glycoside. (Enkhmaa et al., 2005)	76	1	0.01	0.32	1● 2● 3● 4 ●5● 6● 7● 8● 9■ 10 ▲11● 12● 13● 14● 15● 16 ■17● 18● 19● 20●
16	*Morusnigra* L.	Moraceae	Kala toot	Tree	Fruit, Leaves	Extract, decoction	Cough, throat diseases	Flavonoids. (Pawlowska et al., 2008)	50	2	0.04	0.21	1● 2● 3 ●4● 5■ 6● 7 ●8 ●9■ 10▲ 11● 12 ●13● 14 ●15 ●16■ 17 ●18● 19● 20●
17	*Nasturtium officinale* R.Br.	Brassicaceae	Jal dhania	Herb	Leaves	Decoction	Scabies, blood purifier, vermifugl, diuretic	Phenolic compounds.(Bahramikia and Yazdanparast, 2008)	48	4	0.08	0.21	1 ●2● 3● 4● 5● 6● 7● 8● 9● 10● 11● 12● 13● 14 ●15● 16 ●17● 18 ●19● 20●
18	*Ocimumbasilicum* L.	Lamiaceae	Niazbo	Herb	Leaves	Paste	Insects repellent	Phenolic acids (Javanmardi et al., 2002)	29	1	0.03	0.12	1 ●2● 3● 4 ●5● 6 ■7 ●8● 9● 10■ 11 ●12 ●13 ●14 ●15● 16● 17● 18● 19● 20●
19	*Opuntiamonacantha* (Willd.)	Cactaceae	Nag phani	Shrub	Fruits,stem, seed	Ointment	Gonorrhea, syphilis, carthartic, pox ,leprosy, rheumatism	Flavonoids. (Valente et al., 2010)	47	6	0.12	0.2	1● 2● 3● 4● 5● 6 ●7● 8● 9 ●10 ▲11● 12● 13● 14● 15 ●16● 17● 18● 19● 20●
20	*Phyla nodiflora* (L.)	Verbenaceae	Jalnim	Herb	Leaves, young shoots	Extract	Kidneys disorders, digestion problems	Triterpenoids, flavonoids and steroids (Amir et al., 2011)	30	2	0.06	0.13	1 ●2 ●3● 4● 5● 6● 7● 8● 9● 10 ●11● 12● 13● 14● 15● 16● 17■ 18● 19● 20●
21	*Pistaciachinensis* Bunge,	Anacardiaceae	Kakar	Tree	Fruit	Extract	Cough, antidote	Tannins, flavonoids, terpenoids, sterols, poly unsaturated fatty acids. (Limin et al., 2014)	60	2	0.03	0.26	1●2● 3● 4● 5● 6● 7● 8● 9 ●10● 11● 12● 13● 14● 15● 16● 17● 18● 19● 20●
22	*Plantago ovata* Forssk.,	Plantaginaceae	Ispagol	Herb	Fruit bark	Maceration	Dyspepsia, urine problems	Polysaccharides, lipids, caffeic acid derivatives, flavonoids, iridoid glycosides and terpenoids (Samuelsen, 2000)	62	2	0.03	0.26	1■2■ 3● 4● 5● 6● 7● 8● 9 ●10▲ 11● 12 ●13● 14 ●15 ●16● 17 ●18● 19● 20●
23	*Prosopisjulifl*	Fabaceae	Kikri	Shrub	Branch	Paste	Freckless, asthma,	Alkaloids (Nakano et al.,	55	5	0.09	0.24	1● 2● 3● 4● 5● 6● 7● 8●

	ora (Sw.) DC.				es		cough, leucorrhea, pimples	2004)					9● 10● 11● 12● 13● 14● 15 ●16● 17● 18● 19 ●20●
24	*Rhazyastricta* Decne.,	Apocynaceae	Sihar	Shrub	Leaves	Powder	Blood purification, diabetes, skin disease, indigestion menstrual problem, cancer irritation	Alkaloids. (Gilani et al., 2007)	75	4	0.05	0.32	1● 2 ●3● 4● 5● 6● 7● 8● 9 ●10● 11 ●12● 13● 14● 15● 16● 17● 18● 19● 20●
25	*Ricinuscommunis* L	Euphorbiaceae	Harnoli	Shrub	Seeds, leaves	Decoction	Cathartic, paralysis, muscle tonic, inflammation, asthma, cough	Fatty acids and ricinine(Bigi et al., 2004)	57	6	0.10	0.24	1● 2● 3● 4■ 5● 6● 7● 8● 9▲10▲ 11● 12● 13● 14● 15▲ 16● 17 ▲18● 19 ■20●
26	*Rosa damascena* Mill.,	Rosaceae	Gulab	Shrub	Petals and buds	Paste, extract	Cardiac diseases, skin disorders, flue. brain disorder	Volatile essential oils, fats, resins, malic, tartaric and tannic acids.(Achuthan et al., 2003)	51	4	0.07	0.22	1● 2● 3● 4● 5● 6● 7● 8● 9● 10● 11● 12● 13● 14● 15● 16● 17▲ 18● 19● 20●
27	*Rumexdentatus* L.	Polygonaceae	Janglipalak	Wild herb	Leaves, stem	Paste, cooked	Gastric problems, constipation	Alkaloids, saponins, anthraquinones and tannins. (Fatima et al., 2009)	30	2	0.06	0.13	1● 2● 3● 4● 5● 6● 7● 8 9● 10■ 11● 12● 13■ 14 ●15● 16● 17● 18● 19● 20●
28	*Salvadoraoleoides Decne.*	Salvadoraceae	Peeloo	Shrub	Seeds, seed oil, bark, Fruits	Paste	Toothach, diuretic		41	2	0.04	0.18	1 ●2■ 3● 4● 5■ 6● 7● 8■ 9▲ 10 ●11● 12● 13 ●14● 15● 16 ●17▲ 18● 19● 20●
29	*Salvia aegyptiaca* L	Lamiaceae	Khaltara	Shrub	Seeds	Maceration	Gastric troubles, hepatitis	Essential oil, Sterols. (BASAIF, 2004)	43	2	0.04	0.18	1● 2● 3● 4 ●5● 6● 7 ●8 ●9 ●10● 11● 12● 13● 14 ●15● 16● 17● 18● 19● 20●
30	*Salvia moorcroftiana* Wall. exBenth.	Lamiaceae	Gadkan	Herb	Leaves	Poultice	Tonic, bone fracture	Polyphenols (Lu and Foo, 2002)	54	2	0.03	0.23	1 ●2 ●3● 4 ●5● 6● 7● 8● 9● 10● 11 ●12● 13● 14● 15● 16● 17● 18● 19● 20●
31	*Salvia plebeia* R.Br.,	Lamiaceae	Sukh	Herb	Seeds, leaves	Powder	Toothache, male sexual disorders	Caffeic acid, luteolin-7-glucoside, nepetin-7-glucoside, luteolin, nepetin and hispidulin. (Jin et al., 2008)	36	2	0.05	0.15	1● 2● 3● 4● 5● 6● 7● 8● 9● 10● 11 ●12● 13● 14● 15● 16● 17● 18● 19● 20 ●
32	*Senna occidentalis* L.	Fabaceae	Kasundi	Herb	Leaves	Decoction	Dropsy, cough, blindnss	Carbohydrates, glycosides, cardiac glycosides, steroids, phytosterols, gums and mucilages (Arya et al., 2010)	37	3	0.08	0.16	1● 2● 3● 4● 5● 6● 7● 8● 9● 10● 11● 12● 13● 14● 15● 16● 17● 18● 19● 20●
33	*Sesamumindicum* L.	Pedaliaceae	Til	Herb	Seed	Powder	Urinary disease	Oil, lignin (Hu et al., 2004)	71	1	0.01	0.3	1● 2● 3 ●4 ●5● 6● 7● 8● 9● 10● 11● 12● 13 ●14

													●15● 16● 17● 18 ●19■ 20●
34	*Sidaspinosa* L.	Malvaceae	Makhnibooti	Shrub	Whole plant	Powder	Sexual disorders	Alkaloids. (Prakash et al., 1981)	48	1	0.02	0.21	1● 2● 3● 4● 5● 6 ●7● 8● 9● 10● 11● 12● 13● 14 ●15● 16 ●17● 18 ●19● 20●
35	*Solanum alternatopinnatum* Steud.,	Solanaceae	Mako	Herb	Whole plant	Juice, decoction	diuretic, sedative, stomach problems		72	3	0.04	0.31	
36	*Solanum incanum* L.	Solanaceae	Mahori	Shrub	Leaves, fruit	Raw	Belly disease, animal disease	Flavonoids .(Lin et al., 2000)	31	2	0.06	0.13	1● 2● 3● 4● 5● 6● 7● 8 ●9 ●10● 11 ●12 ●13● 14● 15● 16● 17● 18● 19● 20■
37	*Solanum nigrum* L.	Solanaceae	Kanchmanch	Herb	Leaves, Fruit	Decoction, cooked	Diabetes, eye diseases hysteria, tonic, blood pressure	Polyphenols, crude protein, crude lipid, crude fiber and carbohydrate (Jimoh et al., 2010)	44	4	0.09	0.19	1● 2● 3■ 4 ■5■ 6■ 7● 8■ 9■ 10 ▲11● 12▲ 13▲ 14 ●15 ■16 ●17● 18● 19■ 20■
38	*Solanum virginianum* L.	Solanaceae	Mokri	Herb	Fruit, Leaves, stem, roots	Decoction, powder, cooked	Anorexia, toothache, intestinal worms, diabetes, constipation, pimple, typhoid, influenza	Steroids, terpenes, phenolic compounds, saponins, fatty acids, alkaloids. (Khanam and Sultana, 2012)	40	8	0.2	0.17	1 ●2● 3 ●4● 5● 6● 7● 8● 9● 10● 11● 12● 13● 14● 15● 16 ●17● 18● 19● 20●
39	*Tetraena simplex* L.	Zygophyllaceae	Alithi	Herb	Leaves, seeds	Infusion	Eye diseases, leucoma		61	2	0.03	0.26	1●2● 3● 4● 5● 6● 7● 8● 9● 10● 11● 12● 13● 14● 15 ●16● 17● 18● 19● 20●
40	*Tinosporaglabra* (Burm.f.)	Menispermaceae	Gillo	Climber	Leaves, stem	Decoction, extract, milk juice	anorexia, blood purification, sexual tonic, malaria	Alkaloids, diterpenoid lactones, glycosides, steroids, phenolics. (Singh et al., 2003)	63	4	0.06	0.27	1● 2 ●3 ●4● 5● 6● 7● 8● 9● 10● 11● 12● 13● 14● 15● 16● 17● 18 ●19● 20●
41	*Trachyspermum ammi* L.	Apiaceae	Ajwain.	Herb	Fruit and root.	Raw	Sexual disorders, diarrhea, antiseptic, cholera	Essential oils (Kapoor et al., 2002)	35	4	0.11	0.15	1● 2● 3● 4 ■5● 6● 7● 8 ●9 ●10● 11 ●12● 13 ●14 ●15 ●16 ●17 ●18 ●19● 20●
42	*Tribulus terrestris* L.	Zygophyllaceae	Bukhra	Herb	Fruit	Powder, milk juice	urinary problems, colic, leprosy, kidney stone, dyspepsia, gonorrhea	Amide, steroid, Alkaloid. (Wu et al., 1999)	25	6	0.24	0.11	1 ●2● 3▲ 4● 5▲ 6 ●7● 8● 9● 10 ▲11■ 12● 13● 14 ●15▲ 16 ●17▲ 18● 19 ●20●
43	*Vernoniaanthelmintica* (L.) Willd.,	Asteraceae	Kali zeeri	Herb	Seeds	Paste	Skin disease, scabies and white spots.	Flavonoids. (Tian et al., 2004)	41	3	0.07	0.18	1 ●2● 3● 4● 5● 6 ●7● 8● 9● 10● 11● 12● 13● 14● 15● 16● 17● 18● 19● 20●
44	*Viola odorata* L.	Violaceae	Banafsha	Herb	Leaves	Decoction	Chest disease and infections.	Essential oil. (Akhbari et al., 2012)	38	2	0.05	0.16	1● 2● 3● 4● 5● 6● 7 ●8 ●9 ●10● 11● 12● 13● 14● 15● 16● 17● 18● 19● 20●
45	*Vitexnegundo* L.	Lamiaceae	Marvan	Shrub	Whole plant	Ointment, Poultice	Backache, toothache	Flavone, glycoside. (Sathiamoorthy et al., 2007)	52	2	0.03	0.22	1■ 2 ●3 ▲4● 5● 6 ●7● 8● 9● 10■ 11 ●12● 13● 14● 15● 16● 17 ●18 ●19● 20●

46	*Withaniacoagulans* (Stocks)	Solanaceae	Paneer doda	Shrub	Seeds	Powder, maceration	Diabetes	Triterpenoids. (Mirjalili et al., 2009)	51	1	0.01	0.22	1● 2■ 3● 4● 5▲ 6● 7 ●8● 9● 10■ 11■ 12● 13● 14● 15▲ 16 ●17 ●18● 19● 20●
47	*Withaniasomnifera* L.	Solanaceae	Aksin	Shrub	Roots, Leaves	Decoction, Poultice	Dysentery, itching, allergy, Leprosy	Withanolides. (Jayaprakasam et al., 2003)	60	4	0.06	0.26	1● 2■ 3● 4● 5■ 6● 7● 8 ■9■ 10■ 11●12● 13● 14● 15■ 16● 17■ 18 ●19 ●20●
48	*Xanthium strumarium* L.	Asteraceae	Kanda	Herb	Seed, leaves, roots	Powder	Small pox, malaria,	Caffeic acid, phenolic compounds. (Hsu et al., 2000)	51	2	0.03	0.22	1● 2● 3● 4 ●5● 6 ●7 ●8 ●9● 10● 11● 12● 13● 14● 15▲ 16● 17● 18● 19● 20●
49	*Ziziphus nummularia* (Burm.f.)	Rhamnaceae	Beri	Climber	Leaves	Powder, Poultice	Vomiting, hair tonic	Phenolic compounds , flavonoid (Gupta et al., 2011)	55	2	0.03	0.24	1● 2▲ 3● 4 ●5 ●6● 7● 8● 9● 10▲ 11● 12● 13● 14● 15● 16 ●17■ 18● 19● 20●

Plants reported with similar uses▲, Plants reported with dissimilar uses ■, Plants not reported ●
FC*=Frequency of citation (Frequency of Citation of Plants) **UR***= Use Report ***RFC**= Relative frequency of citation ***UV** =Use Value
1.(Iqbal et al., 2011), **2.**(Ahmad and Husain, 2008), **3.**(Ahmad et al., 2010), **4.**(Ahmad et al., 2010), **5.**(Abbasi et al., 2013),**6.**(Bibi et al., 2014). 7. (Mahmood et al., 2012)**8.**(Ahmad, 2006),**9.**(Qureshi et al., 2011), **10.**(Hayat et al., 2009), **11.**(Shinwari et al., 2011)**12.**(Manandhar, 1995), **13.**(Azaizeh et al., 2003), **14.**(Alzweiri et al., 2011)**15.**(Van Wyk et al., 2008)**16.**(Said et al., 2002). **17.**(Shrestha and Dhillion, 2003)**18.**(Aburjai et al., 2007)**19.**(Tene et al., 2007)**20.**(De-la-Cruz et al., 2007).

6. Conclusion

The present ethno medicinal study was carried out for the first time in Tehsil Domel, District Bannu, Pakistan. This study can contribute well in the protection of world traditional heritage as well as the collection of valuable knowledge to document a local record and improve the existing practices. Meanwhile, it's may contributes in writing the pharmacopeia in a better strategy, which is still a question subject. The proposed results demonstrate that the cultivators and local community have a vital and significant understanding showed by the assortment of various species used to treat few disorders, which is considered a worthy hotspot for social event. The Ethnomedicinal and pharmacological data in the region comparatively show fundamentals that how the societies and civilizations affect the entire basin's herbal knowledge in term of science promotion in various field, including medicine, pharmacy, biochemistry and biology in general. Least but not last, it is necessary and needs of time to perform research on the viability and wellbeing of conventional prescriptions with respect to home grown medication alignment with modern drug.

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Inhibition of Patulin-Induced Oxidative Stress in Human Esophageal Epithelial Cells by 3, 3'-Diindolylmethane

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ABSTRACT

To understand the effects of 3,3'-diindolylmethane on oxidative stress induced by patulin in HET-1A human esophageal epithelial cells and the underlying mechanism. HET-1A cells were treated with patulin to establish an oxidative stress model. The results of the CCK-8 assay indicated that low dose of DIM shows no obviously toxic effects on the HET-1A cells. Flow cytometric analysis indicated that DIM could inhibit patulin-induced ROS production in the HET-1A cells. The Western Blotting results showed that the protein expression of phospho-p38 MAPK and phospho-SAPK in the HET-1A cells gradually decreased; In addition, phospho-NF-κB expression also decreased significantly. It can be concluded that the underlying mechanism of DIM reduced the patulin-induced oxidative stress in HET-1A cells might be the inhibition of ROS production and regulation oxidative stress-related proteins such as NF-κB and the MAPK family. These results indicated that DIM might be categorized as an effective drug for treating or delaying the injury of esophageal epithelial cells due to oxidative stress.

KEYWORDS: 3,3'-Diindolylmethane; HET-1A cells; Patulin; Oxidative stress

INTRODUCTION

For healthy humans who are not occupationally exposed to xenobiotics, the main route of exposure to chemical is mouth-digestive tract-gastrointestinal pathway. So, oral epithelial cells (OEC), esophageal epithelial cells (EEC) and gastric mucosa epithelial cells (GMEC) are often the entry of xenobiotics to the body and are often the first layer of barrier or the first target tissue of the chemicals. Exposure to chemical factors such as alcohol, drugs and even dust particles with heavy metals or organic substances, will produce toxic effects to these cells and thereby causing the development of many diseases, including epithelial cell metaplasia, abnormal apoptosis, aging, inflammation and even tumors. Recent studies have found that oxidative stress caused by exogenous factors has a key role in the process of inducing epithelial cells toxicity [1]. Oxidative stress refers to a disruption in the balance between oxidation and anti-oxidation in cells, which thus causes the generation of a large number of reactive oxygen species (ROS). On one hand, free radicals directly react with a variety of biological macromolecules to cause the degeneration of these biological macromolecules, lipid peroxidation, membrane damage, the acceleration of cell aging, and an increased risk of tumor development[2]. On the other hand, free radicals can activate many inflammatory signal moleculars, such as mitogen activated protein kinases (MAPKs) family and nuclear factor-κB (NF-κB), thus causing inflammatory responses in the skin and the degradation of the matrix. Studies have demonstrated that ROS are one of the most important factors that cause oxidative stress [3].

Patulin (PA), also known as penicillium patulum toxin and coral penicillium toxin, is a product of metabolism produced by *Penicillium* and *Aspergillus fungi*. PA shows a broad spectrum of antibiotic characteristic. However, because PA is toxic to human potentially, it is no longer used as a therapeutic drug[4]. Studies indicated that PA from apple and other fruits is mainly derived from the contamination of *Penicillium fungi*. Toxicological tests show that PA has a toxic effect on digestive and reproductive systems [5, 6]. Patulin has teratogenicity, which is harmful to the human body, resulting in the damage of respiratory and urinary system, and leading to nerve paralysis, pulmonary edema and renal failure [4, 7]. Patulin was first discovered in rotten apple and apple juice, and widely exists in many kinds of fruits and moldy silage. PA is a toxic substance, which is mainly absorbed into the digestive tract and entered the blood circulation and distributes in the organs and tissues. The toxicity of PA could be reduced after been catalyzed by the cytochrome P450 enzyme, but the metabolites are toxic, too [8, 9]. PA can lead to the damages to respiratory system, nerve system and urinary system. Many research works show that PA is a potential carcinogen, teratogen, and mutagen [10, 11]. *In vitro*,

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PA could induce DNA damage and gene mutation in various cell strains [12-14]. *In vivo*, hypodermic injection of PA has a carcinogenic effect on mice [14]. The chemical mutagenesis committee of food, consumer goods and the environment of the UK has classified PA as mutagenic substance. So, it is essential to inhibit or prevent the toxicity of PA.

Indole-3-carbinol (I3C) is a confirmed cancer chemopreventive substance and can be extracted from cruciferous vegetables including radish, celery, and cauliflower. 3,3'-diindolylmethane (DIM) is a dimer obtained from the oligomerization of IC3 under acidic conditions, such as in gastric acid. The anticancer and anti-oxidant abilities of DIM have been confirmed in many cell models [15, 16]. However, whether DIM can inhibit patulin-induced toxicity in human HET-1A esophageal epithelial cells as well as the mechanism underlies the toxicity has not been reported. Therefore, in this study, patulin was used as a xenobiotic to observe the antagonistic effect of DIM on patulin-induced toxicities in HET-1A cells and the underlying mechanism of DIM was investigated so as to offer a novel theoretical principal for the prevention and disposal method of toxicity-induced by PA to esophageal epithelial cells.

MATERIAL AND METHODS

Materials

Human esophageal epithelial cell HET-1A was got from China Center for Type Culture Collection. Fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM), streptomycin, penicillin and trypsin were purchased from Gibco (USA). DIM, PA, dimethyl sulfoxide (DMSO) and dichlorodihydrofluorescein diacetate (DCFH-DA) were supplied by Sigma (USA). The rabbit anti-phospho-NF-κB, anti-phospho-p38 MAPK, and anti-phospho-SAPK/JNK antibodies and mouse anti-β-actin monoclonal antibodies were all bought from Cell Signaling Technology (CST,USA). Flasks, culture dishes, and plates were all obtained from Corning (USA).

Cell culture

HET-1A cells were cultured with DMEM containing FBS (10%), penicillin (100 IU/mL), and streptomycin (0.1 mg/mL) at 37°C in an atmosphere with 95 % humidity and a 5 % volume fraction of CO_2. The cell was tested or passaged when the cells reached 80 % - 90 % confluency.

Inhibition of cell proliferation experiment

The Cell Counting Kit-8 (CCK-8) (Beyotime) was used to determine cell survival rates. HET-1A cells at a concentration of 5×10^4 cell/mL were cultured in DMEM for 24 h, and then were treated with DIM for 24, 48, and 72 h. The medium was changed with serum-free medium prior to analyzing the inhibitory effect of PA. After a 2-h treatment with PA, the cells were cultured for another 24 h. After treatment, 10 μL of the CCK-8 reagent was added into the well, and then incubated at 37℃ for 1 h. The Optic Density values (OD) at 450 nm were measured with Microplate Reader (Thermo, USA). The cell survival rates (CSR) were calculated: CSR (%)=$[(OD_{\text{treatment group}}-OD_{\text{blank control}})/(OD_{\text{control}}-OD_{\text{blank control}})]\times100$.

Detection of intracellular ROS content via flow cytometry

HET-1A cells were cultured into 6-well plates for 24 h. The experiment group was treated with DIM (10 μM) for 24 h and then treat PA for 24 h. After treatment, 10 μL DCFH-DA was added into each well, and then cultured for 0.5 h. The cellular ROS content was then detected via flow cytometry (Becton, USA).

Detect the expression of proteins

Cells were lysed with lysis buffer. Total cellular proteins were then extracted, and the concentrations of protein were determined with the BCA method. The amount of protein in each well was 40 μg. Samples were separated using 4 - 12 % NuPAGE precast gels (Invitrogen) and were transferred onto a methanol-immersed polyvinylidene fluoride membrane. The membrane was blocked with skimed milk at room temperature for 1 h and was incubated with primary antibodies (antibodies to phospho-NF-κb, phospho-p38 MAPK, and phospho-SAPK/JNK antibodies at 1:2,000 dilution, the mouse anti-β-actin antibody at 1:1,000 dilution) at 4℃ overnight. After washing with phosphate-buffered saline (PBS)-Tween (PBST) buffer 3 times for 10 min each, the membrane was incubated with goat anti-rabbit secondary antibodies or goat anti-mouse secondary antibodies at room temperature for 2 h and then washed with PBST. The protein bands were developed with chemiluminescence reagents, and the film was developed and fixed. The gray densities of the Western blot results were scanned using the Quantity One software. The relative O.D. value of each band was measured, and the results were compared for the semi-quantitative analysis.

Statistical analysis

The data were statistically analyzed with the SPSS software. All the data were presented as $\bar{x} \pm s$. One-way analysis of variance (ANOVA) and Dunnett's t test were performed. The examination level was α=0.05. The protein expression levels were examined using Pearson's correlation analysis. The difference of statistically significant was defined as P value less than 0.05.

RESULTS

The inhibitory effect of DIM on the proliferation of HET-1A cells

The effect of DIM on the proliferation HET-1A cells was measured to confirm the maximum dose of DIM used in these research works had no toxic effect. Cells were treated with different levels of DIM, and the results indicated that the DIM concentrations and cell survival rates had a dose-response relationship. With 48 h of treatment at concentrations lower than 10 μM, DIM had no obvious toxic effects on the HET-1A cells (P>0.05) (Table 1). Therefore, concentrations of 5 μM and 10 μM were chosen for subsequent experiments.

Table 1. Effects of 1-10 μM DIM on the survival rate of HET-1A cells (%)

group	24 h	48 h	72 h
Control group	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
1 μM	103.41 ± 2,83	102.11 ± 0.99	103.02 ± 3.85
2.5 μM	100.38 ± 2.06	101.65 ± 0.91	104.01 ± 2.87
5 μM	102.29 ± 2.71	99.81 ± 0.91	103.89 ± 3.95
7.5 μM	100.22 ± 2.71	101.31 ± 2.20	96.64 ± 2.29*
10 μM	101.42 ± 4.25	99.37 ± 0.45	86.98 ± 3.57**
F value	1.434	2.958	27.453
P value	0.241	0.057	0.000

Note: * P<0.05, ** P<0.01 compared to the control group.

The inhibitory effect of PA on the proliferation of HET-1A cells

As shown in Table 2, treatment with 50 μM PA for 2 h had significant inhibitory effects on the cells, and the cell survival rate was reduced to 82.25%. With increasing PA concentrations, the inhibitory effect on the HET-1A cell growth significantly increased; after treatment with 200 μM PA for 2 h, the cell survival rate was 71.93%, the difference between 50 and 200 μM PA was statistically significant (F=104.836, P<0.05). Therefore, the exposure condition for the PA treatment was between 50-200 μM for 2 h.

Table 2. Effects of 50-200 μM PA on the survival rate of HET-1A cells (%)

group	2 h
Control group	100.00 ± 0.00
50 μM	82.25 ± 2.80**
100 μM	78.65 ± 2.02**
150 μM	76.63 ± 1.50**
200 μM	71.93 ± 1.58**
F value	104.836
P value	0.000

Note: * P<0.05, ** P<0.01 compared to the control group.

Inhibition by DIM of PA-induced ROS production

As shown in Table 3, with increasing PA concentrations, the concentrations of cellular ROS in the treatment group also gradually increased. After the treatment of HET-1A cells with PA for 2 h, the cellular ROS level was approximately 3.6 times that in the control group. After 24 h of DIM pretreatment, the cellular ROS level distinctly decreased as compared with those in the group treated with PA alone (F=61998.17, P<0.05). This result indicated that DIM could effectively inhibit PA-induced ROS production in the HET-1A cells.

Table 3. Effects of DIM (10 μM) on PA-induced ROS production

group	PA concentration				
	0 μM	1.25 μM	2.5 μM	5 μM	10 μM
Model group	2,459 ± 9.61	4,704 ± 16.44	5,016 ± 12.23	5,780 ± 32.19	11,348 ± 17.52
Treatment group (DIM, 10 μM)	2,039 ± 19.50	3,300 ± 19.08	3,541 ± 14.27	4,467 ± 10.83	7,274 ± 25.63
t value	33.407	96.577	134.429	66.975	227.271
P value	0.000	0.000	0.000	0.000	0.000

DIM inhibit the activation of MAPK and NF-κB induced by PA

Two hours after treating HET-1A cells with PA, the levels of phospho-p38 MAPK, phospho-NF-κB, and phospho-SAPK/JNK were assayed. The results indicated that with increasing PA concentrations, the gray value ratios of the roteins showed an increasing trend (Fig. 1). The Pearson correlation analysis resulted in values of $r>0.946$ and $P<0.05$ for the PA concentrations and expression levels of the oxidative stress-related proteins. This result indicated that PA could induce the increase of the proteins expression. The data presented in Fig. 2, in which cells were pretreated with DIM for 24 h followed by exposure to PA, reveal that with an increase in the DIM concentration and the extension of the PA treatment time (>30 min) the relative O.D. ratio of each protein band exhibited a decreasing trend (statistical values between groups, $P<0.05$). The Pearson correlation analysis between the DIM concentration and the levels of proteins, between the PA treatment time and the levels of the proteins resulted in r values that were lower than -0.742 and -0.936, respectively. Therefore, DIM could significantly inhibit PA-induced phospho-NF-κB, phospho-p38 MAPK, and phospho-SAPK/JNK expression. In addition, when the PA treatment time was longer, the inhibitory effect was more evident.

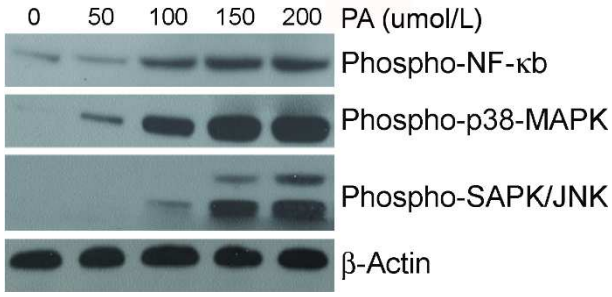

Fig 1. PA increased phosphorylation levels of phospho-NF-κB, phospho-p38 MAPK, and phospho-SAPK/JNK

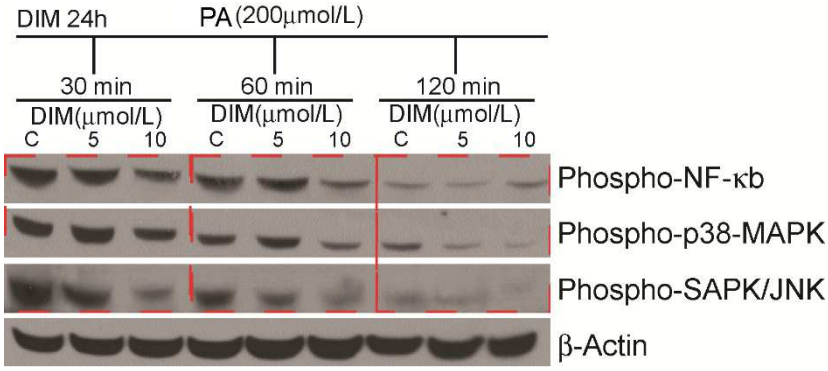

Fig 2. DIM had inhibitory effects on PA-induced activation of phospho-NF-κB, phospho-p38 MAPK, and phospho-SAPK/JNK

DISCUSSION

Many studies showed that cell injury could be caused by ROS-induced oxidative stress [17]. ROS can damage nuclear DNA and mitochondrial DNA. In addition, ROS can activate matrix metalloproteinases in HET-1A cells to damage the extracellular matrix and degrade dermal collagen and elastin, thus causing cell injury [18].

Increasing evidence indicates that PA is one of the important factors causing the amplification of oxidative stress in malignancies [19]. This study successfully established an oxidative stress model with HET-1A cells treated by PA. In the HET-1A cells, PA significantly increased cellular ROS concentrations and activated the expression of oxidative stress proteins.

Many phytochemicals have anti-oxidant functions. I3C is an active ingredient in cruciferous vegetables that has been confirmed to be an effective ROS scavenger [20]. As an important derivative of I3C under acidic conditions, such as in gastric acid, DIM is the major bioactive component that enables I3C to exert anti-oxidant activity. This study found that low doses of DIM could effectively reduce PA-induced ROS production. DIM may inhibit ROS production through a variety of mechanisms [21, 22]. It is known that the anti-oxidant mechanisms of DIM include the inhibition of ROS accumulation to inhibit the expression of the NF-κB signals[23, 24] and the inhibition of oxidative stress through the BRCA1-dependent anti-oxidant signaling pathway[25]. The family of MAPKs, which includes MAPK, ERK and JNK[26], is a cluster of threonine / serine kinases in cells. These family members can transduce extracellular stimulatory signals into cells; therefore, the MAPKs play very important roles in many processes of cell biological reactions. Among these processes, as an important member of the MAPK family, p38 MAPK participates not only in the process of cell growth and development but also in the regulation of cell proliferation; therefore, p38 MAPK is considered to be a hub of a variety of signal transduction pathways[27]. Currently, many studies indicate that the MAPK signaling pathway is associated with oxidative stress. In addition, during oxidative stress, the expression levels of some of the proteins in the MAPK family also increase. However, it has not been reported whether the mechanism by which DIM exerts an anti-oxidant function is mediated by the inhibition of the MAPK signals.

The NF-κB signaling pathway is closely associated with oxidative stress in cells. NF-κB is an induced transformation factor; this factor is very sensitive to ROS produced by oxidation. ROS can directly activate NF-κB in some cells[28]; activated NF-κB can induce cells to express nitric oxide synthase (NOS), different adhesion molecules, and other cytokines. These components act cooperatively together to aggravate the damage produced by oxidative stress. Previous literature has shown that DIM inhibits the accumulation of ROS and inhibits the activation of NF-κB signals, thus exerting anti-oxidant activities. Similarly, the MAPK signaling pathway is also closely associated with oxidative stress. However, whether DIM can inhibit oxidative stress through the MAPK signaling pathway is still unknown. Based on the HET-1A oxidative stress model that was established, this study found that with increasing DIM concentrations, the expression levels of PA-activated oxidative stress-related proteins decreased significantly; this inhibitory function gradually became significant as the PA treatment time increased. Given the combination of results in this study, it is suggested that, in HET-1A cells, DIM could exert anti-oxidative stress functions, the mechanisms underlies which might be attributed to inhibit ROS and activate the NF-κB and MAPKs signals.

In summary, we speculate that DIM could play a specific role in inhibiting the oxidative injury in esophageal epithelial cells, suggesting that DIM might be used as an effective drug for treating or delaying oxidative-stress types of digestive tract injury.

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Housing Adjustment Phenomena in the Coastal Area of Muara Angke, North Jakarta, Indonesia

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ABSTRACT

In the early 1970s, the Government of Jakarta developed Muara Angke, a delta located at the north coast of the city as a new settlement to accommodate housing facilities for the local fishery community members. Muara Angke was an area vulnerable to flooding. During the year 1977 to 2012, ten significant floods had occurred at Muara Angke, causing damages to people's houses and degrading the quality of their lives. Consequently, from time to time the residents had to adjust their houses and surrounding environments to overcome the critical conditions. The objective of this study wasto investigate the housing adjustments made by the residents in response to the threat of flood.This research was carried out using interview and field observation methods. Four housing blocks in Muara Angke, namely block H, L, K and Bermis were selectedas the objects of study.120 households were purposefully selected as respondents, to represent each housing block or sub-population proportionately. The research findings show that the flood incidents had forced the residents to 1) raise the elevation of the ground floor, to avoid water intrusion into their houses, and hence to prevent any damage to the exterior as well as the interior of the house. 2) heighten the ceiling clearance, to anticipate the effect of land subsidence for many years to come. 3) build additional floor levels as family refuge area in case a flood incident occurred.Also, depending on their financial ability, the residents increase the level of privacy and improve the physical standard of their living space, by 1) expanding floor area, 2) improving spatial configuration, and 3) adding income generating unit such as rental rooms and small-shop.
KEYWORDS: housing adjustment phenomena, coastal area, Muara Angke

INTRODUCTION

The coastal flood is a potential problem for Jakarta, the capital city of Indonesia [14]. It occurs due to several factors including land subsidence, sea level rise, tidal waves, storm surges, higher rainfall or water runoff from the hinterland, and sediment deposition on the river bed and estuaries. The rate of land subsidence varied between 3 to 15 cm/year [1]. Meanwhile, sea level rise average was 1.45cm/year during the years of 2005 to 2011 [6].

Muara Angke, a delta locatedat the north coast of Jakarta, is surrounded by Asin River in the east, Adem River in the west, and Jakarta Bay in the north. Initially, this wetland was dominated by mangroves. In the early 1970s,the Government of Jakarta decided to develop Muara Angke as a new settlement to accommodate the local fishery community members, including fishermen, boat owners, crews, and traders [13]. Since then Muara Angke had grown rapidly, covering residential areas, seaport, fish market, seafood center, cold storages, and salted fish production facilities. The concentration of human population and expansion of socio-economic activities had aggravated the land subsidence and increase the vulnerability of the delta, due to excessive groundwater extraction, heavy building structure load, and extensive infrastructure development [12]. Not surprisingly, flood incidents repeatedly struck Muara Angkein the year1977, 1984, 1985, 1994, 1996, 1999, 2002, 2007, 2011, and 2012, with water level ranging from 30 cm to 200 cm.

To mitigate the coastal flood, the government of Jakarta since the year 1984 to 1999had subsequently built a polder system, two retention ponds, two pump stations each fortified by four pumps, and a dike system. However, despite such technical solutions, flood incidents still occurred occasionally but no longer ona massive scale.

Flood incidents had caused disruption to people's daily activity, degraded their quality of life, and instigated significant impacts on property, infrastructure, and well-being of the people (stress, anxiety, trauma) [8].

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When the rainy season comes, people start to worry about flood incidents which might occur and cause damages to their belongings (furniture, electronic goods, glassware, and clothes) [5]. In such critical situation, people are forced to cope with all pressures or threats to achieve a minimum standard of safety and comfort [7].

The critical situation eventually triggers housing stress that demands immediate actions of the residents [2],to bring back housing conditions into new equilibrium [11] that meet their specific needs and cultural norms [4, 10]. In other words, the residents had to make necessary adjustments in order to bring their housing condition back into conformity with family's needs, expectations and aspirations [9].All in all, housing adjustment is a strategy to bridge the gaps between actual conditions and desired conditions. Unfortunately, not all individuals or groups of individuals are able tomake adjustment according to their desires for many reasons, such as psychological barriers, organizational weakness, limitation of resources, market distortions, racial and gender discriminations [3].

The objective of this study isto investigate the housing adjustments made by the residents of Muara Angke, in response to the threat of flood since 1977 to 2017. This study will identify housing adjustment strategies adopted by the residents, and transformation of the physical elements of their houses. As a ground-breaking study, it will focus on physical aspects of housing adjustment, rather than behavioural and cultural aspects of the residents. The impacts of physical aspects on behavioural and cultural aspects, vice versa, will be scrutinized in the next cycle of study. The results of this study will provide accurate empirical data needed by the government and other stakeholders involved in housing development along the north coastal area of Java island.

MATERIALS AND METHODS

This research is carried out using quantitative and qualitative approaches. 120 households were purposefully selected as respondents, to represent each housing block or sub-population respectively. Quantitative method is used to measure and analyze the percentage of physical changes made to the houses. Qualitative method is used to describe and explain the motives and reasons behind the physical changes of the houses. Research data were collected through field observation, building measurement, and direct interview with respondents. Four housing blocks are purposefully selected as objects of study, namely block H, L, K and Bermis in Muara Angke housing estate (Figure 1).

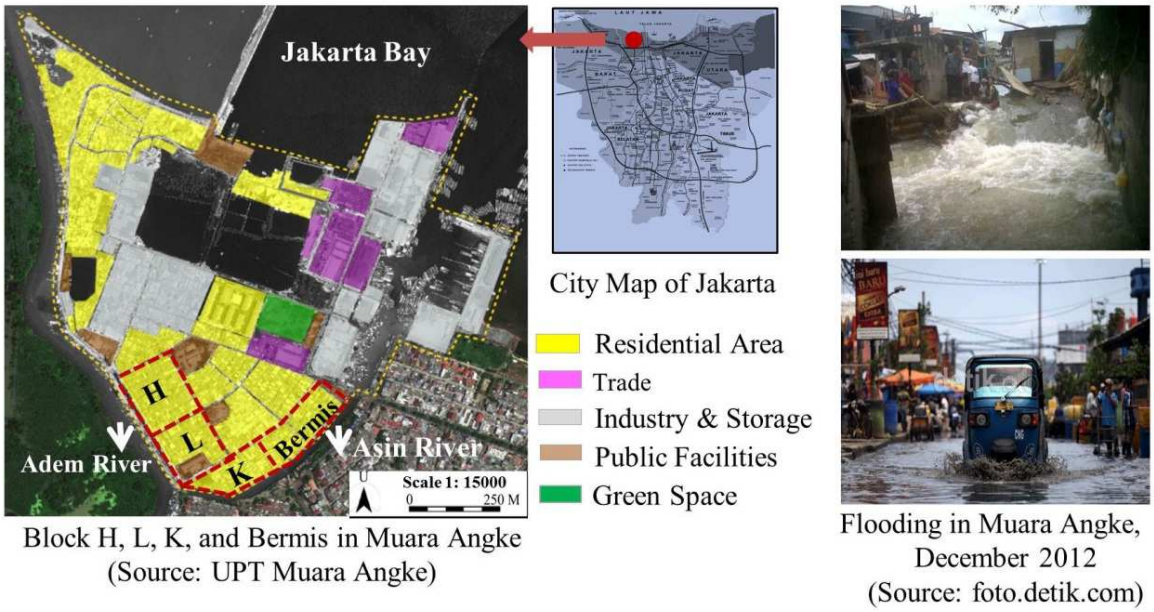

Figure 1. Block H, L, K and Bermis in Muara Angke, North Jakarta

The respondent comprised of 37 households (31.6%) from Block H, 24 (20%) from Block L, 28 (23.3%) from Block K, and 31 (25%) from Block Bermis. The numbers of respondent for each block are determined proportionately according to the total number of housing units in each block (220 in Block H, 140 in Block

L, 164 in Block K, and 184 in Block Bermis). They are selected based on the following sampling criteria: 1) the respondents have occupied their house for more than ten years, and 2) the respondents have renovated their houses at least within the last two years.

Originally houses in block H, L, K, and Bermis were all single-story buildings, but each block had its type in term of architectural design. House in Block H had75 sqm plot with 40 sqm floor area, Block L had 62.5 sqm plot with 30 sqm floor area, Block K had 50 sqm plot with 24 sqm floor area, and Block Bermis had 60 sqm plot with 21 sqm floor area. Each house had front-yard and back-yard, entrance porch, living room, one or two bedrooms, kitchen, small bathroom, with building coverage ranged from 45% to 53% (Figure 2). Building materials used were: stucco and terrazzo for the floor, plastered brick for the wall (exposed brick for block K),and corrugated zinc sheet for the roof. The house had minimum standards of the space requirement to live in, so that the price of the house can be reached by fishery community members.

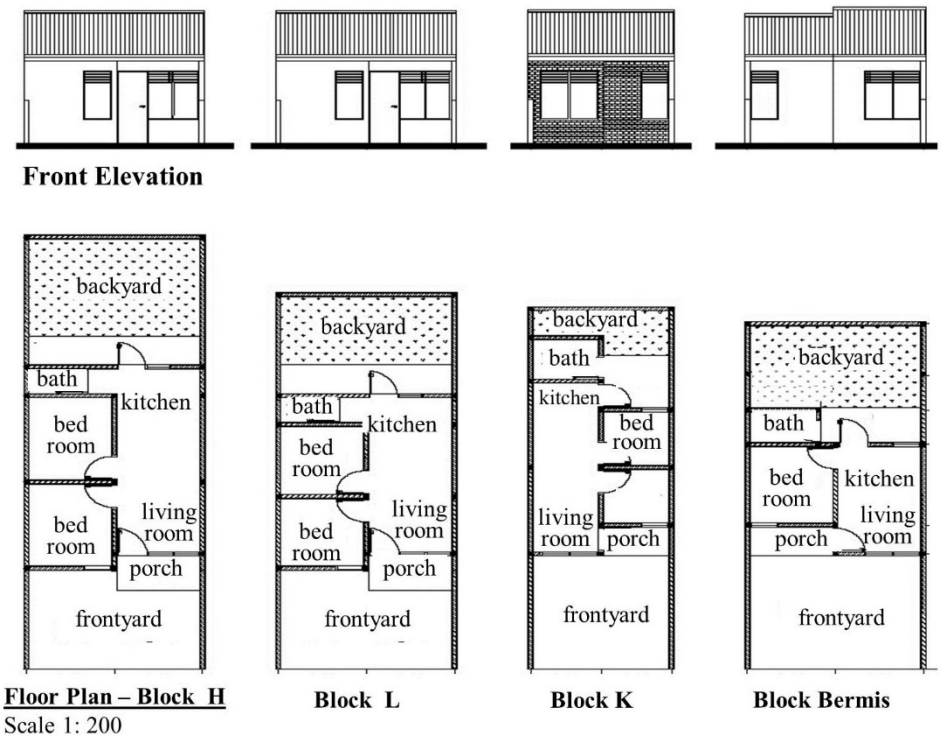

Figure 2. The original type of houses in Block H, L, K, and Bermis

RESULTS AND DISCUSSIONS

The analysis shows that family income level is the most influential factor that determines the ability of the residents to adjust their houses. Families with an income under 2.25 million rupiahs are usually facing financial difficulties in improving their houses (15.8%). Hence their houses belong to the category of "most vulnerable to flood."Family with income between 2.25to 5.25 million rupiahs (64.2%) generally can afford to adjust their house up to the minimum standard hence their houses belong to the category of "safe from flood."Those who have income over 5.25 million rupiahs (20%) can afford to adjust their house up to the maximum standard hence their houses belong to the category "free from flood", while also to improve the safety and comfort of their living milieu.

Educational level of the head of household also plays an influential role in the ability of the residents to adjust their houses. High school and baccalaureate graduates (45%) were more knowledgeable and proficient in managing and executing housing adjustment plans than elementary and junior high school graduates (55%). As far as the cultural backgrounds are concerned, Buginese (32.5%) and Javanese (30%) belong to the dominant ethnic groups, but the analysis shows that socio-cultural attributes have no significant influence on the ability of the residents to adjust their houses.
Based on its motives, housing adjustment phenomena in Muara Angke can be differentiated into two interrelated categories, namely 1) housing adjustment in response to the thread of flood incidents, and 2) housing adjustment to increase privacy and comfort.

Housing Adjustment in Response to the Thread of Flood Incidents

The analysis shows that the thread of flood incidents had forced the residents of Muara Angke to 1) raise the elevation of thegroundfloor, to avoid water intrusion into their houses, and hence to prevent any damage to the exterior as well as the interior of the house. 2) heighten the ceiling clearance, to anticipate the effect of land subsidence for many years to come.And 3) build additional floor levels as family refuge area in case that flood incident occurred (Figure 3 and Table 1).

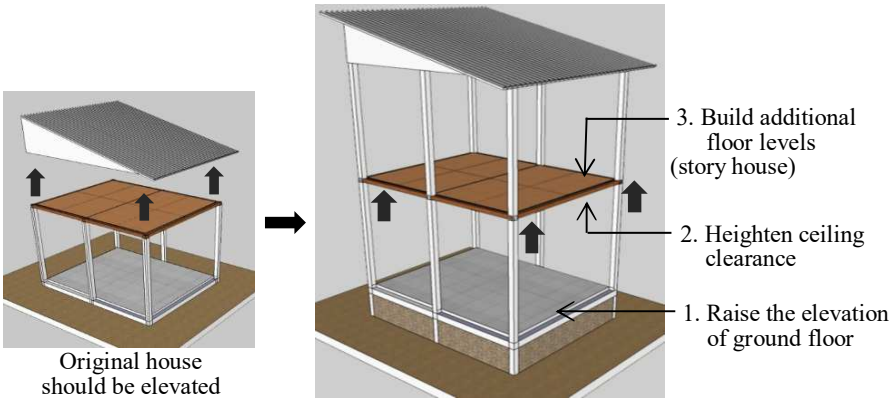

Figure 3. Three responses to the thread of flood incidents

Table 1. Housing adjustment in response to the thread of flood incidents

Housing Adjustment Patterns	Housing Blocks					Total Units	
	Block H	Block L	Block K	Block Bermis	Total	Total (n)	Percentage of 120 units
1) Raise the elevation of the groundfloor							
None (< 0 cm, under street level)	20.8%	25.0%	25.0%	29.2%	100.0%	24	20.0%
1-35 cm (above street level)	42.9%	16.7%	23.8%	16.7%	100.0%	42	35.0%
36-70 cm (above street level)	22.6%	25.8%	9.7%	41.9%	100.0%	31	25.8%
>70 cm (above street level)	30.4%	13.0%	39.1%	17.4%	100.0%	23	19.2%
2) Heighten the ceiling clearance							
≤280 cm	32.5%	17.5%	22.5%	27.5%	100.0%	40	33.3%
281-370 cm	28.8%	26.9%	25.0%	19.2%	100.0%	52	43.3%
371-460 cm	32.1%	10.7%	21.4%	35.7%	100.0%	28	23.3%
3) Build additional floor levels							
None (One-story house)	31.1%	20.0%	26.7%	22.2%	100.0%	45	37.5%
One (Two-story house)	32.8%	19.4%	22.4%	25.4%	100.0%	67	55.8%
Two (Three-story house)	14.3%	28.6%	14.3%	42.9%	100.0%	7	5.8%
Three (Four-story house)	0.0%	0.0%	0.0%	100.0%	100.0%	1	0.8%

1) Raise the elevation of the groundfloor

From the early 1970s to 2016, the residents of Muara Angke had raised the ground floor elevation of their houses up to five times, in response to the raises of street-levelelevation made consecutively by the government in the year 1985, 1995, 2011, and 2014.

The latest flood incident occurred in December 2012with the highest water level of 30 cm. Based on this water level figure, it can be predicted that those houses with the ground floor elevation below street level (20%) are“highly vulnerable to flood.”Those houses with the ground floor elevation 1-35 cm above street level (35%) is“vulnerable to flood.” Those houses with the ground floor elevation 36-70 cm above street level (25.8%) is considered “safe from the flood.”And those houses with the ground floor elevation70 cm abovestreet-level (19.2%)areconsidered as “the safest from the flood”(Figure 4). It should be noted that in general, the ground floor elevation of houses in Block Bermis is higher than houses in other blocks.

For easy maintenance and esthetical reasons, the residents had also replaced the old ground floor materials(stucco or terrazzo) with ceramic tiles (82.5%) and granite tiles (5%).They also provided concrete stairs as regular access from street elevation to the ground floor of their houses, and a concrete ramp in the middle to provide easy access for a motorcycle to enter their houses.

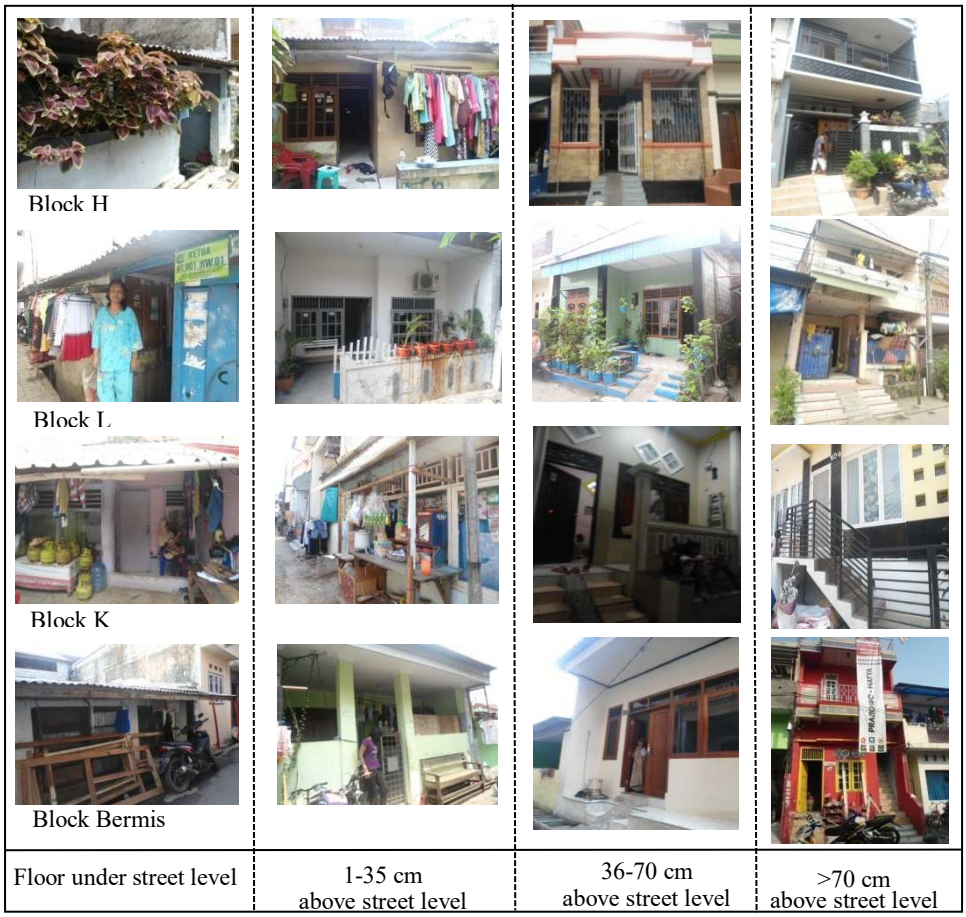

Figure 4. The elevation of ground floor: from "very vulnerable to flood" to "the safest from flood"

2) Heighten the ceiling clearance

To anticipate further land subsidence and street-level heightening project implemented by the government every 5 to 10 years interval, the residents heightened the ceiling clearance of their houses so that thereremainedspare clearance when they had to raise ground floor elevation of their houses in the next years to come. Data recorded during the field observation show conditions of ceiling clearance as follows (Table 1): low ceiling clearance, less than 280 cm (33.3%);high ceiling clearance between 281 to 370 cm (43.3%); and very high ceiling clearance between 371 cm to 460 cm (23.3%). It should be noted that in general, the ceiling clearance of houses in Block Bermis is higher than houses in other blocks.

Figure 5 shows houses with low ceiling clearance (less than 280 cm) compared to houses with high (281 to 370 cm) and very high ceiling clearance (more than 371to 460 cm). The residents can take benefits from higher ceiling clearance regarding thermal comfort, air circulation, and spatial volume.

Low clearance (less than 280 cm) | High clearance (281-370 cm) | Very high clearance (more than 370 cm)

Figure 5. The ceiling conditions: from "low clearance" to "very high clearance"

3) Build additional floor level

The residents had learned some lessons from previous flood incidents that occurred in Muara Angke. They are very reluctant to evacuate to refuge places and abandon their houses because they are concerned with the safety of their property and belongings. The best solution for them is to build additional floor levels so that when the flood incident occurred, they only had to evacuate to the upper floors and wait until the flood ceased. Although the residents just need one additional floor level as a refuge place, but in realities depend on their economic level, some of the residents can afford to build up to 3 other floor levels.

Table 1 shows that 37.5% of the residents cannot afford to build any additional floor level, 55.8% can afford to make one additional floor level, 5.8% can afford to build two other floor levels, and only 0.8% can afford to build three other floor levels. The distribution of other floor levels in four blocks can be seen in Figure 6.

The residents tended to use low-cost and light-weight materials for upper flooring and roofing because such materials can be easily dismantled and re-assembled whenever needed.Preferred materials for upper flooring are thick plywood (38.3%), concrete (18.3%), and steel decking (4.2%), while preferred materials for roofing are corrugated cement board (82.5%), roof tiles (12.5%), and corrugated zinc sheet (2.5%).

Figure 6. Distribution of additional floor levels: from "none" to "three."

Housing Adjustment to Increase Privacy and Comfort

The analysis also shows that depending on their financial ability, the residents were eager to increase the level of privacy and comfort of their living space, by 1) expanding floor area, 2) improving spatial configuration, and 3) adding income generating unit such as rental room and small-shop (Table 2).

Table 2. Housing adjustment to increase the level of privacy and comfort

Housing Adjustment Patterns	Housing Blocks					Total Units	
	Block H	Block L	Block K	Block Bermis	Total	Total (n)	Percentage of 120 units
a) Expanding floor area							
≤ 80 m²	26.7%	20.0%	35.6%	17.8%	100.0%	45	37.5%
81-140 m²	26.8%	19.5%	22.0%	31.7%	100.0%	41	34.2%
141-200 m²	56.5%	17.4%	8.7%	17.4%	100.0%	23	19.2%
> 200 m²	9.1%	27.3%	9.1%	54.5%	100.0%	11	9.2%
c) Improving spatial configuration							
Parallel configuration	27.3%	25.5%	23.6%	23.6%	100.0%	55	45.8%
Un-parallel configuration	33.8%	15.4%	23.1%	27.7%	100.0%	65	54.2%
b) Adding income generating unit							
With income generating	25.8%	22.6%	27.4%	24.2%	100.0%	62	51.7%
Withoutincome generating	36.2%	17.2%	19.0%	27.6%	100.0%	58	48.3%

1) Expanding floor areas

The residents expanded floor areas of their houses to provide ample and convenient spaces for their family member activities, including the addition of new bedrooms, family room, kitchen and bathrooms. Extra spaces are particularly needed when their children had grown up and other extended family members joined in into the house.

Data recorded during the field observation show conditions of floor areas as follows (Table 2): 37.5% of houses have floor area less than 80 sqm; 34.2% have between 81 to 140 sqm; 19.2% have between 141 to 200 sqm; and 9.2% have more than 200 sqm. It should be noted that houses in Block Bermis on average have bigger floor area compared to houses in other blocks, indicating that the residents of Block Bermis relatively belong to higher income group.

The expansion of floor areas of the house indeed can improve the level of privacy and comfort, but unfortunately at the expense of the loss of green open space, particularly the disappearance of front-yards and back-yards.

2) Improving spatial configuration

Originallyparallel spatial arrangement of the houses in Muara Angke did not consider the privacy of their residents. Living and dining activities were accommodated in an open-layout space, adjacent to bedrooms on the left or right side, and service area on the back side. Any guest who comes to visit the family cannot avoid witnessing all kind of happenings in that house. To improve the level of privacy of their space, the residents reorganize their internal living space by making an un-parallel spatial configuration so that more private activities can be concealed from outsiders (Figure 7). More than half of the respondents (54.2%) had succeeded to improve the level of privacy of their houses.

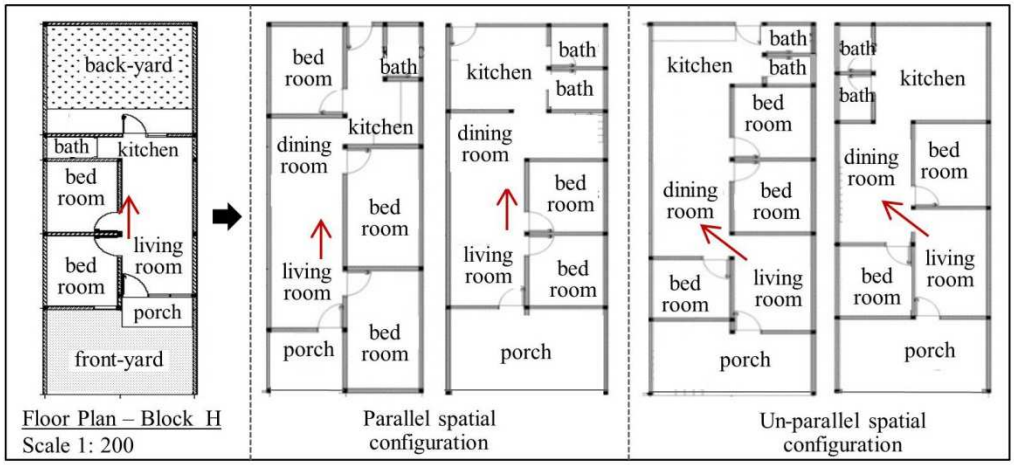

Figure 7. Improvement of spatial configuration

3) Adding income generating unit

In conjunction with the fast-growing commercial and industrial activities in the north-coast of Jakarta during the last few decades, Muara Angke has become an alternative housing accommodation for low-wage migrant workers who find their jobs in the respective areas. The demand for rental bedrooms had increased considerably so that the residents with the financial ability (51.7%) take this market opportunity by adding income generating unit such as rented bedrooms and small-shops while they adjusted their houses (Figure 8). The income generating units provide financial security to the heads of household who already entered the retirement age but still have to support the life of their extended families.84.6% of the heads of household are over 50 years old.

Although this income generating units contributed significantly to the financial sources of the family, however, some of the heads of household complained that the existence of strangers in their houses to a certain degree tend to reduce their level of privacy and security.

Figure 8. Addition of income generating unit: small shop and rental bedrooms

CONCLUSION

Housing adjustment phenomena in Muara Angke characteristically exemplify how the residents who resided in coastal areas vulnerable to flooding had to constantly cope with various environmental challenges and bear the incessant financial burden, in orderto stay put with their only houses. The worse housing adjustment phenomena might also be encounteredin other settlements along the north coast of Java, such as Tambak Lorok in the city of Semarang. Without adequate financial support, the residents are unable to make the necessary adjustment to their houses and accordingly failed to cope with various environmental challenges. Eventually, they will be displaced to other worst locations.

Residents with a higher level of income and education tend to succeed in overcoming various environmental challenges, and increasing level of privacy and comfort of their living spaces. They also tend to have a strong attachment to their houses, community, and environment. By adding income generating units such as small-shop and rental rooms, the heads of household who are already over 50 years old managed to establish a small business and gain some passive income to support their family life.

Muara Angke may not be an ideal settlement to live, but for a certain periodthe residents had already invested their money, energy, emotion,and memory for the betterment of their houses and surroundings.Now, that they have no other choice thanto stay put and continue their livelihood, and hoping for the best to come.Involvement of the government and other stakeholders in public awareness (flood mitigation, environmental health and sustainability, community participation) and financial facilities (banking services, co-op) is a requisite if Muara Angke deserves to have a better future.

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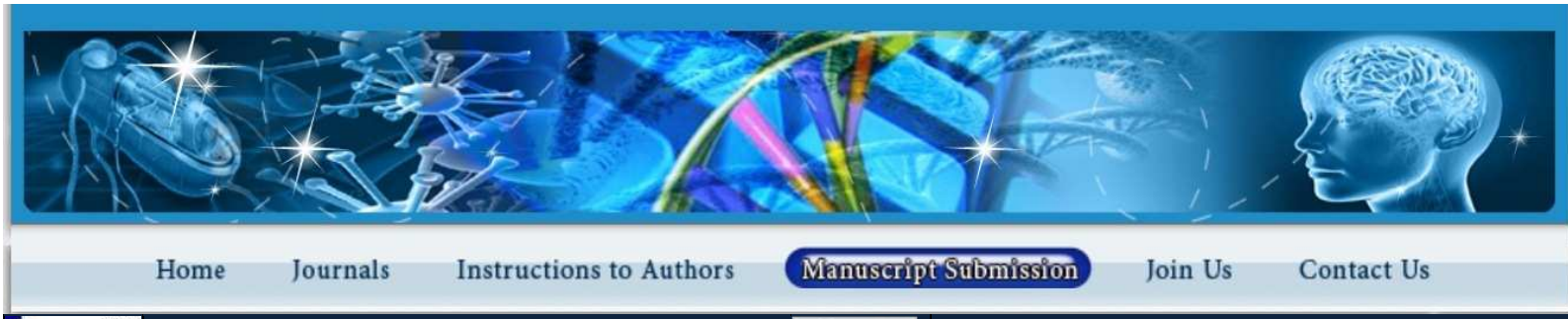

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