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Estimation of some Important Nutritional Chemicals in Hexaploid Wheat Cultivars of Northern areas of Pakistan

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Abstract

Common / bread wheat (*Triticum aestivum* L. genomically AABBDD, $x=7$, $6x=42$) is the most important rabi season crop of Pakistan. Present research was initiated for the first time to estimate biochemical composition in 40 accessions of bread wheat commonly cultivated in Gilgit Baltistan. Total protein, starch and reducing sugar ranged from 8.9 to 12.5% (average 10.59%), 54-75% (average 65.05%) and 4.5-8.9 mg/g (average 6.44 mg/g) in the germplasm used during present study. High protein content lines (Accession numbers 011562, 011581 and 011582) were identified among the 40 germplasm accessions. It is suggested that these lines may be used in breeding programs to improve protein quantity in Pakistani wheat.

Keywords

Triticum aestivum L. biochemical analysis, protein content, starch, reducing sugar.

1. Introduction

Triticum aestivum L. (Common / bread wheat) is one of the most important cereal crop of the world. Area under its cultivation is approximately 220 million hectares with a total production of 723.4 million metric tons (FAO, 2013). With a total of 25.6 million tons annual production, Pakistan ranks at 8th position, area under wheat cultivation in Pakistan is 8.9 million hectares and average yield is 2.87 tons per hectare. In the province of Gilgit Baltistan, wheat is cultivated on an area of 17090 hectares with a total production of 36835 tons giving an average yield of 2.1 tons/ha (MINFAL, 2012).

Major use of common wheat is making bread/nan/chapatti etc. Bread making quality of wheat depends on glutenin proteins. Among glutenins, high molecular weight glutenin subunits (HMW-Gs) play more important role in bread making. In the world 95% of wheat grown is of hexaploid type (FAO, 2013) with only 5% share

by tetraploid wheat. In Pakistan almost all the wheat grown is of hexaploid type (MINFAL, 2012).

Haxaploid wheat contains protein, starch, vitamins (mainly vitamins B1, E and K), minerals (iron and calcium) and dietary soluble fibers. Almost three quarters of wheat flour (71 g out of 100 g) consists of carbohydrates which provide essential energy to humans. Common wheat contains approximately 8-12% proteins in contrast to durum wheat which contains up to 15 % proteins (Khan et al., 2000).

Common wheat contains several medicinal qualities and is one of the most effective protection against diseases like constipation, cardiovascular, colon, appendicitis, obesity and diabetes. Wheat is also valuable in the prevention and cure of pyorrhea and skin diseases. Wheat flour also enhances heart and lung functions, reduce chest and tonsil pain as well as pimples. Wheat bran is also used as a supplemental source of dietary fiber for preventing colon and gastric cancer, irritable bowel syndrome, reducing the risk of hemorrhoids, hernia and hypercholesterolemia, hypertension, breast cancer and gallbladder disease. Wheat is also being used in China to promote female fertility. (Hadjivassiliou et al., 2002). Wheat also contain minerals and vitamins, high levels of antioxidants which is almost equivalent to that in fruit and vegetables. Regular use of common wheat is also considered to reduce risk of cancer, gallstone and diabetes (Anonymous, 2014).

A number of genetic studies regarding biochemical composition have been conducted worldwide in wheat (Colomba and Gregorini, 2011, Al-Fares and Abu-Qaoud 2012, Naghavi et al., 2009 and Gashaw et al., 2007) etc. Since there is no published information regarding

Nutritional value of wheat grown in Gilgit Baltistan, therefore the present studies were undertaken and focused on the biochemical characterization of the hexaploid wheat germplasm belonging to various parts of GB.

2. MATERIALS AND METHOD

Northern areas of Pakistan are the most magnificent mountainous area of the world surrounded by mighty mountain systems of Himalaya, Karakoram, Hindukush and Pamir.

It includes Gilgit Baltistan, Azad Jamu and Kashmir and Chitral. In general, previously a known northern area of Pakistan is now considered a newly established province of Gilgit

Baltistan. Area of GB is 72,496km² (27,991sqmi). with 1,800,000 people. Population density of the province is 25/km² (64/sqmi). Gilgit Baltistan province is divided into 7 districts i.e; Gilgit (head quarter of the province), Hunza Nagir, Ghizer, Astore, Ghanche, Diamer and Skardu. Beautiful historic places of GB include Gindai, Yasin valley, Baltit fort, Sadpara lake, Shangrila, phander etc. Highest peak of the area is K2 (8,611meter) along with Nanga Parbat, Rakaposhi, Masha broom, Gashabroom, Golden peak, Lady finger etc. Latitude of the area is 35.3500 N, 75.9000 E. Common languages of Gilgit Baltistan include Khawar, Shina, Brushaski, Wakhi and Balti etc. Main rivers of GB are river Gilgit, Skardu and Hunza.

Seeds of 40 landraces of hexaploid wheat used during present research were collected during early 80s from northern areas of Pakistan and were preserved in Plant Genetic Resources Institute (PGR), National Agricultural Research Centre (NARC) Islamabad. Seeds of 40 landraces were kindly provided by Dr. Sadruddin, Principle Scientific Officer, NARC

Table 1. List of 40 hexaploid wheat accessions used

S.No.	Accession number	Ar ea / village of collection	District	Province	Altitude (m)
1	000155	Tero	Ghizer	GB	3150
2	000156	Phandar	Ghizer	GB	2750
3	000157	Da malgand	Ghizer	GB	2030
4	000158	Lower Naltar	Gilgit	GB	2400
5	000159	Na ltar Bala	Gilgit	GB	2710
6	011420	Bania	Muzzafarabad	AJK	1090
7	011421	Bania	Muzzafarabad	AJK	1090
8	011422	Sharian	Muzzafarabad	AJK	1250
9	011423	Sharian	Muzzarabad	AJK	1250
10	011480	Hussainaba d	Skardu	GB	2080
11	011481	Thorgopaien	Skardu	GB	0950
12	011561	Gilgit	Gilgit	GB	1450
13	011562	Gilgit	Gilgit	GB	1450
14	011567	M.Abad	Gilgit	GB	1380
15	011568	Shelmish	Gilgit	GB	1320
16	011569	Nom al	Gilgit	GB	1380
17	011570	Nom al	Gilgit	GB	1380
18	011571	Hum ay	Gilgit	GB	1850

S.No.	Accession number	Area / village of collection	District	Province	Altitude (m)
19	011572	Naltar pine	Gilgit	GB	2040
20	011581	Gambus	Skardu	GB	2020
21	011582	Hussainabad	Skardu	GB	2080
22	011583	Hussainabad	Skardu	GB	2080
23	011589	Jutal	Gilgit	GB	1450
24	011590	Chalt	Gilgit	GB	1660
25	011591	Chalt	Gilgit	GB	1660
26	011602	Gitch	Gilgit	GB	1660
27	011603	Gakuch	Ghizer	GB	1760
28	011604	Tawos	Ghizer	GB	2200
29	011605	Ghimsil	Ghizer	GB	2280
30	011607	Siliharng	Ghizer	GB	1970
31	011620	Ghizer	Ghizer	GB	2840
32	011621	Thgmo	Skardu	GB	2120
33	011805	Masoth	Gilgit	GB	1940
34	011806	Masoth	Gilgit	GB	1940
35	011812	Juglot	Gilgit	GB	1300
36	011930	Buber	Ghizer	GB	1730
37	011931	Hatoon	Ghizer	GB	1930

S.No.	Accession number	Area / village of collection	District	Province	Altitude (m)
38	012199	Konchgoal	Chitral	KPK	1855
39	012200	Nagar	Chitral	KPK	1460
40	012201	Chitral	Chitral	KPK	1700

Gilgit Baltistan (GB), Azad Jammu and Kashmir (AJK) and Khyber Pukhtoonkhawah (KPK)

2.1. Extraction of seed storage protein

Seed storage protein of 40 hexaploid wheat landraces were analyzed on SDS-PAGE for High Molecular Weight Glutenin sub units. Standard procedures developed by Payne et al., (1987) was used to extract water soluble seed

storage protein. In brief seeds were crushed in mortar pestle to make a fine powder. Protein extraction buffer (500 ml) and Beta mercaptoethanol (2 mercaptoethanol) (50 ml) were added and proteins were extracted at room temp for 4-6 hrs. During extraction, tubes were vortexed after every hr. At the end of extraction, eppendorf were placed in boiling water for 5 minutes centrifuged (5000 rpm) for 5 minutes, supernatant was transferred to a fresh tube and stored at 4°C till use.

2.2. Extraction of starch

For extraction of starch, procedure described earlier (Khan et al., 1999) was used. In brief, wheat seed were soaked in NaCl (0.5 M) overnight.. Seeds were then ground in NaCl (0.5 M)), the supernatant starch slurries were

pooled and washed through a series of NaCl (4M), 6 M NaCl/50 %, sucrose, 2 % sodium dodecyl sulphate solution, and acetone before being dried over night The resulting dry starch was dissolved in water (1 ml). Carbohydrate solution was mixed with concentrated HSO (3

² ⁴ mL) in a test tube and mixed gently for 30 s. The solution was cooled to room temperature. Finally starch solution was stored at 4°C till further use.

2.3. Extraction of reducing sugar

The reducing sugar were extracted using procedure of Kuila et al., (2011). Wheat seeds were crushed with a mortar pestle and powder was added in an eppendorf tube. Reducing sugar extraction buffer (0.2 M Disodium Hydrogen phosphate, 0.1 M Citrate buffer, pH=4.8, 1ml) was added in the tubes. Reducing sugars were extracted for 4-6 hrs at room temperature. After extraction, tubes were centrifuged (5000 rpm) for 5 minutes and supernatant was transferred to a fresh tube.

2.4. Estimation of protein, starch and reducing sugar

Total seed protein (percent) in seed of wheat landraces were estimated using spectrophotometric procedure described by Grimsley and Pace (2003). Total seed proteins were extracted using standard procedure. UV spectrophotometry was used for the estimation of total seed storage protein, total carbohydrates and reducing sugar.

For estimation of total protein (Grimsley and Pace (2003) following relation was used

ABS of 1.3 at 280 nm = 1 mg protein / ml

The estimation starch concentration (Albalasmeh et al., 2013) was determined as

ABS of 0.4 at 315 nm = 28 % starch

The reducing sugar concentration relationship of ABS of 0.8 at 315 nm = 0.69 mg/g reducing sugar (Albalasmeh et al., 2013) was used.

Basic Statistical analysis (Mean, median,

minimum, maximum, standard deviation, standard error and coefficient of variation) and graphical representation were carried out using computer program PAST (Aleontological Statistics, Hammer, 2016) version 3.11.

3. RESULTS AND DISCUSSION

Mean values of starch, total protein and reducing sugar were estimated in 40 hexaploid wheat accessions collected from northern areas of Pakistan are presented in Table 2, Fig. 1. Basic statistics for 3 biochemical characters is presented in Table 3. Mean starch value for 40 hexaploid wheat accessions was 65.95 % with a range of 54-75 %. Amount of reducing sugar in the germplasm ranged from 4.5-8.9 mg/g with an average value of 6.44 mg/g. Total protein percentage ranged from 8.9 to 12.5 % with an average value of 10.59 % in the germplasm used during present study.

Table 2. Biochemical characters for 40 landraces of hexaploid wheat

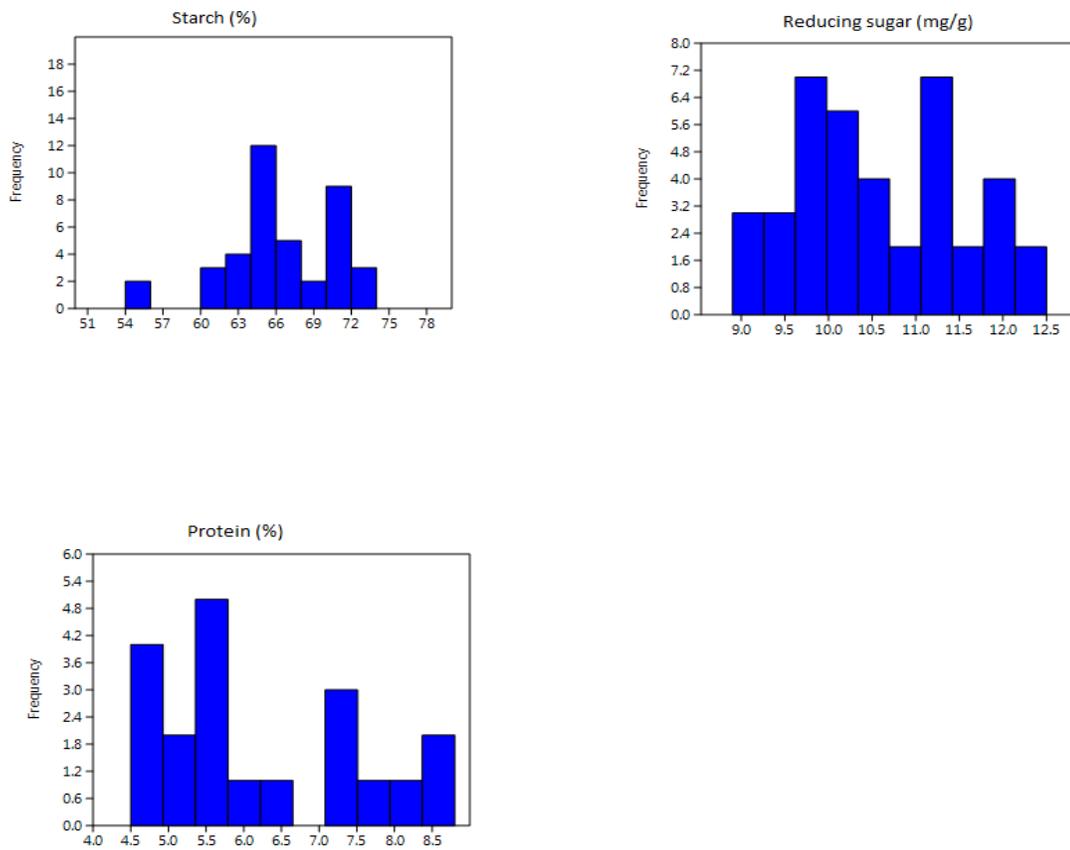
S.No	Accession Number	Starch (%)	Reducing sugar (mg/g)	Protein (%)
1	000155	70	8.9	10.2
2	000156	64	6.4	10.7
3	000157	65	4.5	11.4
4	000158	71	4.7	9.8

S.No	Accession Number	Starch (%)	Reducingsugar (mg/g)	Protein (%)
5	000159	55	6.3	10.3
6	011420	67	7.5	11.1
7	011421	71	6.5	9.9
8	011422	70	5.4	8.9
9	011423	67	6.4	10.2
10	011480	64	7.7	11.2
11	011481	70	7.4	9.7
12	011561	63	4.5	9.9
13	011562	65	6.6	12.5
14	011567	64	7.7	9.2
15	011568	65	5.5	10.7
16	011569	70	7.7	9.7
17	011570	65	5.7	10.4
18	011571	74	8.8	9.9
19	011572	60	7.6	11.3
20	011581	66	7.4	12
21	011582	66	7.4	12.2
22	011583	54	7.7	11.7
23	011589	65	5.7	10.5

S.No	Accession Number	Starch (%)	Reducing sugar (mg/g)	Protein (%)
24	011590	65	5.7	11.8
25	011591	71	8.8	9.9
26	011602	70	8.1	11.6
27	011603	69	5.5	10.3
28	011604	61	5.4	11.1
29	011605	62	5.9	11.1
30	011607	69	4.7	10.3
31	011620	61	5.3	11.8
32	011621	72	4.5	9.3
33	011805	72	7.2	9.5
34	011806	65	4.7	10.2
35	011812	66	7.4	10.9
36	011930	64	4.5	9.2
37	011931	62	5.7	11.9
38	012199	71	8.8	9.5
39	012200	65	6.4	10.9
40	012201	62	5.2	11.1

Table 3. Basic statistics for biochemical characters in 40 hexaploid wheat germplasm

	Starch (%)	Red sugar (mg/g/)	Protein (%)
Minimum	54	4.5	8.9
Maximum	75	8.9	12.5
Mean	65.95+0.70	6.44+0.21	10.59+0.15
Median	65	6.4	10.45
Coeff.var	6.7	21.1	8.7

**Fig 1:**

Percent of starch, reducing sugar and protein in 40 hexaploid wheat landraces from Northern areas of Pakistan

For biochemical characterization of local germplasm, starch, total protein and reducing sugar were estimated in 40 hexaploid wheat accessions collected from northern areas of Pakistan. Mean starch value for 40 hexaploid wheat accessions was 65.95 % with a range of 54-75 %. Amount of reducing sugar in the germplasm ranged from 4.5-8.9 mg/g with an average value of 6.44 mg/g. Total protein ranged from 8.9 to 12.5 % with an average value of 10.59 % in the germplasm used during present study. Earlier studies reported 11.1% protein and 67.39% starch in Serbian wheat bread (Milovanovic et al., 2014). In another study 67.5-69.5% starch, 5.3-8.6 mg/g reducing sugar and 9.1-10% protein were reported in Bangladeshi hexaploid wheat varieties (Rahman and Kader, 2011). In another study Gunashree et al., (2014) reported crude protein content in bread wheat ranging from 6.39 to 11.88%.

It is suggested that hexaploid wheat germplasm collected from northern areas of Pakistan specially Gilgit Baltistan has a unique and important value because it belongs to high altitude (at least more than 1500 meters above sea level) may be value able for genes responsible for cold and frost tolerance. Present research is first documented attempt to estimate genetic diversity of hexaploid wheat germplasm from High mountainous areas surrounded by mighty mountains Himalaya, Karakorum, Pamir and Hindukush ranges.

4. CONCLUSION

Hexaploid wheat were analyzed including starch, total protein and reducing sugar in the 40 hexaploid wheat accessions.

Present results indicated that sufficient level of genetic diversity exists among landraces of hexaploid wheat collected from northern areas of Pakistan. It is recommended that these landraces should be used in future breeding/hybridization programs aimed at increasing quality, quantity, cold tolerant of wheat production.

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Analgesic Practice through Morphine: A Prospective Study regarding Prescribing Pattern & ADRs

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Abstract

The present investigation was designed to analyze therapeutic stratagem of pain management among ICU patients and post-operative cases in tertiary care hospital. The therapy through morphine is a general practice to reduce the postoperative pain. WHO has established guiding principles to minimize the probable medication inaccuracy in analgesic therapy, however the morphine potentially accountable for hormonal imbalance, prolong constipation and behavioral psychological disturbances furthermore addiction is another potentially considerable hazard. The present research based on a prospective cross sectional study of patients under the treatment in ICU and post-operative cases. The prescription for alternative analgesics and their number were analyzed for the physicians, surgeons and duty doctors to avoid predictions. In addition, broad assessment was made to verify the implementation of pain intensity assessment tools

or pain rating scale before prescribing analgesic drugs. In the study (n=128), 53.33% male and (n=112), 46.6%) female, morphine was prescribed to 180 patients. The result outcomes of study showed overuse of morphine for the hospitalized patients by healthcare practitioner even though in the conditions where the pain could be controlled by NSAIDs. In present work, poor management of pain was observed among the patients admitted in a tertiary care hospital of Karachi, Pakistan. The analgesics or pain killers were prescribed without estimating the pain intensity.

Keywords

Morphine, analgesic, hormonal imbalance, addiction, NSAID.

1. INTRODUCTION

Morphine is used for the management of severe pain through its action on central nervous system (Tattersall, 1981). A significant pain

Management is observed at standard dose for analgesic. In prolonged use some short-term adverse effects were observed and others adverse effects may be long-term treatment (Dennis & Melzack., 1983; Gourlay *et al.*, 1986). The drug accounts for affecting gastrointestinal functions and resulting in abdominal cramps, constipation, nausea and vomiting, opioid receptors (morphine) are located in the GIT and their response may lead to a slow movement of gut causing the constipation furthermore the long-term use leads to severe constipation (Hanna *et al* 1990). Morphine is responsible for nausea and vomiting by stimulating the vomiting centre in the brain. There may be drying of the mouth as well (Hanks, 1991). The most prominent effect of its morphine is sedation and its long term effect associated with addiction (Hanks, 1991; Portenoy *et al.*, 1991). The prolong treatment may directly release histamine that in turns causes warmth of skin, redness of skin and urticaria. Sometimes upper derma may develop hypothermia, shrunken pupils and uneven respiration as the respiration rate can be disturbed due to low blood oxygen (Foertsch *et al.*, 1995). In healthy persons, there is fall and rise in blood O₂ and blood CO₂ respectively, the drive for breathing increases (Cleeland *et al.*, 1996; van Dorp *et al.*, 2006). Although, morphine accounts for suppression of drive in the brain, potentially leading to dangerous zone and the condition, respiratory depression or hypoventilation, is highly associated with large doses. Starting doses lead to euphoria although undesirable symptoms at higher dosage includes hallucinations, delirium, dizziness and confusion leading to headache and memory loss (Grace and Fee, 1996; Minami *et al.*, 2009). Morphine overdose is responsible for biliary colic

and subsequent severe abdominal pain. Myoclonus manifest may develop due to muscle rigidity. Psychological dependence on the drug is the prominent side effect and withdrawal symptoms due to its addiction effect include pain, loss of sleep, nausea, vomiting, diarrhea, cold and hot flashes, goose bumps and intense craving. The prolonged treatment causes development of tolerance, the condition when a patient tolerate at large doses to attain the same level of euphoria or pain relief as prior (Bruehl *et al.*, 2014; Kesavan, 2015; Berger *et al.*, 2017). Hormonal imbalance is one of the other hazards as well. Recent researches strongly reveals a reduction in testosterone, estrogen and other sex hormones after a prolonged treatment of opioids specifically morphine. Furthermore tolerance for addiction is observed on at a similar rate to both morphine and heroin (Berger *et al.*, 2017; Sverrisdottir *et al.*, 2015). According to an advanced research, adenosine receptors act as a modulator in withdrawal and drug dependence, consequently administration of the A1 receptor agonist markedly declines the withdrawal effects and accounts for rise in dopamine and noradrenaline concentrations in the condition of opioid withdrawal with controlled dosing. (Furukawa *et al.*, 2017).

2. MATERIAL AND METHOD

Present cross sectional prospective study was performed from August 2018 to February 2019 at a tertiary care hospital and the ICU and post-operative cases were taken as data collection source. Convenience sampling technique was used for data selection.

2.1. Ethical considerations

The survey was directed according to

Declaration of Helsinki, 2008. After verbal consent, the data was gathered and definitely conserved the confidentiality of information.

2.2. Data collection

Data was collected using specified performa, developed by segregating into three major domains including patient's demographics, medical history and pain assessment tools for evaluating prescriptions. During the research patients were interviewed and the effectiveness of pain management therapy was designed. Further prescriptions were reviewed to analyze the prescribing pattern of analgesics. In the study adult patients hospitalized in ICU and post-operative cases were included. Pregnant women, patients below age of twenty were excluded from the study.

2.3. Data Analysis

The software of statistics (SPSS) version 20 was used. Descriptive analysis including frequencies and percentage were calculated. All respondents included in the present study either male or female were informed and their consent was taken.

3. RESULTS AND DISCUSSION

A total of 240 patient prescriptions (53.33% male and 46.66% female) were evaluated. Patient's ranged in age of 20-60 years. The main reason for admission in hospital was hepatic cirrhosis and stomach cancer. While breast cancer and heart patients accounted for minimum number of hospital admission. The duration of hospitalization might reflect the severity of complication. For instance, around 25% of the patients were hospitalized for 3 days, while 4 days and 5 days hospitalization

was for 31% and 18% of the patients was receiving respectively. The duration of above hospitalization above 5 days was least frequent. In poly pharmacy per prescription 60%, 30%, 10% patients received 10-14, 6-10 and 15-18 drugs respectively, As an analgesics were concerned 53% prescriptions had two analgesics while 3 and 4 analgesics per prescription were seen in 27 % and 17 % prescriptions, respectively. Only 3% cases had received a single analgesic (Table 1). Moreover, ketorolac and ibuprofen was prescribed only in 16% and 9%, while morphine was prescribed to 180 patients. Adverse effects of morphine included high addiction; hormonal imbalance and reinforcement disorder were observed (Figure 1). In majority of cancer patients morphine was prescribed (Figure 3). Engrossingly, the preferred treatment regimen of analgesics was not effective for pain management. Pain intensity was not assessed in any patient before prescribing analgesics.

Present study determined that pain was untreated in most of the patients and without any concern of pain intensity same analgesic regimen were prescribed. Another study also reported similar in orthopedic and cancer patients and was linked with insufficient knowledge of health care provider's knowledge (Crow, 2016).. In current study lack of assessment of intensity of pain was identified as a main cause of incomplete analgesia in most of patients. Effective communication between health care providers and patients could improve the pain assessment. The WHO analgesic ladder appears to be most suitable for selection of analgesics depending upon intensity os pain. Ealth pain assessment numerical score is a standard tool used in many developed countries for analgesic

selection. However, in our studies these guidelines were not followed completely. Additionally the choice of dosage form in the patient were also not managed. In most of the patients, morphine and many other potent analgesics were administered only intravenously, even though these drugs are available in oral dosage forms. Ketorolac and ibuprofen were common analgesics prescribed, while other analgesics were seldom prescribed.

The WHO has declared the presence of one pharmacist for fifteen beds in hospital

pain management was investigated to be followed.

Pain management is an intense area of treatment and collaborative team work, trained pharmacist to elaborate with physicians to decide the correct analgesic with accurate doses according the status of patient. The pharmacist should have knowledge of drugs-drug interactions, drug pharmacokinetics and mechanism of action that will allow the individualize analgesic regimen for effectiveness with minimal adverse drug reactions (Crow, 2016).

Table 1: Pattern of Prescribing Analgesics in Prescriptions

No. of Analgesics per prescriptions	No. of Prescriptions (%)
1	7.2(3)
2	127.2(53)
3	64.8(27)
4	40.8(17)

4. CONCLUSION

In current study, deprived method for in tertiary care hospital of Karachi, Pakistan. The analgesics for relieving the pain are Prescribed without analyzing the pain intensity. There should be standard prescription pattern of analgesic regimen. The dosing of morphine according to the condition of patient, consequently the health care system should be designed to minimize ADRs associated with morphine via following

WHO guidelines and deploying proper professionals in hospitals and clinical set.

Conflict of Interest

The authors declare that there is no conflict of interest.

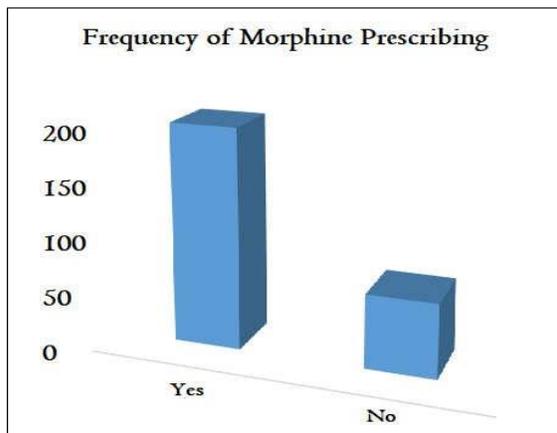


Fig 1: Frequency of Prescribing Morphine among Patients

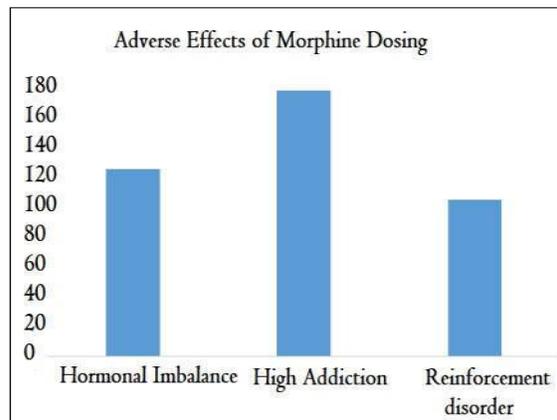


Fig 2: Adverse Effects of Morphine Dosing among Patients

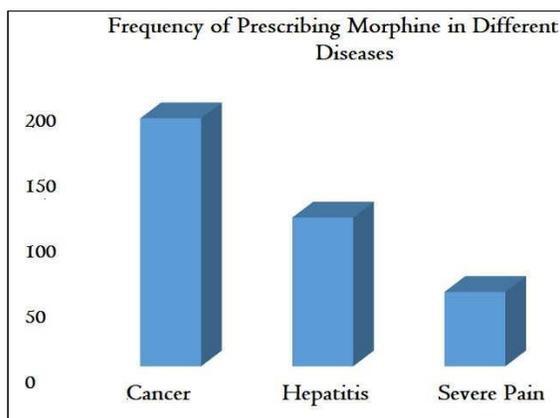


Fig 3: Frequency of Prescribing Morphine among Patients in Different Diseases

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Virulent Epidemics of Zika: Clinical Manifestation, Challenges and Prevention

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Abstract

A new facet for medical practice has shifted toward the awareness and imperative plan to combat the virulent disease associated with widespread epidemics of Zika virus. The mutual multidisciplinary efforts are also needed for managing this imminent threat in a safer and effective manner with suitable and efficient diagnostic and treatment approaches. Exploring the various strains of the virus and evaluating the major areas of human immunology are main stream actions to be taken for formulation of the vaccine for Zika virus. This review article encompasses the epidemics, etiology, clinical demonstration, mode of transmission, therapeutic rational and prevention strategies.

Keywords:

Zika virus, clinical demonstration, mode of transmission, epidemics, prevention, immunology

1. INTRODUCTION

There is an episode of another mosquito borne flavivirus i.e. Zika infection. Zika infection has turned into a point of convergence for general wellbeing and wellbeing experts. This broad infection needs a most basic practice like medicinal services camps, by directing classes and through handouts. The recently emerging infection belongs with the family Flaviviridae and the genus Flavivirus. The broadly rising infection topographically dispersed in the territory going from eastern and western Africa, South Asia alongside East Asia, Micronesia and in addition Yap Island [1]. The infection has been demonstrated in two noteworthy families, Asian and African family [2].

1.1. Outbreak and current epidemics

In Uganda, the Zika infection was first recognized in monkeys in 1947 however later in 1952 the instances of Zika infection was

accounted for people. Zika infection was along these lines recognized in Uganda as well as in United Republic of Tanzania [3]. Close to postulations zones, it was additionally found to change in a few distinct main lands of world, for example, Asia, America and Africa. In Nigeria, the Zika infection was additionally identified in human specimens in 1968 [3-5]. The infection that associated with causing yellow fever, dengue fever, encephalitis, West Nile and Japanese encephalitis have been likewise required with Zika sickness. They share same neurotic and clinical results and introduction. In the year 1966, serological confirmations in human demonstrate that Zika has spread to Asia and has turned out to be endemic in East and West Africa. On Yap Island (Pacific Ocean) which situated in the Southwest portions, a direct issue was spotted which was recognized by unbearable pain in joints, swelling of conjunctiva of eye, skin eruption demonstrating soonest disclosure of that infection outside of Africa and Asia in 2007 [6]. Moreover, the rankling overall conveyance of Zika Virus has been affirmed by the proliferation of the infection in more prominent than 13 nations of Americas [10]. Zika has secured a boundless zone of infection conveyance since the year 2013 insect it has been demonstrated by the cases announced by Caledonian [10].

1.2. Presence of virus

The key factors that affecting the infection in Pakistan are; expanded inhabitants rate, poor sanitation, swelling in populace, unhygienic sustenance and water, deficient medicinal and pharmaceutical care units, lack of literacy rate, poor immunization and prevention practices. There is a wide assortment which has been

found among a few unique genera of the infection. Indoor condition is appropriate living space for *Aedes aegypti* they can without much of a stretch develop in blossom vases, water tanks and vehicle tires and holders. The hard covers of coconut, spaces between trees, steel boxes are paramount lodgings for *Aedes-hensilli* and *Aedes*. Polynesians which are potential vector components for Zika [11-12].

1.3. Cause of the disease:

The infections which have a place with the Flaviviridae family are the principle causative operators of Zika infection and different irresistible maladies. The expansion of infection relies on the spread of infection. There are 2 standard vectors of dengue i.e., *Aedes Aegypti* (most pervasive) and *Aedes albopictus* (the most intrusive and distending mosquito around the world), *Aedes* mosquito is able to nibble in day time and a night. From the hereditary viewpoints, Zika infection is firmly identified with dengue fever, japons encephalitis, yellow fever, or West Nile [11]. Ribonucleic acid have 10.8 kilo bases. A film glycoprotein assumes a vital part in the connection of infection. Likewise, with different individuals from family of flaviviridae, film proteins are key for mounting defensive component in contrast to viral sickness [11-12, 26].

1.4. Disease Propagation and clinical Manifestation:

Zika is basically spread by the female *Aedes aegypti* mosquito, which is dynamic for the most part in the daytime. Zika infection is principally transmitted to individuals through the tainted mosquito from the *Aedes* family, basically *Aedes aegypti* in tropical areas. *Aedes*

Mosquitoes more often, than not nibble amid the day, topping amid early morning and late evening. Sexual transmission of Zika infection is additionally conceivable. Different methods of transmission, for example, blood transfusion are being examined [13]. Investigations of Foy et al additionally recommended the spread of Zika infection by performing physical contact [14-15]. Zika infection imparts its clinical outcomes to different flavivirus issue like joint throb, rashes over body, ophthalmic issues and disturbance. The manifestations showed up for 2-7days. The fundamental method of infection transmission is blood transfusion [16].

1.5. Pathophysiology

It is exceptionally hard to illuminate the fundamental pathology and system of the ailment caused by virus (Zika) infection [17-18]. The ailment victory in Polynesia in 2013 and then in the year 2015 in Brazil has laid anxiety that there is a plausibility of the ailment result, for example, harm to sensory system and debilitating resistance. Wellbeing specialists have been bringing up the neurological manifestations in Brazil which impersonates therefore expanding number of instances of Guillain Barré Syndrome, alongside babies having Microcephalus (brain growth abnormality). The scientists have been discovering approaches to find interfaces in the middle of the infection causing infection and microcephaly. statically information demonstrates that many the infection casualties roughly around 70 were found with Guillain Barré Syndrome and in addition with immune system issue, for example, Immune thrombocytopenic Purpura. Extra examinations are in pipeline to announce the infection cause, how they spread and other optional convolutions [19-20].

1.6. Observation

The observation framework for Zika infection must be started and overseen in a same way as the framework produced for different flavivirus maladies like Dengue and Chikungunya yet should concentrate on the variety among their clinical outcomes and case conditions [21]. As per proposals, patients who are experiencing fever and joint torment like joint pain with no etiologies, their blood test ought to be gathered and monotone. Cross cynicism can without much of a stretch be controlled by serological test. To recognize incalculable strains of Zika infection and to survey the malady control, it can wind up noticeably simpler through prior recognition of the infection. Moreover, perceiving dangers attending with the infection, open presentation to contaminations, co-morbidities are fundamental to oppose the infection victory [22].

1.7. Incubation period for Zika virus disease

Data about the Zika infection malady hatching period can help distinguish chance periods and nearby infection transmission. In 2015–2016, information from 197 symptomatic voyagers with late Zika infection contamination showed an expected hatching time of 3–14 days. For symptomatic people with manifestations >2 weeks after travel, transmission may be not travel related generally.

Sub-atomic methodology, for example, RT-PCR (reverse transcriptase polymerase chain response) fill in as steady device to recognize viral RNA amid the intense period of the viremia cycle. Besides, a measure for the infection nonspecific sequencing for viral genome can assume to be a definitive part [22-23]. After

the indications, have showed up, the serological test for setting up immunoglobulins; IgM and IgG against Zika infection performed were positive following 5-6 days. Raised immunizer titer levels even following two weeks may likewise fortify the analysis and the test is additionally ensured by plaque decrease balance test (PRNT). On the off chance that the patient had already determined to have Dengue, cross-reactivity can be discovered particularly if there should be an occurrence of dengue and yellow fever infections which displays a fourfold high titer esteem for antibodies and in such situation, one must be cognizant abundant to distinguish any artifacts. The infection energy of Zika infection has been analyzed and comes about uncovered that following 10 days of the sickness beginning, examples of the patient urine samples were positive for ZIKV which is not at all like serum trials of the patient that uncovered negative outcomes. It has turned out to be certain that one of the huge symptomatic devices for Zika infection could be urine test of the ailing patients [23-27, 38].

1.8. Treatment

Zika infection has been associated with microcephaly and Guillian Berri Syndrome which has created the infection deadlier and consequently it has been in limelight for medicinal sciences. For the treatment of Zika infection drug treatment has not been built up but rather according to supports it is essential to regard patients according to exhibiting indications. Treatment of the malady caused by (ZIKV) is for the most part symptomatic centering to diminish fever, agony and rashes. It is exceedingly supported not to commence treatment by the utilization different dose of

non-steroidal anti-inflammatory drugs (NSAIDS) and Aspirin as acetyl salicylic acid (ASA). Since they light on to deliver heighten injurious impacts in patients having illnesses activated by flavivirus, for example, Dengue and other viral diseases. Also, these restorative medications have identified with the upgrade of hemorrhagic obstacles and Reye Syndrome (is an uncommon however genuine condition that causes swelling in the liver and mind). In general No particular antiviral treatment for the Zika infection is recommended. Treatment is planned for diminishing symptoms with to have rest, plenty of liquid intake to get hydrated and medication for symptomatic relieve, including acetaminophen to relieve joint discomfort and fever. Vaccines to anticipate the Zika infection still not reported though a very few immunizations programs are under clinical investigation [25-27].

2. DISCUSSION

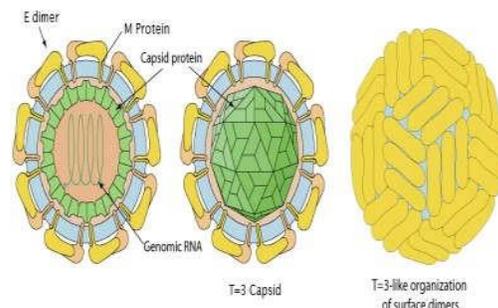


Fig. 1 Structure of Zika Virus [40]

Icosahedral-shaped is mainly depicted in various literature for zika virus. The diameter was found 18-45nm in enveloped manner. The Zika genome contains positive RNA strand along with membranous capsid. Total nucleotides and encoded aminoacids in RNA were reported to be 10,794 and 3419 respectively [40].

Zika in total is associated with various clinical complications for example, microcephaly, if affirmed to be identified with the contamination in prenatal period, would be a deprived result for the infant. Furthermore, vision variations from the norm and Guillain-Barré disorder may happen. Viral disease begins with fever and migraine took after by inflammation and irritation of palms and soles skin and in addition summed up body ach. As the ailment advances, fever progresses high and skin rash vanishes subsequently [28]. The viral illness instigates pyrexia and joint agony starved of exanthema (a skin rash going with a malady or fever) in its intense condition. Zika infection may have demonstrated its connections with intrinsic malady of infant which brings about immature brains, a condition known as microcephaly. Similarly, Zika has uncovered its envelopment with neural Guillain Barré disorder [32-33], Stomach obstruction, discharge, stoppage and additionally ophthalmic disablement portrayed by retro orbital torment alongside inflammation are uncommon articulations [7, 34-39].

Aversion and Vaccination

In current days, two worldwide unhampered immunization have been documented i.e., a recombinant antibody and an inactivated immunization protocol for ZI. The stride of antibodies aligned with ZI as a challenge is accepted in countering the acceleration of immunization but need its trials to be conclusive in couples of years. In view of the fact that this disease could be consequence of various obscure strains of ZV; the greatest target is to conceal all classes of infection while planning antibodies [41-43]. Essentially immunization planning for ZV requires a decade for FDA approval, however it has been

foreseen that the procedure of antibody advancement should be an expedient procedure as a concern of general wellbeing crisis; so human trials of some vaccinated products are under Phase 1 clinical examinations [44-45].

3. CONCLUSION

Indicative managing guideline is dependent on research facility testing that is not broadly accessible. Understanding treatment administration frameworks alongside solid arrangement of standards for maladies control and foundation of principles strategies can serves to be turning point in acknowledgment, control and obstacle related to viral ailment eradication.

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Perception, Knowledge, and Attitude of Herbal Medicines Utilization in Various Gastrointestinal tract Diseases among Undergraduate Students

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Abstract

A demographic survey was conducted to find out the trend of use traditional medicines against various gastro intestinal diseases (indigestion, gastritis, nausea, vomiting, acidity, diarrhea, constipation, and heart burn etc) among the undergraduate students. showed that 83% participants used traditional medicines while 17% did not use these medicines. Regarding the safety concerns only 33% participants were aware about the side effects of using herbal drugs while 67% had misperception that herbs are safe and free from any side effects. This study will be helpful in creating awareness among undergraduate pharmacy students regarding the need of conducting pharmacovigilance, quality control standards, and safety issues of complementary and alternative system of medicines / green pharmacy, parallel to the pharmaceutical control that is mandatory in the allopathic system of medicines.

Keywords:

Use Traditional medicines, gastrointestinal diseases, undergraduate perception

1. INTRODUCTION

Use of traditional medicines has deep cited roots in the history in order to provide relief to mankind from various illnesses. Since prehistoric times nature has been serving as the strongest pillar of the health care that provided foundation and search engine for the modern synthetic medicines with the advent of conventional allopathic medicines, the utilization of herbs and other natural remedies apparently declined in the field of therapeutics. However currently people are moving back towards nature oriented green pharmacy for better and healthier quality of life. According to World Health Organization (WHO) 70-95% of the developing world (Asia, Africa, Latin America, and Middle East) and 70-90% of the developed countries (Canada, France, Germany, and Italy) rely on traditional

medicines under the of Complementary and Alternative (CAM) or Non-conventional medicines [1,2].

In Pakistan both conventional and Traditional system of medicines are being practiced for primary health care 3-7. However traditional system of medicine has been neglected as pharmacovigilance of traditional medicines have been rarely seen [3, 11- 14]. Like other cities of Pakistan, in Karachi habitant's use of herbs and herbal products is very popular in various forms especially against various gastrointestinal (GIT) diseases.

On scientific grounds Medicinal plants and related herbal medicinal products contain specific natural phytochemical constituents i.e. alkaloids, glycosides, flavonoids, tannins, saponins, and volatile oil etc that are responsible for the therapeutic activity justifying its medicinal use. Due to the presence of such phytochemicals the uncontrolled/misuse of traditional medicines not only provide severe health concerns but the concurrent use of herbs and conventional allopathic medicines may also cause herb-drug interactions [8-10]. Moreover presence of environmental contaminants, heavy metals, pesticidal residues, and microbial contaminants have also been documented in crude drugs and related herbal medicinal Products (HMPs) 11-14.

On the basis of above mentioned scientific grounds it is the need of time to collect proper data on the herbal drug usage and perception of general public regarding its safety and efficacy by the local population. Phytochemicals in the traditional medicines may interact with the allopathic medicine therefor herb drug interaction should also be taken into account.

The objectives of this survey was to gather a small scale data regarding the knowledge of Pharm D undergraduate students regarding various aspects of herbal medicines like prescription and nonprescription based use of crude herbs and ready to use herbal formulations, concurrent use of herbs along with conventional medicines, and the perception about the safety, toxicity, herb-herb, and herb-drug interactions of the traditional medicines.

2. MATERIAL AND METHODS

A demographic survey was performed on involving undergraduates (n = 450) of Jinnah Sindh Medical University Karachi. Data was collected through a structured questionnaire. Each questionnaire contained questions regarding herbal drug utilizations in various gastrointestinal diseases, safety concepts, information regarding concurrent use of herbal medicines with other medicines.

Statistical evaluation of data was performed using Pearson correlation (p 0.01) and correlations among different variables were also conducted using SPSS v 19.

3. RESULTS AND DISCUSSION

The findings of current demographic survey of participants use traditional medicines, forms in which these herbs are taken i.e. nonprescription herbs/crude drugs (in form of home remedies and self-medication of over the counter available ready to use herbal formulations) or acquiring prescribed herbal drugs after proper checkup and diagnosis of traditional healer, effectiveness of herbal treatment (Figure 1 a and 1b). Table 1 contained the names of some herbs that are commonly used in G.I.T diseases.

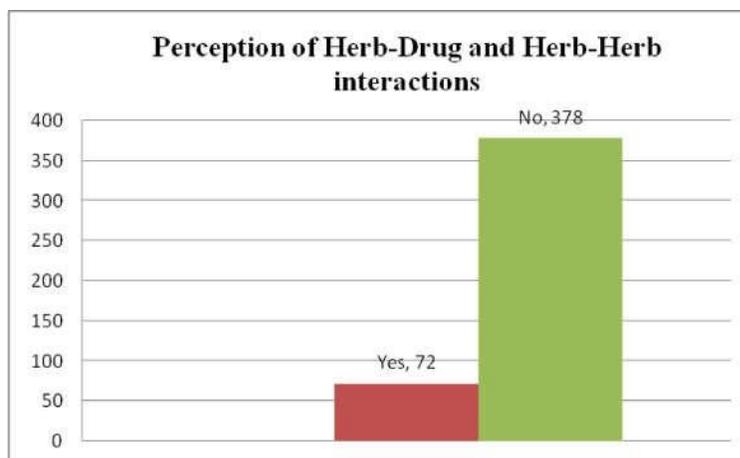


Figure 3b: Perception of respondents for herb-drug and herb-herb interaction informed about simultaneous use of herbs and HMPs with allopathic medicines

The prevalence of gastrointestinal diseases (Nausea, vomiting, constipation, diarrhea, heart burn, acidity, ulcer etc) is very common in Karachi city. Both conventional and traditional systems of medicines are being practiced for this treatment. According to the current scenario of Karachi, Pakistan mainly Conventional system of medicines is used with less attention on the use of traditional /complementary and alternative medicines. The quality standards of crude drugs and herbal formulations, effects of concurrent use of herbs with other systems of medicines, control of self medication of herbal formulations along with statistics on the percentage of local population using traditional medicines [6, 10, 12, 13]. Therefore was conducted a small scale demographic survey to gather data about the percentage of pharmacy undergraduate having belief on traditional

related issues.

According to the survey 83% of participants rely on traditional medicines to help out GI tract diseases. Among which 71% people use these drugs without getting any advice from registered herbal practitioner. They usually opt for taking crude drugs as home remedy/folk medicines and ready to use over the counter available herbal formulations of population admitted that they use herbs and herbal Medicinal Products (HMPs) after acquiring prescribed medicines from herbal practitioners after proper visits and check-up. The herbal drug users have strong belief that these drugs are very effective and usually they get relief after using these medicines.

Table-1 describes the most commonly used herbs against acid reflux regarding the safety perception of Traditional medicines 67%

population have misperception that these drugs are safe and free from side effects as they believed that these drugs are obtained from nature. There is no concept that natural active ingredient present in these drugs may produce ill effects if wrongly taken or used for longer therapeutic durations. These drugs may also produce antagonistic or agonist effects with the other herbs or conventional drugs used simultaneously. But unfortunately these concepts are not well developed not only at the level of general population but other health care professional who are also usually unaware of these parameters. This is a great concern as people are not only using these medicines on their own without the advice of any herbal practitioner but also inclined to use herbs and other conventional medicines simultaneously. Moreover we also got information regarding the concepts about the safety issues of herbs and herbal medicinal products, concurrent use of traditional medicines with conventional medicines, names of herbs used in the treatment and sharing of information of taking herbal drugs with their general practitioner and other health care personals.

This mal practice may lead to many complications to the patients. The public use (67%) other system of medicines especially allopathic medicines along with traditional medicines simultaneously among which 77% people even don't inform the doctors or pharmacists about the concurrent use of herbs with allopathic drugs.

According to the statistical evaluation using pearson correlation coefficient a Positive correlatin (0.791) was obtained between people that inform practitioners about using herbal remedies also have concept of herb and drug

interaction. It was also noted that most of the people simultaneously using herbs with allopathic medicines donot inform their practitioners (Weak correlation 0.241) Participants of the study became well aware about the potential risks that may be associated with the misuse of crude herbal drugs and over the counter available herbal formulations. Moreover, they were educated that drugs of natural origin have some phytochemicals constitute that may be responsible for the therapeutic action and may safely consumed but under expert supervision. Since the participants are Pharm D under graduate students understood clear perception regarding the herbal medicines safety and efficacy. In future they will have better knowledge of these issues as clinical or community pharmacist.

Most of the younger generation including students usually prefer junk food having rich protein and fat contents. This high caloric food intake causes disturbs the natural harmony of immune system and ultimately leads to weakness, poor memory, and different physiological, psychological and infection born diseases. This problem can be minimized by carrying out physical activities, consuming balanced diet maintaining the efficacy, quality and safety parameters of herbs and herbal formulation is a great challenge for pharmacist and other health care professionals. This goal can be achieved by building the concept of Pharm. D students to treat drugs of pharmacognostic origin (herbs and related herbal formulations) according to the pharmaceutical standards. Unnecessary use of synthetic medicines to treat various gastro intestinal diseases should be avoided. Taking healthy diet having normal balance of carbohydrate, protein

fat, mineral, vitamin, and dietary fibre with proper physical activity usually keep gastrointestinal tract healthy and prevent it from different diseases like constipation, acidity, and

Use of remedies and drugs of herbal origin should be the first line of treatment in the management protocol to treat aIT related ailments.

gastritis. **Table 1: Home remedies for treatment of acid reflux, heart burn, gastritis, indigestion, and constipation**

Source/ Scientific Names	Pictures	Local names	English Names	Families	Actions and Uses
<i>Foeniculum vulgare</i>		Saunf	Fennel	Apiaceae	heart burn, gastritis, indigestion, carminative
<i>Menthe piperata</i>		Podina	Mint	Apiaceae	Acid reflux, heart burn, gastritis, indigestion, nausea
<i>Cinnamomum zeylanicum</i>		Darchini	Cinnamon	lauraceae	Acid reflux, heart burn, gastritis, indigestion
<i>Phychotis ajwain</i>		Ajwain	Bishop	Apiaceae	Acid reflux, heart burn, gastritis, indigestion
<i>Glycyrrhiza glabra</i>		Mulethi	Licorice	Leguminosae	Acid reflux, heart burn, gastritis, indigestion
<i>Ricinus communis</i>		Roghane Arand	Castor oil	Euphorbiaceae	Constipation
<i>Prunus domestica</i>		Alobukhara	Plum	Roseaceae	Acid reflux, heart burn, gastritis, constipation, nausea, vomiting

Source/ Scientific Names	Pictures	Local names	English Names	Families	Actions and Uses
<i>Punica granatum</i>		Anar dana	Pomegranate	Lythraceae	Acid reflux, heart burn, gastritis, indigestion
<i>Coriandrum sativum</i>		Dhanya	Coriander	Apiaceae	Acid reflux, heart burn, gastritis, indigestion
<i>Cuminum cyminum</i>		Zeera	cumin	Apiaceae	Carminative, Acid reflux, heart burn, gastritis, indigestion
<i>Plantago ovate</i>		Isabgol	Phyllium husk	Plantaginaceae	Acid reflux, heart burn, gastritis, indigestion
<i>Ficus carica</i>		Injeer	Fig	Moraceae	Constipation
<i>Nigella sativum</i>		Kalongi	Black Cumin	Apiaceae	Acid reflux, heart burn, gastritis, indigestion
<i>Apis mellifera</i>		Shehad	Honey	Apidae	Acid reflux, heart burn, gastritis, indigestion, constipation
<i>Phoenix dactylifera</i>		Khajoor	Date	Arecaceae	Acid reflux, heart burn, gastritis, indigestion,

Source/ Scientific Names	Pictures	Local names	English Names	Families	Acti ons and Uses
<i>Zingibar officinalis</i>		Adrak	Ginger	Zingibera ceae	Acid reflux, heart burn, gastritis, nausea, indigestion,carminati ve
<i>Citrus lemon</i>		Lemo	Lemon	Rutaceae	Acid reflux, heart burn, gastritis, indigestion, constipation
<i>Eletteria cardamomum</i>		Elachi	cardamom	Zingibera ceae	Acid reflux, heart burn, gastritis, indigestion
<i>Allium sativum</i>		Piyaz	Onion	Amaryllid aceae	Acid reflux, heart burn, gastritis, indigestion
<i>Curcuma longa</i>		Haldi	Turmeric	Zingeber aceae	Acid reflux, heart burn, gastritis, indigestion
<i>Eugenia corylyphylis</i>		Long	Clove	Myrtacea e	Acid reflux, heart burn, gastritis, indigestion
<i>Cucumis sativus</i>		Khera	Cucumber	Cucurbit aceae	Acid reflux, heart burn, gastritis, indigestion
<i>Sodium bi carbonate</i>		Khanay ka soda	Baking soda	NaHCo3	Acid reflux, heart burn, gastritis, indigestion

Source/ Scientific Names	Pictures	Local names	English Names	Families	Actions and Uses
<i>Ocimum tenuiflorum</i>		Tulsi	Holy basil	Lamiaceae	Acid reflux, heart burn, gastritis, indigestion
<i>Prunus amygdalis</i>		Roghan e badam	Almond oil	Roseaceae	Acid reflux, heart burn, gastritis, indigestion, constipation
<i>Sodium Chloride</i>		Namak	Salt	Nacl	Acid reflux, heart burn, gastritis, indigestion
<i>Acetic acid</i>		Sirka	Apple cider vinegar	CH ₃ COOH	Acid reflux, heart burn, gastritis, indigestion
<i>Triticum aestivum</i>		Gundum ka atta	Whole wheat flour	Poaceae	Constipation
<i>Hordium vulgare</i>		Jau	Barley	Grasses	Constipation
<i>Nux gallica</i>		akhroot	Walnut	Juglandacea e	Constipation
<i>Zea mays</i>		Makai	Maize	Gramineae	Constipation

4. CONCLUSION

The survey regarding the traditional medicine utilization in Karachi, Pakistan, according to the result use of traditional medicines and herbs is very common against gastrointestinal diseases especially in the form of home remedies or self medication generated awareness about the green pharmacy especially at the level of Pharm D who will be future drug specialist. They are expected to educate the society about safety and efficacy concepts especially in community and clinical pharmacy sessions. Concurrent use of herbs and crude drugs are also practiced which may interfere with each other producing agonist and antagonist effects and needs to be discouraged. Society or general public should be educated regarding usage of herbal medicines/home remedies of crude drugs by the health care provider for the maximum benefits with minimum harmful effects associated with these drugs.

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***In vitro* Free Radical Scavenging Activity of Some Myanmar Traditional Plant Extracts**

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Abstract

The present study was devised to estimate the antioxidant activity of six ethanolic extracts of dried leaves of *Piper betle* L., flowers of *Dolichandrone Spathacea*, fruits of *Embllica officinalis* Gaertn, flowers of *Haplophragma adenophyllum* (wall) Dop., *Oroxylum indicum* Vent. and *Boscia variabilis* Coll. & Hemsl.. The antioxidant activities of aforementioned plants were evaluated *in vitro* assays to compare antioxidant effects using DPPH (1,1- diphenyl-2-picrylhydrazyl), potassium ferric cyanide, superoxide anion and nitric oxide. Total phenolic contents of selected extracts were also determined. Among all six plant extracts, *E. officinalis* possessed high antioxidant activity and total phenolic content. The crude extract of *E. officinalis* showed significant DPPH free radical scavenging activity with IC₅₀ value of 3.8 µg/mL. Its reducing power was found to be maximum with the absorbance value of 1.3 at 100 µg/mL concentration. Moreover, it also had the highest scavenging activity on superoxide

anion and nitric oxide radicals with an average IC₅₀ values of 3.28 µg/mL and 15.62 µg/mL respectively. Extracts of other selected plants could scavenge DPPH, superoxide anion and nitric oxide radicals to a certain extent. Hence, raw extract of natural antioxidant from *E. officinalis*'s fruits may be very effective in the management of diseases caused by oxidative stress.

Keywords

Plant extracts, Total phenolic content, Reducing power, Radical scavenging.

1. INTRODUCTION

Free radicals such as superoxide ions (O₂⁻), hydroxyl radicals (OH) and hydrogen peroxide (H₂O₂) are various forms of activated oxygen which may be produced endogenously or exogenously (Yildirim *et al.*, 2001). These radicals play an important role in degenerative or pathological processes, such as aging,

cancers, coronary heart diseases, Alzheimer's disease, neurodegenerative disorders, atherosclerosis, cataracts and inflammations (Huang *et al.*, 2005). Excessive production of free radicals can induce various diseases under the condition use of anti oxidant rich compounds are most likely to antagonize free radicals production and or action thereby minimizing the harmful effects of oxidative stress (Grice, 1986).

Medicinal plants are well known to botanic herbs known to have diversified type of secondary metabolic compound such as tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids (Edoga *et al.*, 2005). These components of plants have able to remove not only free radicals scavengers but also involve in chain reaction pre version oxidative enzyme inhibitors, factors as anti oxidants in reducing the risk of chronic diseases including cancer and heart disease (Kadhun *et al.*, 2011). These compounds are associated with antioxidant potential (Grice, 1986). Since ancient times, medicinal plants have been used and documented to promote human health. Although modern medicines are abundant in the market, traditional medicines are still popular in the developing world and people still prefer to improve health by using locally grown medicinal plants. They are of great importance to the health of particulars and communities. Many of these primitive medicinal plants are used as spices and for food plants. plant samples are presented in **Table 1**

Most quality assays used for antioxidant evaluation with antioxidants as reductant or as scavengers of radicals (Wolfe and Liu, 2007). Since the antioxidant potency can differ significantly according to the physical and chemical framework of the systems used for their identification, many systematic techniques are important to assess the prospective antioxidant functions of components in natural plant extracts (Zhou and Elias, 2013). Quantification of radical scavenging capacity or reducing activity alone provides guidelines for their final evaluation in human intervention studies (Lund *et al.*, 2007).

Hence, present study was undertaken to compare radical scavenging activity of six traditional edible medicinal plants from central region of Myanmar in order to evaluate their potential as a natural antioxidative source. The antioxidant activities were determined by *in vitro* assays to compare their antioxidant effects using DPPH (1,1-diphenyl-2-picrylhydrazyl), potassium ferric cyanide, superoxide anion and nitric oxide. Additionally, total phenolic contents of the extracts were also detected.

2. MATERIAL AND METHOD

2.1. Plant Materials and Chemicals

All plant materials were carried out from Mandalay Division, the central region of Myanmar. Botanical identifications were conducted in Pharmaceutical Research Department, Department of Research and Innovation, Yangon in Myanmar.

Table 1. Some common plants of Myanmar

No	Botanical Name	Myanmar Name	Family Name	The Parts Used
1	<i>Piper betle</i> L.	Kun	Piperaceae	Leaves

2	<i>Dolichandrone Spathacea</i>	Tha-khut	Bignoniaceae	Flowers
3	<i>Emblica officinalis</i> Gaertn	Zee-phyu	Phyllanthaceae	Fruits
4	<i>Haplophragma adenophyllum</i> (wall) Dop.	Phet-than	Bignoniaceae	Flowers
5	<i>Oroxylum indicum</i> Vent.	Kyaung-sha	Bignoniaceae	Flowers
6	<i>Boscia variabilis</i> Coll. & Hemsl.	Tha-mone	Capparaceae	Flowers

Chemicals such as gallic acid monohydrate, Folin-Ciocalteu's reagent, 1,1-di-phenyl-2-picrylhydrazyl (DPPH), ferric cyanide, FeCl₃, EDTA, sodium nitroprusside, sulphanic acid, naphthalene diamine dihydrochloride, nitro blue tetrazolium (NBT) and riboflavin were purchased from HiMedia Co. Ltd., India. Trichloro acetic acid was obtained from Sigma-Aldrich Company. Chemicals and solvents used in all assays were of analytical grade.

2.2. Extraction

The selected plant samples were collected, cleaned, air-dried, powdered and stored in air-tight containers to be used in experiments. Each sample was percolated with ethanol (95%). After 30 days they were subjected to filtration and filtrate collected were concentrated by using rotary evaporator. The concentrated plant

extracts were hoarded at cool place below 25°C for more experiments. All experiments were conducted at Pharmaceutical Research Department, Biotechnology Research Department (BRD) under Ministry of Education in Kyaukse in Myanmar.

2.3. Total Phenolic Content

The total phenolic content (TPC) of the crude extract was estimated by Folin-Ciocalteu method (Prior 2005, Ainsworth 2007). Briefly each extract 100 µL was mixed with 10% Folin-Ciocalteu reagent 500 µL. After 5 mins, 7.5 % sodium carbonate was added. The mixture was shaken and allowed to react in the dark at room temperature for 30 mins. The absorbance was measured at the wavelength of 760 nm using SPECTROstar Nano microplate reader. All phenolic components of plant was calculated as gallic acid equivalent (GAE) by the following

equation:

$$T = C \times V/M$$

T is the total phenolic content (mg g⁻¹) of the extracts in GAE, Concentration (C) of gallic acid established from the calibration curve in mg ml⁻¹, the volume of the extract solution (V) in ml and the weight of the extract (M) in g (Ainsworth 2007). All samples were tested with three replications.

2.4. DPPH Scavenging Activity Assay

The inhibition of tested plant extracts on DPPH radical was carried out and quantified by using 96-well micro titer plate and measured using a spectrophotometer (Tepe *et al.*, 2005). Different concentrations (100µL) of the extracts were added into 96 well micro titer plate and then 0.2mM DPPH in methanol solution (100µL) were added to each well. The reaction mixture was allowed to proceed in 30 min in dark at room temperature. The methanol was used as blank while DPPH without extract represented control. After incubation for 30 mins the activity was measured using SPECTRO star Nanomicroplate reader at wavelength 517 nm. The radical scavenging activity was calculated as described below:

Percentage scavenging = [Absorbance of Control - Absorbance of Sample / Absorbance of Control] × 100

IC₅₀ value was calculated representing the inhibitory concentration by 50%. Ascorbic acid served as positive control. All the conducted tests were carried out in triplicate.

2.5. Reductive Potential Assay

The reductive potential of the extracts were measured using a modification of the method described earlier for their reductive potential

(Oyaizu 1986). Various concentrations (100, 80, 60, 40, 20 µg/mL) of the plant extracts (250

µL) were mixed with phosphate buffer (250 µL) and potassium ferric cyanide K₃Fe(CN)₆ (250 µL). This mixture was kept at 50°C in water bath for 20 mins. After cooling, trichloro acetic acid (250 µL 10%) was added and centrifuged (3000 rpm) for 10 mins whenever it is required. The upper layer solution (600µL) was mixed with a freshly prepared ferric chloride solution (120 µL). Then, the absorbance was measured at 700 nm in 96 wells microplate. Control was prepared in similar manner excluding samples. Ascorbic acid at various concentrations was used as standard. A higher absorbance of the reaction mixture indicated a greater reductive potential power.

2.6. Nitric Oxide Radical Scavenging Assay

The nitrite can be determined by using Griess reagent (Govindarajan *et al.*, 2003). The assay was carried out in a flat-bottomed 96- well micro titer plate. The concentrations of extracts prepared in DMSO with methanol using the stock (10mg/mL). Gallic acid was diluted with water and served as standard antioxidants. Different concentrations (10 µL) of each extract, potassium phosphate (20µL, 0.1M) buffer and 10mM sodium nitroprusside (70µL) was added in each well and the mixture was incubated at 25°C for 90 mins similar with amount of reaction mixture with DMSO, without extract, served as blank control. After incubation, plate was pre-read at 540 nm. Then sulphanilic acid (50µL, 0.33%) dissolved in glacial acetic acid (20%) was added. The plates were placed without disturbance to complete of diazotization.

After 5 mins, naphthalene diamine dihydrochloride (50 µL 0.1%) was added and

mix. Then plate was incubated (25°C) for 30 mins, the plate was mix and measured at 540 nm. Same procedure carried out with gallic acid which was used as a standard compound for comparison with tested extracts. Percent inhibition was calculated by the following equation:

$$\text{Percentage scavenging} = \left[\frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \right] \times 100$$

IC₅₀ which is an inhibitory concentration of each extract that reduce 50% of nitric oxide formation was determined. The IC₅₀ value was calculated from the plot of inhibition percentage against extract concentration. All tests were carried out in triplicates.

2.7. Superoxide Radical Scavenging Assay

This assay was assessed as described earlier.

The assay is based on the ability of extracts to inhibit formazan formation by scavenge thereby inhibitory formazon formation. In the present study it was modified regarding reagents concentration and volume to re-adjust for 96-microtitter plate.

Different concentration (10µL) of tested

Table 1.

No	Botanical Name	Myanmar Name	Family Name	Parts of the plants Used
1	<i>Piper betle</i> L.	Kun	Piperaceae	Leaves
2	<i>Dolichandrone Spathacea</i>	Tha-khut	Bignoniaceae	Flowers
3	<i>Embllica officinalis</i> Gaertn	Zee-phyu	Phyllanthaceae	Fruits

extracts, EDTA (15µL of 12mM), NBT (10µL,1.2mM), and riboflavin (5µL of 0.5mM) were added in each well and reaction mixture was diluted up to 200 µL with phosphate buffer(50mM). In control assay DMSO was added instead of extracts. Plates were shaken and pre-measured at 560nm in spectrophotometer followed by illumination for 30 mins and measured at 560 nm (Winterbourne *et al.*, 1975, Patel Rajesh and Patel Natvar, 2011). Super oxide scavenging activity was obtained as the difference in absorbance before and after illumination value. Gallic acid was used as standard control. The IC₅₀ values for each extracts and gallic acid standard were calculated as below:

$$\% \text{ scavenging} = \left[\frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \right] \times 100$$

3. RESULTS AND DISCUSSION

Six medicinal plant extracts were analyzed total phenolic contents and radical scavenging activity. A list of medicinal plants, tropical species along with the parts is presented in **Table 1.**

4	<i>Haplophragma adenophyllum</i> (wall) Dop.	Phet-than	Bignoniaceae	Flowers
5	<i>Oroxylum indicum</i> Vent.	Kyaung-sha	Bignoniaceae	Flowers
6	<i>Boscia variabilis</i> Coll. & Hemsl.	Tha-mone	Capparaceae	Flowers

Table 1. Selected Myanmar Traditional Plants

In Myanmar, the leaves of *P. betle* and *H. adenophyllum* are mainly used as medicines for few diseases. The *E. officinalis* fruits while flowers of *D. Spathacea*, *O. indicum* and *B. variabilis* are also edible and consumed as seasonal food by local people in Myanmar. The antioxidant activity of plant extracts were determined using stable radicals such as DPPH (1,1-diphenyl-2-picrylhydrazyl), reducing

power, superoxide anion and nitric oxide. The amount of total phenolic content was determined spectrometrically according to the Folin-Ciocalteu procedure and calculated as gallic acid equivalents (GAE). Gallic acid used to prepare the standard curve and all extract samples were analyzed in triplicates. The linear calibration curve of gallic acid was in the range of 1 to 1176 mg/L with correlation coefficient

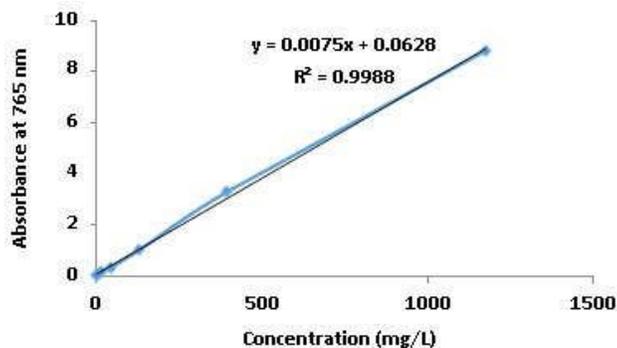


Fig. 1 Standard Curve of Gallic Acid (Concentration of gallic acid used: 0, 1.7, 5, 15, 44, 131, 392 and 1176 mg/L)

The results are presented in **Figure. 2**

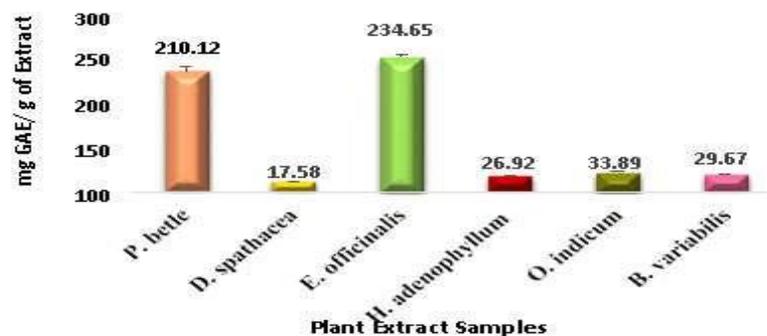


Fig. 2: Total Phenolic content of plant extracts determined by Folin - Ciocalteu assay

It is clear that the total phenolic contents of samples ranged between 18 to 235 mg gallic acid equivalent / g of extract. Among all the plant extracts, *E. officinalis* demonstrated the highest amount of total phenolic content (234.65 mg GAE/g of extract) while the lowest content (17.58 mg GAE/g of extract) was observed in *D. Spathacea*. The variation of total phenolics

in extracts may be due to the existence of different phenolic compounds.

In order to determine the antioxidant activity of plant extracts or specific compound, DPPH assay is an easy, rapid and sensitive way. Antioxidant activity measured by DPPH method were calculated in the form of IC_{50} values of different extracts.

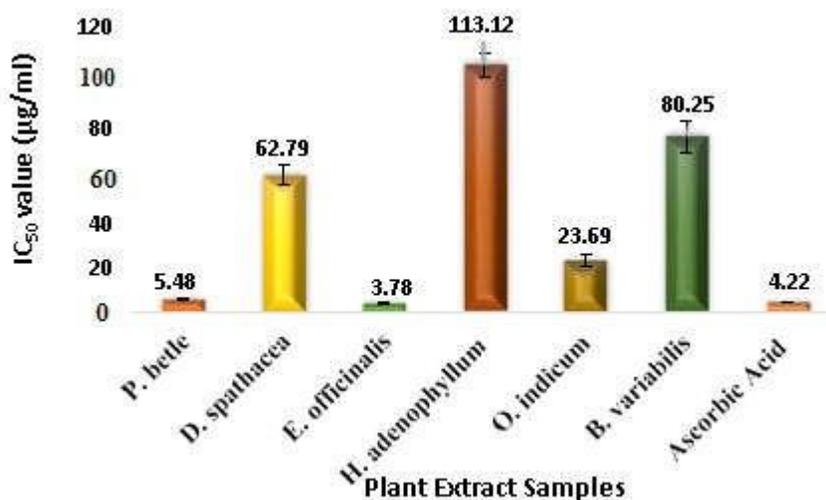


Fig. 3: IC_{50} value of DPPH Scavenging Activity of Selected Plant Extracts

IC₅₀ values of different extracts were ranged from 3.78 to 113.12 mg/mL. The highest antioxidant activity in scavenging DPPH was demonstrated by *E. officinalis* which was greater than the ascorbic acid, the standard compound. Ascorbic acid is a strong reductant and could readily donate a hydrogen atom to a free radical, thus terminating free radical reactions (Shimada *et al.*, 1992). As DPPH scavenging activity of *E. officinalis* was higher than that of ascorbic acid, suggesting that it contains antioxidant compounds at much higher quantities.

P. betle had comparable reducing power towards ascorbic acid. The extracts of *D. Spathacea*, *H. adenophyllum*, *O. indicum* and *B. variabilis* can scavenge the DPPH radical some extent. It is established that DPPH scavenging activities increased with increasing phenolic components and depended on plant type and extraction solvent used. Previous studies demonstrated the existence of strong correlation between total phenolic contents and scavenging activity (Piluzza and Bullitta, 2011). Thus the phenolic compounds in the extracts are mainly responsible for antioxidant activity.

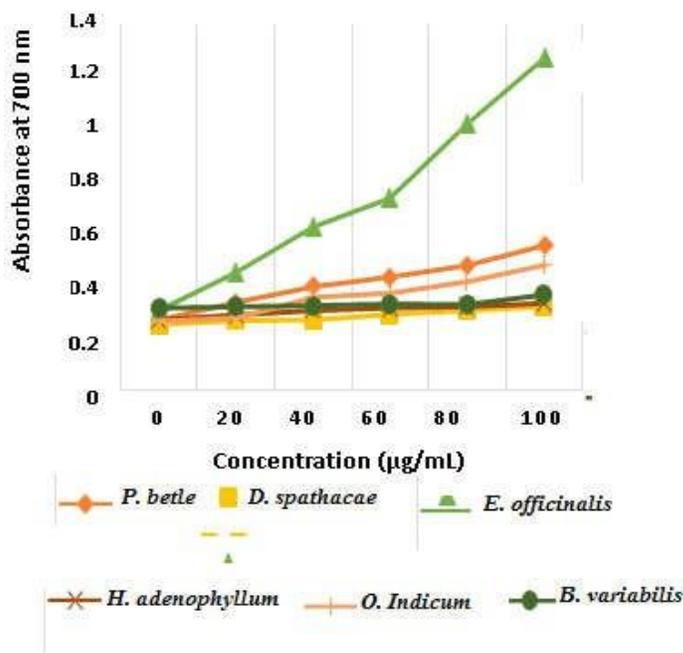


Fig. 4: The Fe³⁺ reductive potential power of solvent extracts. Spectrometric detection was at wavelength 700 nm.

Previous study used Fe^{3+} reduction as an indicator of electron donating activity (Nabavi *et al.*, 2009a). The reducing power of the extracts were determined and mean values were calculated for each extract.

Like DPPH assay, *E. officinalis* demonstrated a better reducing power compared to other plant extracts. Extracts of *P. betle*, *D. Spathacea*, *H. adenophyllum*, *O. indicum* and *B.variabilis* the higher their reducing power. Generally also ad reducing power activity. A linear positive relationship was noted between the reducing power and total phenolic contents of tested plant extracts. The higher the total phenolic contents of the tested plant extracts, the higher their

reducing power. Generally reducing power of a compound is associated to their electron transfer ability. As phenolic compounds associate easily donate hydroxyl hydrogen due to resonance stabilization, the reducing power of such possibly extracts might be pre-dominately related to hydroxyl hydrogen (Meir *et al.*, 1995)

As indicated in our result, the reducing power assay may be used as a indicator for determining potential antioxidant activity.

It is well established that being super oxide radical uses a major biological source of radical oxidative species, is one of the most frequently generated reactive oxygen species in the cells.

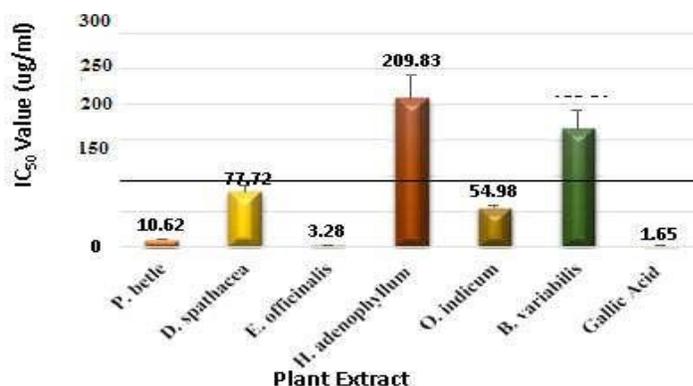


Fig. 5: IC₅₀ value of Super oxide Scavenging Activity of Selected Plant Extracts Compared with Gallic Acid. Results were the average of triplicates with \pm standard deviation

The superoxide scavenging activity of ethanolic extract of *E. officinalis* was an average IC₅₀ value of 3.28 ± 0.26 and higher than that of gallic acid. Except *E. officinalis*, *P. betle* had the second highest SO radicle scavenging activity

The moderate scavenging activity was found in the extracts of *D. Spathacea* and *O. indicum* while *H. adenophyllum* and *B. variabilis* had the lowest scavenging activity, with an approximate IC₅₀ values of 203.83 $\mu\text{g}/\text{mL}$ and

166.61 $\mu\text{g}/\text{mL}$, respectively.

Nitric oxide is generated in biological tissues by specific nitric oxide synthesis (NOSs) and responsible for altering the structural and functional behavior of many cellular components.

Like other radicals, excessive levels of nitric oxide is associated with several diseases (Ialenti and Di Rpsa, 1993). **Fig. 6.** The comparative nitric oxide scavenging activity of the selected plant extracts demonstrated.

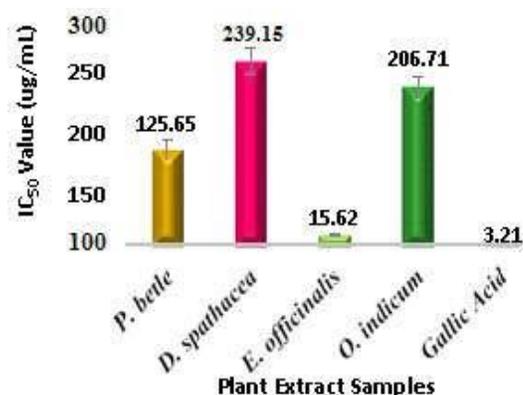


Fig. 6: IC₅₀ value of Nitric oxide Scavenging Activity of Selected plant Extracts Compared with Gallic Acid. Results were the average of triplicates with \pm standard deviation.

The extracts of *H. adenophyllum* and *B. variabilis* may have nitric oxide scavenging activity because of their lower color intensity.

However, the absorbance could not be detected because of the precipitation during reaction. Therefore the data of two extracts for nitric oxide scavenging was not measurable.

4. CONCLUSION

In all tested plant extracts, fruits of *E. officinalis* demonstrated highest amount of total phenolic contents. Moreover, it inhibited the maximum radical scavenging activity in DPPH. In addition, the highest reducing power was also observed. Extracts from leaves of *P. betle* and flowers of *D. Spathacea*, *H. adenophyllum*,

O. indicum and *B. variabilis* scavenged the free radicals at same concentration. Hence, based on these results, fruits of *E. officinalis* may contain active constituents that can be used as promising candidate for further research leading to natural antioxidants for treatment of free radicals associated diseases.

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PAM caused by *Naegleria fowleri*, An Alarming Situation in Pakistan:

A Meta-Analysis Study

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Abstract

Primary Amoebic Meningo-encephalitis (PAM) is a CNS disease and *Naegleria fowleri* is an independent breathing pathogen is responsible for this disease. The disease is caused by infecting the central nervous system and since called brain eating amoeba. The disease occurs worldwide leading to diagnostic challenges as quick and specific identification in clinical samples could be prove helpful for proficient case management. In present study the mortality ratio due to *Naegleria* is analyzed in Pakistan, further the lacking in community awareness and facilities are also described that are comorbid with the wide spread of disease. Despite of advancement in antimicrobial chemotherapy, no improvement of fatality rate due to the amoeba was observed in last decade as like in USA the ratio of survival was verified three out of 133. The amoeba lives in soil near warm water, also in poorly chlorinated or unchlorinated swimming pools, ponds, torpid water and polluted water discharge from industries. The initial symptoms are fever, headache, stiff neck

leading to rapid progressive illness, coma and then death. From 2008 till 2017, in Karachi, a metropolitan city of Pakistan, mortality rate from the PAM was recorded 108 ($n = 108, 100\%$). The precarious condition and high stats of mortality rate urge to develop strong therapeutic weapons to fight the disease and beneficial compounds to transmit through blood-brain to target and kill the living pathogenic moiety, present in the brain tissue. However awareness regarding associated risk of PAM in developing regions can prove significant against the over spread of disease.

Keywords: *Naegleria fowleri*, free-living amoeba, central nervous system.

1. INTRODUCTION

The worldwide fatality rate due to primary amoebic Meningo-encephalitis (PAM) was notified 95% statistically interestingly, in spite of having advanced antimicrobial moieties (Marciano-Cabral and Cabral., 2007; Visvesvara *et al.*, 2007;

Diaz., 2012). *Naegleria fowleri* (Figure 2), mostly found in temperate fresh water such as lagoon, springs, canals and rivers that are the most of

contributing sources of infection, responsible for transferring the living moiety in human body that invades the central nervous system (Figure 1).



Fig 1: *Naegleria fowleri* (*N. fowleri*) Ameba

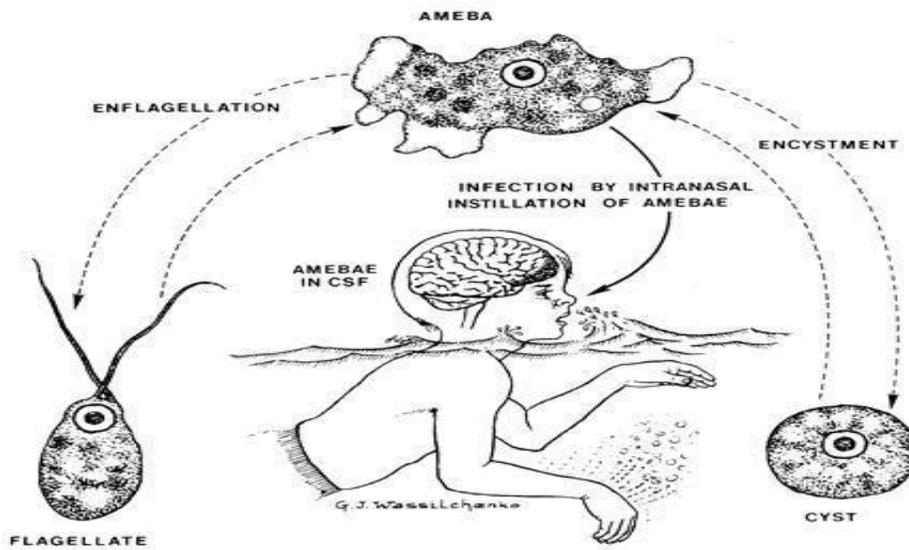


Fig 2: The mechanism of entrance amoeba via into the brain through forceful pushing up into nostrils in contaminated water.

The epidemiology reveals that the occurrence of infection was first Virginia, 1937, however was reported initially from Australia, 1965. The rise in the happenings was observed with the passing years may be due to high temperature as a consequent of global warming, specifically the regions of hot prolonged summer months are found more susceptible as the warm temperature provides more encouraging environment for the amoeba to grow and multiply. The consumption of water is the major source for the amoeba to be transmitted into human body.

Accordingly the quality of water matters and responsible to more extent. Improperly disinfection lead to high chances of contamination and deterioration in the water supply are the factors responsible for dispersion of disease. (Siddiqui and Khan, 2012; CDC, 2015; SAAL, 2015; Shakoor *et al.*, 2011; Cope *et al.*, 2015). Furthermore waste water from industries, amusement parks, electrical power plants and health resort are source of contamination and infections (Marciano-Cabral *et al.*, 2013) (Figure 3a)



Fig 3a: The chances of getting infected from PAM

The amoeba enters into human body and approach to the CNS travelling through the olfactory nerve. (De Jonckheere, 2011). In Pakistan, numerous cases of the disease have been testified from the metropolitan capital Karachi and unfortunately none have stayed alive thus far. A research demonstrated in Karachi reveals the existence of PAM amoeba in 8% of drinking water samples (Yousuf *et al.*, 2013). The figure can be explained for many apparent causes as the source of water

is the fresh water that have poor disinfection and also mixing of dirty water that is a potential supply of *N. fowleri*. Further the leakages in water and also sewerage pipes, inappropriate cleaning and disinfecting procedures of supply tanks may also be the factors that favors the growth of the pathogen (Yoder *et al.*, 2010; Visvesvara., 2010; Marciano-Cabral and Cabral., 2007; Hunteet *al.*, 2013).

2. MATERIAL AND METHOD

All data relating to the *N. fowleri*-regarding deaths in Pakistan were searched from Google Scholar, PubMed, Web of Science, ScienceDirect, and local database and news agencies such as

Dawn.com, the Express Tribune, and the News. Search was conducted using the key words like “*Naegleria fowleri*” along with “Primary amoebic meningo-encephalitis”, “diagnosis”, “epidemiology”, “pathogenic” and “olfactory”. Accordingly, in present review an up to date data regarding the pathogenic nature of *Naegleria fowleri* is covered with further aspects are also discussed for future and prevention against the fatal infection.

3. RESULTS AND DISCUSSION

In Pakistan, the death ratio due to PAM is startling since has resulted in the death of 98 individuals during a short period of 8 years from 2008 to 2015. In the year 2012, Pakistan had the highest number of cases reported when as many as 22 cases were recorded. This study highlights the risk factors of infection, especially in Karachi, that has witnessed the emergence of this pathogen at an alarming rate. Karachi is situated on the southern coast of Pakistan and experiences a hot and humid climate (The Express Tribune, 2012; Main 2015; Khan *et al.*, 2010). The high population and low sanitary facilities are highly accountable for the wide spread of disease. Accordingly numerous cases of PAM has been recorded and reported as like in June 2008, a 30-year-old,

with a good health history was admitted to the Aga Khan University Hospital with complain of high--grade fever for last forty eight hours, brutal headache, and seizures. After the initial examination of primary amoebic meningo-encephalitis, he was put on an intravenous amphotericin B plus oral rifampin and fluconazole, unfortunately he could not survive and expired after 48 hours of admission (Shakoor *et al.*, 2011). One more case was also to mention here of 24-year-old man with complain of pyrexia, neuralgia and vomiting. When motile trophozoites were identified on a wet mount of cerebrospinal fluid, the treatment was started with intrathecal amphotericin B, fluconazole and rifampicin. He also could not survive and expired on the sixth day of hospitalization. Another patient of 30-year-old man hospitalized with three-day history of high grade fever, vomiting and agitation. He received intrathecal amphotericin B, fluconazole and broad spectrum antibiotics when motile trophozoites on wet mount were observed. Once again, the patient’s disease condition did not improve and he died on the eighth day of admission (Saleem *et al.*, 2009). Another case of 42 years old patient who was employed in poultry farm and he died within one week of hospital admission. *Naegleria fowleri* was detected in the sample of cerebrospinal fluid of patient. This is a serious problem and requires instant measures to prevent general population to get affected by this fatal neurological infection (Shariq *et al.*, 2014). A detailed report of *N. fowleri* cases in the country is presented in [Table 1] below:

Year	Reported Cases of <i>N. Fowleri</i>	SOURCE
2008	02	Shakoor <i>et al</i> 2011
2009	11	Shakoor <i>et al</i> 2011

Year	Reported Cases of <i>N. Fowleri</i>	SOURCE
2010	20	Siddiqui and Khan, 2014
2011	13	Mahmood, 2015., Hassan 2014
2012	22	Alam 2012, Ilyas 2012, Shariq <i>et al</i> 2014, Malik 2012, Abbasi 2012, The Express Tribune 2012
2013	03	Zaheer and Bhurgri, 2013, Hasan 2014, The express tribune 2013.
2014	14	Hasan 2014, Dawn .com 2014, Ilyas 2013, The Express tribune 2014.
2015	13	Tanveer Ahmed 2015, Dawn.com 2015, Hasan 2015, Mandhro 2015, The Express Tribune 2015, The News Tribe 2015.
2016	5	Health Department officials, 2016
2017	5	ROBERT HERRIMAN ¹⁴ th April, 2017, August 2017 National Courier

TABLE 1: SUMMARY OF *N. FOWLERI* CASES REPORTED IN PAKISTAN

Most cases reported healthy young adults with acute, fatal meningitis. Despite the fact that improvement in early detection of PAM may have contributed toward the high rise in cases however early detection cannot be accountable for life saving in PAM, but may contribute for the awareness and preventive measures (Jamilet *al.*, 2008; Ahmed *et al.*, 2007). The public health officials reported that the patient was initially treated at some private hospitals for malaria and then for meningitis and finally the family shifted him to the ICU where his condition never improved and doctors pronounced him dead today". Four different cases were also reported from Karachi including a teenage girl, a middle-aged woman, a 37-year-old man and a 16-year-old boy and grievously all of them passed away. The only patient who had contracted the disease from an area out of Karachi was a 40-year-old man from Thatta (Hasan, 2014).

Health officials in Karachi have reported the first such case of 2017 (Herriman, 2017). Initial symptoms, start within the few hours of infection are severe headache along with nausea and vomiting, high grade fever specifically and rarely stiff neck complain. Seizures and hallucinations may also be observed but at a later stage (Laghari, 2016). Swimming is considered to be one of the key risk factors that causes *Naegleria*. So stay away from public pools with improper arrangements. And if it's so necessary for you to swim, try to keep nostrils closed. Chlorination is a useful method to execute the germs. So, chlorinate your water tanks. Chlorine tablets are available at medical stores. Use boiled water for cleaning nose as the pathogen may enter through the nostrils and may assault the brain. The brain-eating amoeba does not survive in clean water, so regularly clean water tanks, especially underground tanks, in your house or residential

apartment. Imams (prayer leaders) should be advised to the use of chlorine in water being used in mosques for ablution since the amoeba approaches brain through nasal space (Laghari, 2016). PAM is highly associated with freshwater swimming (Craunet al., 2005) specifically in poorly chlorinated swimming pools (Shenoy et al., 2002). Anyhow, no association was found with swimming in all reported cases from Karachi. Lavation is an essential and mandatory spiritual formalistic procedure performed by Islamic religious persons, before prayers. It involves nasal irrigation with water (Siddiqui and Khan, 2012; Shariq et al., 2014). The Karachi's municipal water supply is highly accountable due to many reasons for the maximum possible finding of *N. fowleri* amoebae. The authorities do not adequately process of chlorination in Karachi lacks, an original water source for natives, and two suburban resources of freshwater as well (Dawn News, 2009). Moreover, the pathetic situation of numerous leaks in water and sewage pipes cause seepage of sewage into the water supply that work as a potential reservoir for pathogen *N. fowleri* (Dawn News, 2009; Lawandeet al., 1979).

Current temperature records from Karachi have also shown a temperature flow and that may lead a further rise is expected in prospect summer season (Sajjad et al., 2009). Several factors may be accountable for this epidemic as an increase in the number of pathogens (due to rising temperatures), changes in the ecosystem of the lakes, and further decline in the quality of water treatment and distribution systems. Another study reported that close observation of the cases reported reveals that all the deaths caused by that *N. fowleri* occurred in April- September when most part of the country experiences pronounced spells of hot weather.

Pakistan is experiencing changing climatic conditions. Karachi in particular has been experienced soaring temperatures, and summer temperatures are projected to continue rising. It is feared that this increase of temperature may be one of the factors potentially contributing to the recent upsurge in the number of infection cases in the city (Naqvi et al., 2016). The alarming mortality rate due to this amoebic infection is more than enough to explain the treacherous situation in region. The majority population being Muslim observe and practice Islamic rules of cleanliness including nasal irrigation as part of ablution and other necessary measures for the proper sanitation despite of the fact that water supply is not well chlorinated.

For the purpose public awareness lectures and other activity through electronic, print and social media can significantly make the people alert and conscious for taking preventive measures. Proper treatment of water, either by chlorination or boiling, should be further carried out to ensure the low risk of infection, otherwise due to negligence cases of primary amoebic Meningo-encephalitis will continue to rise in Pakistan.

4. CONCLUSION

Pakistan has been seen as a developing country, with a high rate of illiteracy and many people living below the poverty line with unsatisfactory level of health systems. The country where facilities regarding healthcare are still nonexistent and unreachable for many. Lack of potable water supply and proper sanitation, with lack of awareness, further augments such problems of epidemics of water borne diseases. All facts and figure reveals the present time high for authorities to identify the health problems of masses and take concrete measures to salvage the desolation.

Further investigations are also required to work on the role of environmental factors, figures and causes in the increasing cases of Naegleria infection.

Conflict Of Interest

The authors declare that there are no conflicts of interest.

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Phytochemical and Physico-chemical Properties of *Zanthoxylum armatum* DC. Seeds.

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Abstract

The presence of phytoconstituents makes the plant useful for treating different diseases and also helpful for manufacturing drugs for human use. *Zanthoxylum armatum* DC. belongs to family Rutaceae, traditionally used in the treatment of abscesses, arthritis, bruises, gastritis and swellings. The present study was aimed to investigate the physicochemical and phytochemical screening of *Z. armatum* seeds in four different solvents (Acetone, Chloroform, Methanol and Water). The results revealed the moisture content was 9.04% and total ash content was 7.14%. Extractive value was higher in methanol (7.8%) and lowest in chloroform (3%). The phytochemical constituents such as phenolic compounds, flavonoid, terpenoids, fixed oil and natural compound (carbohydrate, protein and amino acid) were present in all solvents extract. While alkaloid and glycoside were absent in acetone and chloroform extracts. These secondary

metabolites are important for their therapeutic properties as presence of phenolic compounds indicate the antioxidant and free radical scavenging activity of plant, thereby justifying the importance of *Z. armatum* DC. seeds in ethnomedicine.

Keywords:

physicochemical, phytochemical, phytoconstituents, seed, *Zanthoxylum armatum*.

1. INTRODUCTION

Plants have been reported for their therapeutic utility for thousands of years and now have important position in allopathic, herbal medicine and homeopathy (Sneider, 2005). Medicinal plants are considered as one of the main sources of biologically active materials for prevention and cure of various diseases. According to the World Health Organization, 80% of the population uses plant-based remedies as their primary form of health care. Plant chemicals are regarded as secondary

metabolites because the plants that manufacture them mostly have minimum need for them. Many classes of important chemical compounds have been evaluated from the plants e.g. alkaloids, flavonoids, terpenoids, glycosides, phenolic compound using pharmacognostic tools (Edoga *et al.*, 2005). Phytochemical constituents of medicinal plants are helpful for healing as well as for curing of human disease (Anti-inflammatory, anti-malarial and anti-bacterial). Many important drugs in particular Artemisinin from *Artemisia annua* are derivatives of traditional medicinal plants (Pan *et al.*, 2013; Nostro *et al.*, 2000).

Zanthoxylum armatum DC. commonly known as timbar belongs to family Rutaceae. The genus *Zanthoxylum* comprising 20-30 species distributed in East Asia, Philippines, East Malaysia and North America. Represented in Pakistan by single species *Zanthoxylum armatum* DC. The plant is 2-4 m tall, small tree or shrub, leaves are compound, flowers usually unisexual, axillary, minute and petals absent (Hassan-ud-Din and Ghazanfar, 1980). Leaves, roots and seeds have been also used in traditional medicine. The seeds are used in the treatment of digestive impairment, piles, heart diseases, hiccups, cough, throat disorders, asthma, and dental diseases (Shinwari *et al.*, 2006). Its decoction is used in the treatment of arthritis, bruises, gastritis, swellings etc. (Duke and Ayensu, 1985) while its paste is used to relieve toothache. The root decoction used as an anthelmintic while leaves paste is applied externally to treat leucoderma (Manandhar, 2002). Therefore, the present study aimed to explore the physicochemical and phytochemicals analysis of the seeds in four different solvents.

2. MATERIAL AND METHODS

2.1 Collection of Plant Material:

Seeds (200 g) of *Zanthoxylum armatum* DC. were collected from Kashmir and Hazara. Pakistan. Herbarium sheets were prepared (Fig. 1) deposited in the Karachi University Herbarium, Centre for Plant Conservation. Healthy plants were chosen with high quality seedpods and fruits for seed collection. The fresh fruits were washed under running tap water to remove dust, pulp was separated, seeds obtained were washed thoroughly, air dried and grounded by using a mechanical blender.



2.2 Macroscopic Study

Morphological characters including color, shape, size and weight were noted from the fresh and dried seeds and also observed under the stereo microscope.

2.3 Physicochemical Analysis

Seeds (100 gm) of *Zanthoxylum armatum* were ground well using a mechanical blender into fine powder. Physicochemical analysis included percentage of loss on drying moisture content, ash values (Total acid insoluble and water soluble ash).

i). Determination of Ash Contents

Seed powder in crucible was placed in furnace at 450°C until grayish white powder free from carbon compound was obtained, followed by cooling and weighed again (Gupta, 2003 and Indrayan *et al.*, 2005). The total ash was calculated by using following formula:

$$\text{Total ash} = \frac{\text{weight of ash} \times 100}{(\%) \text{ weight of powder}}$$

Water Soluble Ash

Total ash was boiled with water (5 ml) for 5 minutes, filtered on ash less filter paper and ignited. The water soluble ash was calculated as follows:

$$\text{Water soluble ash} = \frac{\text{Weight of total ash} - \text{Weight of insoluble ash} \times 100}{\text{Weight of sample taken}}$$

Acid Insoluble Ash

Total ash obtained from the powder was boiled with 2M HCl (25ml) for 5 min, and the insoluble matter was collected on ash less filter paper and ignited, cooled and weighed. The water soluble ash was calculated using following formula:

$$\text{Acid insoluble Ash} = \frac{\text{Weight of Acid Insoluble Ash} \times 100}{\text{Weight of the sample taken}}$$

ii). Moisture Content

Powdered sample (2 g) was weighed accurately in crucible and heated (105°C) in furnace till the constant weight. Cooled and the difference in the weight was calculated as moisture content by using the following formula:

$$\text{Moisture content} = \frac{W1 - W2}{W1} \times 100$$

(%)

W1= Weight of the sample before drying

W2= Weight of the sample after drying

2.4 Extractive Value

Powdered sample (10gm) was soaked in different solvents (200 ml) separately and was placed for 48 hrs for continuous shaking. Solvents used were acetone, chloroform, methanol (BDH Laboratory Supplies) and water. After 48 hrs continuous shaking extracts were filtered using filter paper Whatmann No. 1 paper and left for complete evaporation. Dried material obtained was collected and the extractive value was calculated by weighing.

2.5 Phytochemical Analysis

Powdered seeds sample (15 g) was soaked in four different solvents and placed for continuous shaking for 48h in shaker. It was filtered after shaking and the filtrate was evaporated. The four different extracts obtained were subjected to various phytochemical analysis according to standard methods (Harborne, 1998).

2.5.1. Test for Alkaloids

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

i. Wagner's Test

In filtrate (2ml), added 2-3 drops of Wagner's reagent (0.63 g iodine and 1 g potassium iodide in small amount of water and raising volume up to 50 ml with distilled water).

Formation of reddish brown precipitation showed the presence of alkaloids.

ii. Mayer's Test

To filtrate (1ml) few drops of Mayer's reagent [Potassium iodide (5 g) dissolved in water (10 ml) and mercuric iodide (1.358 g) dissolved in water (60 ml). Both solutions were mixed and made up the final volume with water (100 ml)]. Dense white precipitation confirms the presence of alkaloids.

2.5.2. Test for Carbohydrates

Extract (1g) was dissolved in water (2.5 ml) and filtered. The filtrate was used for following tests (Ramakrishan *et al.*, 1994).

i. Benedict's Test

Aqueous extract (1 ml) and Benedict's reagent (1 ml) Sodium citrate (17.3 g), sodium carbonate (10 g) dissolved in distilled water (80 ml) and boiled to acquire solution. Copper sulphate (1.73 g) dissolved in distilled water (10 ml) was then added. The mixture was heated on water bath for 2-3 min. Formation of precipitation indicated the presence of carbohydrates.

2.5.3. Test for Amino Acid and Protein

Extract (100 mg) was dissolved in distilled water (10 ml), The filtrate was used to test for proteins and amino acids (Fisher, 1968; Ruthmann, 1970)

i. Biuret Test

Filtrate (3 ml) was treated with copper sulphate solution (2 drops) followed by addition of 2 ml ethanol (95%) and several pellets of potassium hydroxide. Pink coloration in ethanolic layer confirmed the presence of proteins. Ninhydrin test was used for detection

of amino acid.

ii. Millon's Test

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

2.5.4. Test for Phenolic Compound

i. Lead Acetate Test

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

ii. Ferric Chloride Test

Filtrate (2 ml) was treated with ferric chloride solution (2 ml 10%). Dark brownish green color indicates the presence of phenolic compounds (Evans, 1997).

2.5.5. Test for Flavonoids

In the aqueous filtrate (1 ml) was treated with ammonia (2 ml 10%) and concentrated H_2SO_4 (1ml) was added. Development of yellow color confirms the presence of flavonoids (Ramakrishan *et al.*, 1994).

2.5.6. Test for Glycoside

Borentrager's Test

In filtrate (2 ml) chloroform (3 ml) was mixed and shaken, 10% solution (1 ml) of ammonia was added. Pink coloration indicate the presence of glycoside.

2.5.7. Test for Fixed Oils (Spot Test)

A small quantity of dried powdered seed samples was pressed between two filter papers. Oil spot on the paper is an indicative of presence

of fixed oils.

2.5.8. Test for Terpenoids

Extract (0.5 gm) was mixed with chloroform (2 ml) then add H_2SO_4 (3 ml). Appearance of brownish red color indicate the presence of terpenoid (Evans, 1997).



2.5.9. Test for Saponin

Extract (0.5 g) was dissolved in autoclave distilled water (20 ml), shaken continuously for 15-20 min. Formation of soap layer indicate the presence of saponins.



Zanthoxylum armatum

3. Results and Discussions

3.1 Seed Morphology

The *Z. armatum* seed was 2-2.5mm in diameter, 0.01g, ellipse or oval, black shiny, hilum (5mm), marginal (Fig. 1 A, B, C). Its leaves, roots, and seeds are used for medicinal purpose. In Ayurveda, the seeds are used in treatment of digestive impairment, piles, heart diseases, hiccups, cough, throat disorders, asthma, and dental diseases (Shinwari, 2006).

The results of physico-chemical analysis of *Z. armatum* given in Table 1. Ash of seeds was fine powder and grayish white in color, total ash content was 7.14%, water solubility of ash was 42% and acid soluble ash was observed 46.5%, moisture content was 9.04%. Extractive value was higher in methanol i-e 7.8% and lowest in chloroform 3%, while extractive value in acetone 3.5% and in water 4% was calculated.

Table 1: Physicochemical analysis of seeds of *Zanthoxylum armatum* DC.

S.No.	Parameter	Inference (%)
1.	Physical state of ash	Fine powder
2.	Colour of ash	Grayish white
3.	Loss on drying	9.04
4.	Ash content	7.14
5.	Water soluble ash	42

S.No.	Parameter	Inference (%)
6.	Water insoluble ash	58
7.	Acid soluble ash	52
8.	Acid insoluble ash	48
9.	Acetone soluble extractive value	3.5
10.	Chloroform soluble extractive value	3
11.	Methanol soluble extractive value	7.8
12.	Water soluble extractive value	4

The therapeutic properties of medicinal plants are associated with the presence of various secondary metabolites such as alkaloids, flavonoids, terpenoids, glycosides, phenols, saponins etc. Phytochemical investigations of various extracts of seeds of *Z. armatum* revealed alkaloid, carbohydrate and glycoside

are absent in chloroform and acetone extract, while the other natural compound like carbohydrate, protein and amino acid, phenolic compounds, flavonoids, terpenoids, fixed oil was present in all solvents extract. The results of preliminary phytochemical analysis are presented in Table 2.

Table 2. Qualitative phytochemical screening of seeds of *Zanthoxylum armatum* seeds in different solvents

S.NO	Phytochemical test	Acetone	Chloroform	Methanol	Water
1.	Alkaloid				
	Wagner's reagent	-	-	+	+
	Mayer's reagent	-	-	+	+
2.	Phenolic compounds				
	Lead acetate	+	+	+	+
	Ferric chloride test	+	+	+	+
3.	Flavonoids	+	+	+	+

S.No	Phytochemical test	Acetone	Chloroform	Methanol	Water
4.	Glycoside Borntrager's test	-	-	+	+
5.	Terpenoids	+	+	+	+
6.	Saponin Foam test	-	-	-	-
7.	Carbohydrate Benedict's test	-	-	+	+
8.	Protein and amino acid Biuret test Millions test	- +	- +	- +	- +
9.	Fixed oil Spot test	+	+	+	+

The presence and absence of phytochemical are represented by (+) and (-) respectively

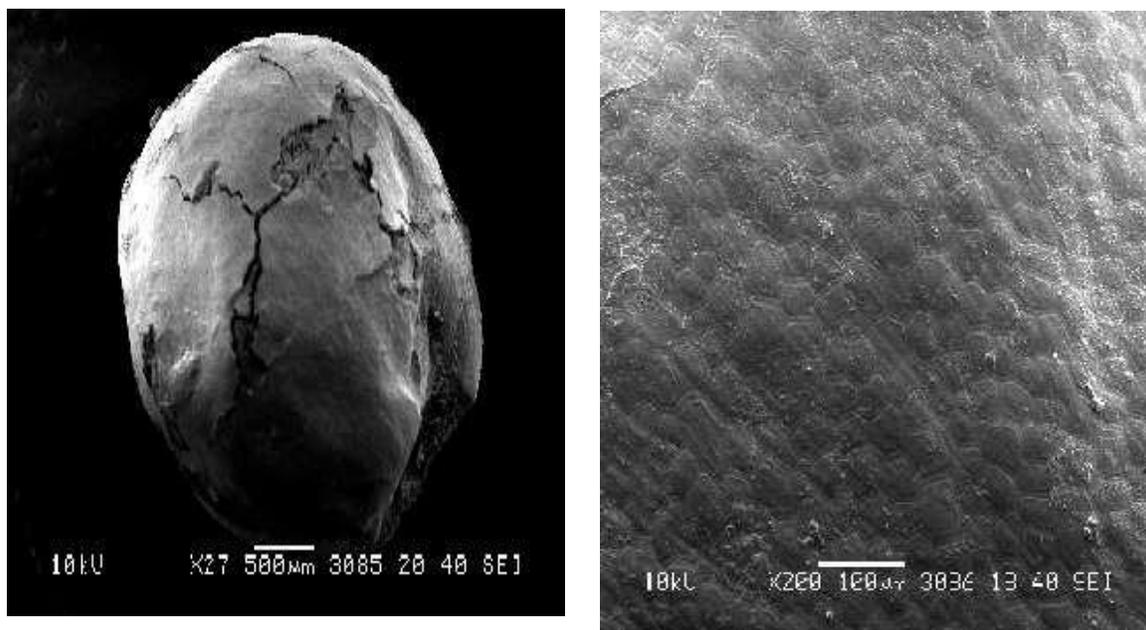


Fig. 2:

Scanning electron micrograph of *Zanthoxylum armatum*; (A) Entire seed (B) Surface (Scale bar: A= 500µm; B=100µm)

The phytochemical analysis of different plant extracts indicates the presence of various phytochemicals, which are considered as active medicinal constituents (Sofowra, 1993).

Ash represents the inorganic part of the plant due to ashing all the organic material present in sample are destroyed. Ash of the seed was observed greyish white fine powder. About 42% of ash was soluble in water and 52% was soluble in acid. Highest extractive value was obtained in methanol while it was lowest in chloroform. Phytochemical analysis in the present study revealed the presence of alkaloids, carbohydrates, phenolic compounds, flavonoids, glycoside, proteins and fixed oil in the seed extracts. Therapeutic properties of *Z. armatum* seed may be due to the presence of phytoconstituents in four different extracts. Alkaloids have been linked with medicinal uses, several workers have reported their common biological properties including cytotoxicity, antibacterial and analgesic (Harborne, 1973; Nobori *et al.*, 1994; Okwu, 2004). Several studies also revealed plants rich in phenolic compounds possessing antioxidant properties (Brown & Evans, 1998; Krings & Berger, 2001). Plant phenolics are a major group of compounds that are considered primary antioxidants or free radical scavengers. Since these compounds were present in the extracts and might be responsible for the potent antioxidant capacity associated with *Z. armatum*. Secondary metabolites and other chemical constituents of plants are important for their medicinal value. For instance, different studies demonstrated that glycosides are helpful in lowering blood pressure and are also used in the treatment of cardiac arrhythmia and congestive heart diseases (Nyarko & Addy, 1990). The terpenoids has

the blood sugar lowering properties. The presence of glycosides and terpenoids in seed extract may play an important role in cardio protective action of *Z. armatum*.

4. CONCLUSION

The physicochemical analysis revealed that seed of *Z. armatum* contained 9.04% moisture, 42% ash soluble in water and 52% ash soluble in acid. It also showed the presence of carbohydrate and protein along with alkaloids, phenolics, flavonoids, terpenoids, glycosides and fixed oil. All these group of phytochemicals are collectively and accountable for pharmacological and therapeutic effects justifying its ethnomedicinal properties. Thus, the seed of *Z. armatum* can be used in the preparation of alternative or new drugs against different diseases.

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