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MADINAT AL-HIKMAH
City of Education, Science and Culture

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

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INFORMATION FOR CONTRIBUTORS

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Title Page Should Contain: Title of the manuscript, Short title, Authors names with superscripts representing their affiliation clearly marked *corresponding author with Telephone, Fax and E-mail address.

Abstract: On a separate page (200-250 words) with Keywords (3-5).

The Text For Articles Should Contain: Main headings in caps and bold as:
1. INTRODUCTION, **2. MATERIALS AND METHODS** (2.1 sub-headings [in ***bold italics***] describing animals, chemicals, preparation of extract, experiments and statistical analysis), **3. RESULTS AND DISCUSSION, 4. CONCLUSION** and **ACKNOWLEDGEMENTS** (if needed) before References, **5. REFERENCES.**.
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References: Follow the pattern shown below: References should be in alphabetical order.
(Articles) 1. Quinton, R. (2012). The increase in the toxicity of yohimbine induced by imipramine and other drugs in mice. *Brit. J. Pharmacol.* **21**:51-66.
(Books) 1. Gaill, W., Jon, A.W. (1995). *Manual of Clinical Microbiology*. ASM Press, Washington, DC. 6th Edn., pp. 1327-1332.
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Prevalence of Anemia and Its Various Types in Early Marriages in Gilgit and Adjacent Areas

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Abstract

Early marriages of women (under the age of 18 years) is a common practice in Pakistan especially in some districts of Gilgit Baltistan. Such marriages have serious adverse effect on women health. The present study was undertaken to estimate prevalence of anemia in married women of Gilgit and adjacent areas. Data regarding various blood components including hemoglobin percentage (Hb), total leukocytes count, neutrophil, lymphocytes, esonophils, monocytes and platelets counts, were obtained and analyzed. About 140 of women married before and 860 married after the age of 18 years (n=1000). Average values of percent hemoglobin, total leukocyte, lymphocytes, and platelets were less in women married under 18 years as compared to women married over the age of 18 years.

The present study indicated that it is not adviseable for women to marry under 18 years of age as it is a causitive factor leading to anaemia.

1. INTRODUCTION

Blood is an essential component of heamopoietic system along with iron a key component of blood in human. Most important functions of blood are to carry oxygen and nutrients to various parts of body and also to transport waste products from the cell. An adult person has approximately 4.7 liters of blood in his/her body (Lee, 1998). Blood contains around 4000 of different types of cells which are specific for their functions. Among 4000 of different types of cells which are specific for their function forming two portions i.e. elements and plasma. Eements composed of 45% of blood and consists of red blood cells (Erythrocytes), white blood cells (Leukocytes) and platelet (Thrombocytes) (O'Neil, 2012; Schmaier, 2008).

Major portion of blood iron is involved in many essential functions, especially production of hemoglobin. An adult human being contains 2.5 g of iron, 70% present in RBC which is

associated with hemoglobin (Helmer and Emerson, 1934). Anemia is a common problem in humans associated with deficiency of iron, especially in women during pregnancy (Ijlal *et al.,* 2000). Common symptoms of iron deficiency include yellow skin color, fatigue, shortness of breath, rapid heartbeat, headache etc. Clinically iron deficiency is diagnosed by complete blood count (CBC). Persons with iron deficiency commonly have low hemoglobin, low cellular volume, low serum iron and low iron saturation. Such conditions may be overcomed in patients by using food like meat, poultry, fish, leafy green vegetables, legumes, iron rich grain foods and cereals etc. In some cases, patients with iron deficiency are treated by given intravenous injection of iron dextran, iron sucrose and ferric gluconate. In extreme cases, anemic patients are given blood as well.

In normal (healthy) individual, concentration of hemoglobin ranges from 14.0-17.5%. Average hemoglobin concentration in male is 5 g/dL while in female it is 12.3-15.3 g/dL. The RBC count and amount of haemoglobin increase at high altitudes. Individuals having lower values of hemoglobin than normal are said to be anemic. It has been estimated that world wide, 55.5% females have Hb level lower than 11.1 g/dL hemoglobin (Basu and Kulkarni, 2014). During a recent study, Afzal (2015) reported that mean hemoglobin percentage in women population of Gilgit and adjacent area was 12.02% (ranging from 5.4-16.6 %). Raza *et al.,* (2011) reported that rate of anemia was higher in pregnant women as compared to other women in Hazara division, Pakistan. In a previous study Ijlal *et al*., (2000) reported that 43.1 % of female in Gilgit had anemia. Baig (2015) reported that 85% mothers in Gilgit Baltistan had anemia. It was also reported that 25-40 years old mothers were among the most susceptible to anemia. Mostly diet of the people in GB comprised wheat, chopped meat, tomato, onion, butter, apricot oil. The report also emphasized that a large majority of anemic women had poor dietary habits including use of hygienically unclean water for drinking, excessive use of tea, low consumption of red meat and iron rich fruits and vegetables. Due to no intake of iron supplements and frequent abortions are also important reasons of anemia among the women of Gilgit Baltistan.

In many eastern countries, traditionally people arrange early marriages due to lack of education and awareness. It is a social problem in many underdeveloped countries including in this region too. Early marriages lead to increased number of children that may be one of the factor for directly increasing the population of the country. In Pakistan in general and especially in some parts of Gilgit Baltistan early marriages (marriages of female below 18 years of age) is a serious problem. Early marriages not only have serious social psychological concerns but also impose adverse effects on woman health. One of the most important physiological/medical consequences of early marriages is high level of anemia in the early married females. Present research is the first documented attempt to study effects of early marriages with special reference to the prevalence of anemia in Gilgit and adjacent areas.

2. MATERIALS AND METHODS

Gilgit Baltistan (GB) also called “jewel of nature” of Pakistan (Fig. 1). Previously the area was included in Northern areas of Pakistan and is situated at 35°552193"N (Latitude) and 74°182313"E (Longitude). Average elevation of Gilgit is 1464 meters (4803 ft) above sea level. Gilgit district has an area of 38,000 km^2

(15,000 sq miles) and population of 243,324. Present work was carried out under supervision of Dr. Abdul Latif, Chief Pathologist, Pathology Department, District Headquarter Hospital, Gilgit. Laboratory work conducted from September, 2015 to February, 2016. Proper procedure was followed for conducting this research and all the necessary approvals from the concerned authorities (including District Head Quarter Hospital, Gilgit and Karakoram International University, Gilgit) were obtained. Blood samples from: (1) Female patients admitted in DHQ hospital, Gilgit and (2) Females attending Out Patient Department, DHQ, Gilgit were obtained. Analysis of blood samples (n=1000) females were carried out among which 140 of the women were married under the age of 18 years while 860 women were married above the age of 18 years. The females included in present study belonged to all background of life and most parts of Giligt Baltistan including Gilgit city, Yasin valley, Nagar, Hunza, Jugloot, Haramosh, Chilas, Ghizer, Astore, Ashkomin and Skardu areas.

Fig. 1: Map of Pakistan and Gilgit Baltistan

2.1. *Blood Analysis Procedure*

Blood sample (5 ml) was drawn from each female and was immediately processed using Sysmex machine. The blood cells including neutrophils, eosinophils, leucocytes, and lymphocytes were quantified using Olympus microscope. Blood samples were vortexed well and centrifuged at 1500 RPM. In 5 ml of Drab kin reagent (Sigma Aldrich) blood samples (20 µl) were added in the tube and mixed well and incubated for 5-10 minutes at 37°C. Samples were then placed in chemistry analyzer machine Sysmex for haemoglobin (%) estimation.

2.2. *Statistical Analyses*

Data was arranged using Microsoft Excel. Basic statistics including mean, median, minimum, maximum, standard error and coefficient of variation were calculated using PAST (Palentological statistical software, ver 3.11) computer program (Hammer, 2016). Graphical representation of data were carried out using computer program (Create a graph).

3. RESULTS AND DISCUSSION

Analysis of blood samples from married female (n=1000) was carried out at the Department of Pathology, District Head Quarter Hospital, Gilgit. Complete blood profile including hemoglobin percentage, total leukocyte count, neutrophils, lymphocytes, eosinophils, monocytes and platelets was recorded. Individuals tested during present study ranged between 14-70 years. All these females belonged to districts of Gilgit Baltistan viz;. Astore, Diamer, Ghanche, Ghizer, Gilgit, Hunza Nagar and Skardu.

In women married after 18 years of age, hemoglobin percentage, total leukocyte count, neutrophil, lymphocytes, eosinophils, monocytes and platelets counts ranged from 5-15.8%, 1000-83000, 20-95, 1-93, 1-16, 1-9 and 100000-982000, respectively Table 1. Average hemoglobin percentage, total leukocyte count (TLC),

Table 1: Blood Analysis for Women Married Under and Above 18 Years of Age

S.No.	Parameters	Min	Max	Mean	Median	Coefficient of Variation
1.	Hemoglobin (%)	5 (5.1)	15.8 (16.2)	11.8±0.1 (11+0.2)	12 (11)	15.81 (23)
2.	Total leukocytes	1000 (1000)	83000 (44000)	8441±153 (7811±318)	8200(7350) (7350)	53 (48)
3.	Neutrohphils	20 (24)	95 (91)	67±0.41 (67±1)	69 (68)	17.81 (17.5)
4.	Lymphocytes	1 (8)	93 (69)	29±0.4 (28±1)	28 (27)	43.4 (41.2)
5.	Eosinophils	1 (1)	16 (10)	4±0.1 (4±1)	4 (4)	61.5 (58.8)
6.	Monocytes	1 (1)	9 (3)	1.8±0.03 (1.7±0.05)	2 (2)	52 (31.4)
7.	Platelets	100000 (22000)	982000 (492000)	259572±3553 (217492±7390)	247000 (208000)	40.14 (40.21)

Top values represents woman married above 18 years while values within parenthesis referes to females married under 18 years of age

neutrophils, lymphocytes eosinophils, monocytes and platelets were 11.8, 8441.0, 66.9, 28.7, 3.903, 1.8 and 259572.1, respectively. For women married under age (under 18 years), hemoglobin percentage, total leukocyte count, neutrophil, lymphocytes, eosinophils, monocytes and platelets counts ranged from 5.1-16.2, 1000-44000, 24-91, 8-69, 1-10 and 22000-492000, respectively Table 1. Average hemoglobin percentage, TLC, neutrophils, lymphocytes eosinophils, monocytes and platelets counts for the women married under the age of 18 years were 10.6, 7810.7, 66.9, 27.54, 3.97, 1.67 and 217492, respectively. The Fig. 2 represents scatter diagrams of age and haemoglobin

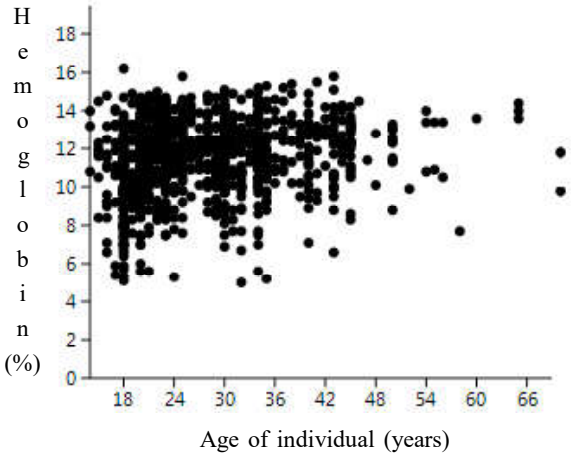

Fig. 2: Scatter diagram of age of the individual and hemoglobin percentage

percentage. Most of the values were clustered towards lower left side of scatter diagram indicating that young aged individuals have lower mean hemoglobin percentage.

4. CONCLUSION

The present study indicated that it is not adviseable for women to marry under 18 years of age as it is a causitive factor leading to anaemia.

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ANNOUNCEMENT

Due to a number of reasons, the *Hamdard Medicus* issues Nos. 3 & 4 of 2018 would be published as a combined issue (comprising pages of both the issues) which in progress. Regrets for inconvenience.

(Editor)

Phytochemical and Biological Reports of *Ficus carica* L. Stem Bark – A Review

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Abstract

This review focused on *Ficus carica* stem bark therapeutic importance as it is traditionally being used by people of rural areas in several countries as an analgesic, anti-diabetic, anti-oxidant, anti-obesogenic, anti-inflammatory, anti-pyretic, anti-bacterial, anti-fungal, anti-diarrhoeal, anti-cancer, anti-viral as well as used scientifically for the reduction of gold and silver nanoparticles (Green nano-technology). Various research studies confirmed the presence of poly-phenolic compounds, flavonoids, cardiac glycoside, tannins, saponins, lipids and proteins in *F. carica* stem bark. Documented data available regarding the chemical constituents of *F. carica* stem bark are limited and there are only few known anti-microbial compounds previously isolated from it. These chemical compounds include ingenol, stigmasterol, campestrol, taraxerone, hexacosanate, hentriacontanol and euphorbol. The purpose of present paper is to investigate the pharmacological and phytochemicals significance of *F. carica* stem bark.

Keywords

Ficus carica, Stem bark, Phytochemicals, Taxonomy, Pharmacological activities.

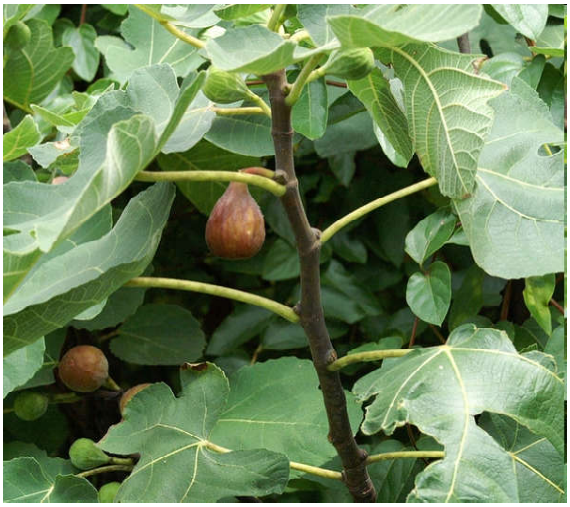
1. INTRODUCTION

Medicinal plants are the oldest valuable source of traditional medicines for different ailments worldwide (Aziz *et al.,* 2018). There are approximately 30,000 to 75,000 of known medicinally active plants. Indigenous medicinal herbs are being used in different remedies against many human health disorders, prescribed by 50,000 registered practitioners (Ahad *et al.,* 2010, Piero *et al.,* 2012). Both local practitioners as well as local people of rural areas of developing countries greatly depend upon medicinal plants for cure against severe infectious diseases (Edziri *et al.,* 2018).

Present review is based on the information about a common and beneficial medicinal plant specie *F. carica* L. which belongs to kingdom Plantea of subkingdom Viridiplantea, order Rosales, and family Moraceae (Mulberry). The vernacular names include: Teen (Arabic), Wuhuaguo (Chinese), Common Fig (English), Carique (French), Echte (German), Fico (Italian), Higo (Spanish), Muhwagwanamu (Korean), Anjir (Hindi and Malaysia) (Al-Snafi, 2017). It is known by 135 different names in the world (Badgujar *et al.,* 2014). Followings are synonyms of *Ficus carica, Caprificus rugosa* (Miq.) Gasp., *Caprificus insectifera* Gasp.,

Ficus praecox Gasp., *Ficus deliciosa* Gasp., *Ficus carica* var. riparium Hausskn., *Ficus communis* Lam., *Ficus colombra* Gasp., *Ficus caprificus* Risso., *Ficus neapolitana* Miq., *Ficus polymorpha* Gasp., *Ficus dottata* Gasp., *Ficus colombra* Gasp., *Caprificus oblongata* Gasp., *Ficus silvestris* Risso. and *Ficus hyrcana* Grossh (Gilani *et al.,* 2008).

Ficus is a large genus of angiosperms comprising more than 800 species, includes shrubs and trees. This genus provides species of good nutritional value and economic importance for people living in tropical regions (Mawa *et al.,* 2013). Among the different species of this genus about half of it is dioecious and *F. carica* L. is an important member. It is a large shrub growing to a height of 7-10 meters. Bark of fig is smooth and dull-white or silver grey, having irregular and round flakes on its surface, its young twigs are glabrous having soft hairs on it. The leaves are almost 12 cm long grooved petiole lamina which is variable in shapes and sizes, having rough hairs on upper surface and soft hairs on lower surface. Flowers are tiny and invisible because they are clustered inside the green fruit. Pollinating insects attain access to the flowers by opening at the apex of synconium. Fruits has tough peel (Green in color), inside is a white inner rind having jelly like flesh (Lansky *et al.,* 2008). The seeds may be large, medium and small range in number from 30 to 1600 per fruit. The seeds are hollow and pollinated due to which it exhibit nutty taste (Joseph and Raj, 2011).



Plant of *Ficus carica* L.

F. carica is widely distributed from South Asia to Eastern Mediterranean. This specie is abundantly located in Pakistan, Turkey, Italy, Germany, Spain, Syria, Iran, Afghanistan, Nigeria, Kenya and in wild arid areas of India. In Pakistan it is cultivated on small scale in different areas of Sindh, Punjab as well as Khyber Pukhtunkhwa (Dueñas *et al.,* 2008, Ahad *et al.,* 2010, Singh and Bhakat, 2012).

1.1. *Traditional Uses*

Generally *F. carica* has been identified by various traditional systems of medicines including Ayurvedic, Traditional Chinese medicines (TCM), Unani and Siddha (Kasote *et al.,* 2017, Sen and Chakraborty, 2017). *F. carica* has been used locally in many countries of world, it helps in treatment against cancer hence it is used in stomach, prostate, colon, liver and testicular cancer (Pathania *et al.,* 2017). Beside anticancer activity, it is also used as anti-pyretic (Amol *et al.,* 2010), anti-inflammatory (Patil and Patil, 2011), anti-spasmodic, anti-platelet (Gilani *et al.,* 2008), anti-helminthes (Das *et al.,* 2011), anti-oxidant or anti-aging (Vinson, 1999), anti-platelet and anti-diarrheal anti-viral, anti-bacterial, (Pathania *et al.,* 2013).

Specifically *F. carica* stem bark is locally used as anti-oxidant, anti-fungal and in treatment against diabetes (Mopuri *et al.,* 2017), it is also used as as stomachache, anti-inflammatory (Ramazani *et al.,* 2010) anti-depressant

(Badgujar *et al.,* 2014) in treatment of piles, skin infections, dysentery and stomach ulcer.

1.2. *Phytochemical Analysis*

Plants produce secondary metabolites for their survival and acclimation (Zandalinas *et al.,* 2017) as well as to perform its various functions (Stevenson *et al.,* 2017), such chemical constituents are useful for human beings as it can help them to combat against different diseases (Jadhav *et al.,* 2015). Such metabolites provides a good source of new drug in pharmaceutical research (Seca and Pinto, 2018). *F. carica* is highly nutritious and its reported phytochemicals confirms the presence of phytosterols, flavonoids, tannins, phenols, alkaloids, coumarins, glycosides, terpenes, volatile oil sugar, proteins and minerals (Al-Snafi, 2017).

The dried fruits of *Ficus* containing vital vitamins, minerals, carbohydrates, fiber, polyphenols, sugars and other phenolic compounds (Vinson, 1999, Vinson *et al.,* 2005). Leaves of fig contain flavonoids, organic acid, sterols, phenolic compounds and tri-terpenoids (Oliveira *et al.,* 2009, El-Shobaki *et al.,* 2010). The occurrence of fatty acids, phytosterols, amino acids and volatile compounds in latex (Oliveira *et al*., 2009). Bark contain cardiac glycosides, tannins (Azam *et al.,* 2013), polyphenols and flavonoids (Mopuri *et al.,* 2017).

Quantitative phytochemical screening of the fig bark confirmed the presence of polyphenols in ethanol and aqueous extracts as 48.36±11.25 mg/g and 28.60±2.03 mg/g (Gallic acid), respectively, flavonoids content 7.66±1.02 mg/g, 26.32±2.40 mg/g (Quercetin acid) and 19.25±1.11 mg/g (Quercetin acid) in hexane, ethanol and aqueous extracts, respectively (Mopuri *et al.,* 2017). Total phenolic content of fruits were 10.90 µg, saponins (0.59 g/100 g) dry weight, total flavonoids (2.75 µg) and crude alkaloids (9.6%/100 g) dry weight (Oliveira *et al.,* 2009). Nutritional analysis of fig leaves demonstrated protein (5.90%), lipids (0.81%), moisture (65.90%), ash (5.30%) and fibers (4.50%) (El-Shobaki *et al.,* 2010).

Six major organic acids, i.e. oxalic, citric, malic, quinic, the shikimic and fumaric acids (Oliveira *et al*., 2009) were also found in the plant along with ficins and cysteine isolated from fig. Ficins isolated from fig includes A, B, C, D_1, D_2 and E (Devaraj *et al.,* 2008). Volatile compounds including 2,3-butane-diol, caryophyllene, octadecane, caryophyllene-oxid and apiol (Ficsor *et al.,* 2013) while psoralen, sitosterol, lupeol, gallic acid, rutin, syringic acid, catechin,epicatechin and chlorogenic acid (Veberic *et al.,* 2008) were also present in fig as an important components.

Keeping in view the literature survey of fig, there is little documented data available regarding pure phytochemicals isolated from its stem bark (Mawa *et al.,* 2013). Compounds such as stigmasterol, campesterol, sitosterol, taraxerone (Jeong and Lachance, 2001), hexacosanate, ingenol, hentriacontanol and euphorbol have also been isolated from stem bark of *F. carica* L. Dried seeds containing fixed oil (30%) which is edible, used as lubricant and containing arachidic acid (1.05%), oleic acid (18.99%), palmitic acid (5.23%), stearic acid (2.1%) and linoleic acid (33.72%). Leaves contain proteins (4.3%), pentosans (3.6%), fat (1.7%), ash (5.3%), tyrosine and fiber (4.7%). Many oxidizing enzymes such as lipase, rennin, diastease, malic acid, sugar, albumin, catalase and resins are also present in plant latex (Joseph and Raj, 2011).

1.3. *Pharmacological Effects*

1.3.1. *Anti-oxidant Activity*

The anti-oxidant potential of fig bark

residing in ethanol extracts showed 25.63% activity at 0.1mg/ml using DPPH free radical scavenging assay (Rawat *et al.,* 2012).

1.3.2. *Anti-diabetic Activity*

Keeping in view traditional use of *Ficus carica* bark, its ethanol extracts, hexane as well as aqueous extracts were examined for their abilities to treat diabetes mellitus adopting α-amylase and α-glucosidase assay. Each extract (250 µl) was incubated with α-amylase (500 µl of 1.0 u/ml) in phosphate buffer (100 ml), same concentration of α-glucosidase were used with crude extracts. The results of the inhibitory activity (IC_{50} (µg/ml) of *F. carica* hexane, ethanol and aqueous extracts against α-amylase were 9998.256±30.165 µg/ml, 642.05±54.032 and 638.601±32.086 µg/ml, respectively. The IC_{50} values against α-glucosidase were 43131±116.93 µg/ml, 441.08±25.404 µg/ml and 710.974±31.32 µg/ml, respectively. These results indicates that stem bark of *F. carica* possesses high anti-diabetic activity (Mopuri *et al.,* 2017).

1.3.3. *Anti-obesogenic Effect*

Anti-obesogenic effect of fig bark was confirmed following pancreatic lipase assay. Mixture of pancreatic lipase and bark hexane, ethanol and aqueous extracts were incubated for 30 min. Hexane, ethanol and aqueous extracts showed 276085.1±1403.4 µg/ml, 688.56±69.9 µg/ml, 842.70±151.34 µg/ml, respectively. It was revealed that bark possess moderate anti-obesogenic activity as compared to that of its fruits (Mopuri *et al.,* 2017).

1.3.4. *Anti-pyretic Activity*

In traditional medicine, *F. carica* bark was used to relieve body temperature, in order to justify its local use, the ethanolic bark extracts were investigated for anti-pyretic activity at 100 mg/kg, 200 mg/kg and 300 mg/kg (body weight), its effect extended upto 5 h after drug administration and compared with paracetamol used as standard drug (150 mg/kg). It significantly lowered body temperature at 100 mg/kg while its effect was maximum at 300 mg/kg (27.1±0.2°C to 25.0±0.2°C) in a dose dependent manner (Badgujar *et al.,* 2014).

1.3.5. *Analgesic and Anti-inflammatory Activity*

The analgesic and anti-inflammatory activity were conducted and comparised with standard drug indomethacin. The potential of fig bark to recover the pain and inflammation with relatively less side effect (Modi *et al.,* 2012).

1.3.6. *Acute Toxicity Studies*

Toxicity of fig bark were determined following animal toxicity assay. Mice were injected intraperitonially with different concentrations of bark extract (50 mg/kg, 100 mg/kg, 150 mg/kg, 200 mg/kg and 250 mg/kg) and control group was treated with sterile saline solution. After one week, mortality was observed. Thereby, indicating that bark of *F. carica* do not exhibit any toxicity at doses tested (Nguyen *et al.,* 2000).

1.3.7. *Anti-bacterial Activity*

Methanolic extracts of *F. carica* bark were evaluated for their anti-bacterial potential in comparison with streptomycin using agar diffusion method against human pathogenic bacterial strains, including *E. coli, P. aregunosa and S. aureus.* The bark of fig did not show any detectable anti-bacterial effect (Azam *et al.,* 2013).

1.3.8. *Anti-fungal Activity*

Anti-fungal activity of methanolic extracts of fig bark was performed following agar tube

dilution assay. Amphotericin-B was used as standard drug against human pathogenic fungal strains i.e. *F. solani* and *A. flavus.* It showed negative response indicating that bark of fig does not exhibit antifungal activity (Azam *et al.,* 2013).

1.3.9. *Heavy Metals Contents*

Heavy metals like Fe, Mn, Zn, Cd, Cr, Cu, Ni and Pb etc were assessed in Fig bark and leaves using absorption spectrophotometer. Heavy metals in stem bark of fig were in an acceptable range of WHO guidelines. It indicates the safe use of bark of fig because excess of heavy metals accumulation leads to severe health problems (Ugulu *et al.,* 2016).

1.3.10. *Green Synthesis of Au and Ag Nano-particles*

Green synthesis of gold and silver nanoparticles from *F. carica* stem bark using wet chemical technique (Toxic and flammable chemicals were used). Bark extracts were added to an aqueous solution of $HAuCl_4$ (1mM) and $AgNO_3$ (1 mM), respectively acting as reducing as well as capping agent to reduce gold ions and silver ions. Color change and UV-VIS spectroscopic technique were used to confirm synthesis of gold and silver nano-particles (Singh and Bhakat, 2012).

1.3.11. *Other Miscellaneous Uses*

F. carica bark is used locally to relieve kidney stone, intestinal pain, inflammatory and anti-depressant in Pakistan, and for inflammation in Iran (Ramazani *et al.,* 2010).

1.2.12. *Uses of Fig Parts*

Besides bark, other parts of fig like leaves, fruits and roots have also been used in the treatment of different disorders. Fruits are edible and used for abdominal pain in Italy (Idolo *et al.,* 2010), anti-helmentics, antiseptic burn and emollient (Jaradat, 2005) and cardiac troubles (Khan and Khatoon, 2007). It also helps to relieve kidney stone, liver diseases, piles and chronic ulcer (Akter *et al.,* 2012) in Pakistan, (Idolo *et al.,* 2010), to treat anemia in Iran, ascites in Khouzestan (Ramazani *et al.,* 2010),

Leaves are used to treat hemorrhoid in Turkey, kidney stone, as well as laxative in Pakistan (Zaman-Allah *et al.,* 2011) , Pain and sedative in Eucador (Tene *et al.,* 2007) and anti-septic in Italy (Idolo *et al.,* 2010). Leaves of fig are also effective anti-diabetic (Khan *et al.,* 2011), anti-hemorrhoids (Genç and Özhatay, 2006), and as laxative (Zaman-Allah *et al.,* 2011), in menstrual pain and to induce sedation (Tene *et al.,* 2007). It can also be used in management of jaundice (Krishna Mohan *et al.,* 2007).

Roots are used for leucoderma in india, latex used for wound in Nablus (Jaradat, 2005), warts in Turkey (Ugulu *et al.,* 2016), anti-depressant, laxative and for buns (Pakistan) (Zaman-Allah *et al.,* 2011). Roots are used as anti pyretic and purgative (Mopuri *et al.,* 2017), in treatment of leucoderma as well as ringworms. Its milky latex is helpful in removal of warts (Joseph and George, 2011).

2. CONCLUSION

F. carica L. is one of the oldest and nutritious known medicinal plant specie in the world. Its history goes back to ancient times and people from different fields of life have been using its (leaves, fruits and bark) for both nutritious and medicinal purposes. Keeping in view the current review, fig bark can also be useful in management of various disorders but advanced scientific approaches are required for bio-assay guided isolation of different phytochemicals present in not only the bark but also in entire plant.

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Survey Based Studies on Pakistani and Saudi Arabian Populations Using Different Brands of Asmad (Antimony Sulphide)

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Abstract

The eye is most prominent, beautiful, attractive and sensitive part of human organs. It is one the most critical and valuable part of human body which associated with vision. There are several aesthetic ophthalmic preparations available in market for beautification and for medicinal use. Asmad/antimony Sulfide/Kohl/ Surma is one of the eye preparations known to have anti-infectious, strengthening, brightening, cleansing and soothing action along with some therapeutic values.

The main objective of current study is to provide scientific findings regarding the beneficial and toxic effects of different Asmad products to the frequent users through conducting detailed survey based study and checking eye responses after Asmad application.

The most frequent used Antimony Sulfide/ Asmad 10 brands were collected from Pakistan and Saudi Arab markets. Five samples belonging to Pakistan i.e. PHS1, PHS2, PLS, PMS and PSS while other five samples belonging to Saudi Arabia i.e. SBS, SAS, SHS, SMS and STS. The prior approval was taken from Bioethics Committee for conducting the eye response study on 100 random individuals (n=100) with healthy eyes from Pakistani and Saudi Arabian populations. Other than this study, random survey based study was also performed on 50 individuals of Asmad frequent users of both the countries. It is concluded that the eye preparation like Asmad of different regions have beneficial effects for improvement of vision and have anti-infectious properties due to containing nutritive and therapeutic ingredients.

Keywords:

Asmad/antimony sulfide, Kohl, Ophthalmic preparation.

1. INTRODUCTION

The eye is one of the sensitive and precious part of human body. It consists of rods and cones, rods provides scotopic vision which useful in low light while cones provide photopic vision which useful in bright light. It is estimated that our photopic vision can distinguish

about 10 million colors (Judd and Wyszecki, 1975).

Eye preparations are available in different dosage forms including eye drops, lotions, eye shadow, eye liner, mascara, cleansers and powders cake etc. Eye cosmetics are part of everyday life around many parts of the world. In western society, eye cosmetic is considered as a factor in facial attractiveness (*Mulhern et al.,* 2003). Asmad is one of the eye preparation since long time is well known in Islamic and other literatures its external application and availing lots of benefits. Asmad or antimony sulfide/Kohl is the most popular eye product reported in almost every human civilizations used to keep the eyes cool and clean for the prevention and treatment of eye diseases such as, blepharitis, trachoma, chalazion, pterygium, cataract, conjunctivitis, ectropion, as well as for the prevention of recurrence of trichiasis (Sweha, 1982).

It is a traditional powder-like mixture, such as powdered antimony sulfide, used both as cosmetic eyeliner and to treat eye diseases in many Middle and Far East countries (Al-Hawi, 1986). Asmad or kohl exists under different names depending on the origin from the civilizations or different countries, such as collyrium, ancient Egyptians, kollurion (Romans and Greeks), Kohl or kuhl/kahal Arabs and Egyptians and shurma, surmi and surma Indo-Pakistan subcontinent (Zaheer *et al*., 1991).

According to the Unani/Ayurvedic and Greko-Arabic system of medicine, Asmad is ultra-fine powder containing one or more ingredients (such as galena, herbs, pearls, gemstones etc.) to be used in the eyes for prophylaxis and treatment of various eye disease (Nadkarni, 1954; Kaushal, 2008).

Archaeological and historical studies indicate that antimony and its mineral sulfides have been used by humans for at least six millennia. In ancient times, powdered stibnite, the most common mineral form of antimony sulfide, along with lead sulfide, a principal ingredient of kohl, the thick black paste used by the Egyptians and others as a cosmetic for coloring eyebrows and lining the eyes (Li, 1953). Antimony (III) Sulfide, Sb_2S_3, was recognized in predynastic Egypt as an eye cosmetic (kohl) as early as about 3100 BC, when the cosmetic palette was invented (Shortland, 2006).

Asmad has also been used as cosmetic in Asian, African and Middle East countries since long time. In addition, mothers apply kohl to their infants' eyes soon after birth. Some did this to strengthen the child's eyes, and others believed it could prevent the child from being cursed by the evil eye (Hardy *et al*., 2004).

The current study followed by earlier studies indicating that Asmad or antimony sulfide contains multiple elements or minerals in variable amounts. These elements required by human body in major or in trace amounts for the functioning of different physiological system. Some of these elements also have key functions in ophthalmic system. In contrast, Asmad preparation reported to contain lead (Pb) or galena (PbS) as major components. Despite of harmful effect of lead, the beneficial effects also reported. Asmad has been used since long back because of its cosmetic and therapeutic value including eye cleansing, strengthening and brightening and for the resistance to possible cause of infections. Asmad several studies (Zaheer *et al.,* 1991; Awan, 1956; Levey and Al-Khaledy, 1967) show that it improves the vision, strength and keeps the eye healthier.

Lead-based compounds were used in cosmetics, particularly in Asmad or kohl, used to outline eyelids. Analytical chemistry has revealed that two lead chlorides, laurionite and phosgenite, were manufactured to form fine

powders present in eye lotions and makeup. Contrary to modern beliefs of the toxicity of lead, the study suggested that submicromolar concentrations of lead ions may play a role in promoting immunological defenses action (Tapsoba *et al*., 2010).

Lead containing Asmad preparation, produce desirable effects including defense via providing protection to eye by the reflection of sun light and therefore, protects them from harmful UV rays emitting from the sun (Cohen, 1999; Heather, 1981; Kathy, 2001).

The dark or black colour of Asmad is due to the presence of lead or galena in large amount. Darkening around the eyelids also provided relief from the glare of the sun. The earliest historical record of eyeliner use appears in the ancient Near East and Egypt (Keville and Green, 1995; Cohen, 1999).

Asmad benefits from Islamic prospects are well documented, Ibnul Qaiyum narrates that the Prophet Muhammad (ﷺ) said, "Ithmid is the black Kohl stone the Kohl tends to be cold and dry and it is beneficial to the eye as it strengthens the eye nerve. Ithmid dissolves excessive flesh around ulcers and healing wound while cleansing the area around them" (Eng. trans. *Medicine of the Prophet* (ﷺ) by Imam Ibnul Qaiyum, p. 251). The Prophet Muhammad (ﷺ) also told, "Ithmid is the best type of Kohl used for the eye, especially for old people whose eyesight has weakened (Eng. trans. *Medicine of the Prophet* (ﷺ) by Imam Ibnul Qaiyum, p. 251).

2. MATERIALS AND METHODS

For current research study, ten (10) famous Asmad (Antimony Sulfide) locally available brands were collected from market of Pakistan and Saudi Arabia. Five sample belonging to Pakistan named as PHS1, PHS2, PLS, PMS and PSS while other five brands from Saudi Arabia were named as SBS, SAS, SHS, SMS and STS.

Survey based research study was performed on randomly selected individuals from Pakistan and Saudi Arabia region having healthy eyes. These ten selected brands were applied to healthy eyes of 100 individuals (n=100) living in Pakistan and Saudi Arab. Each brand was applied to ten individuals. Pakistani samples PHS1, PHS2, PLS, PMS, PSS applied to 50 individuals living in Pakistan Saudi Arabia samples SBS, SAS, SHS, SMS and STS applied to 50 individuals living in Saudi Arabia. The approval of this study was taken from the Institute of Bioethics Committee, University of Karachi, Pakistan (Approval Reference # IBC KU 45). After apply action of Asmad on healthy eyes (without having any eye infection or eye disease), like itching, redness, burning, drying, watery, swelling and blurry vision etc. were recorded. Each individual was kept under observation for one hour, just within 5 -10 second after application of Asmad. The eye response was also recorded through designed proforma covering individual detailed profile history including general information, medical history and lifestyle. The follow-up feedback of each individual was taken after 15 days and 30 days to evaluate the infection or any other issue observed after application of Asmad/Antimony Sulphide.

In addition, random survey based research study was also conducted on the frequent users of Asmad or Antimony Sulfide. The survey based study was conduction on 50 individuals of Pakistan and Saudi Arabia. 25 individuals were selected from Pakistan and 25 were selected from Saudi Arabia.

3. RESULTS AND DISCUSSION

Eye is as organs of vision with complex optical highly organised system that receives

light from the surrounding environment, regulates its intensity through a diaphragm, focuses it through an adjustable assembly of lenses to form an image, converting its image into a set of electrical signals, and transmits these signals to the brain through complex neural pathways that connect the eye via the optic nerve to the visual cortex and other areas of the brain (Land and Fernald, 1992). There are different eye preparations available on cosmetic and health store for human, having cosmetic and some therapeutic values respectively. Asmad/ Antimony sulphide is one of the famous eye preparations have been used since long time period in Asian and Middle East countries for healthy, beautiful and perfect human eyes condition for the purpose of cleansing, strengthening, brightening and vision improvement of eye.

There are several scientific debates between two schools of thoughts for the lead (Pb) toxic and beneficial effects. Fist thought believes that Lead either organic or inorganic both are harmful and toxic substance which might contaminate the Asmad products, hence it should not be the part of ophthalmic preparation while other believes that only organic lead are responsible for toxicity while inorganic lead are not toxic and has been using since long back because of its biomedical importance.

Thus Asmad is being used since long back in both the countries because of Islamic and historical importance. As the Pilgrim travelling to Saudi Arab from all over the world for performing *Umrah/Hajj* and when they are returning to their home countries they are bringing Asmad/antimony sulfide or surma as gift or tabarrukat. The Asmad manufacturing is a huge business in Saudi Arab and are very famous due to the spiritual and Islamic heritage aspects. While in Pakistan most of the cosmetic industries are also manufacturing Asmad as common useful eye cure product.

The subjected research topic was selected because Asmad formulation is an ultra-fine powder and applying directly on the human eye which may cause harmful or unwanted effects. The objective of current study is to provide valuable information to the Asmad frequent users related to beneficial and harmful effects on the basis of its physical, chemical and pharmacological investigations.

The other chemical composition/ elemental composition investigation was also performed by using advanced Energy Dispersive X-rays Spectroscopy (EDS or EDXS) technique. This technique revealed the occurrence of organic and inorganic elements in the samples of Pakistan and Saudi Arabia. Both the countries samples contain multiple elements like Calcium (Ca), Carbon (C), Oxygen (O), Sodium (Na), Magnesium (Mg), Chlorine (Cl), Aluminum (Al), Silicon (Si), Sulphur (S), Lead (Pb), Copper (Cu), Zinc (Zn), Bismuth (Bi), Potassium (K), Molybdenum (Mo), Iron (Fe) and Chromium (Cr) while each sample scanned from different locations. These elements are very important for human body's physiological system either in major or in trace amounts. Among these elements few of elements i.e. Fe, Zn and Cu play vital role in ophthalmic system besides other human system. Zn plays an important role in the metabolism of the retina and the lens of the eye (Karcioglu, 1982). Although, samples of both countries contain all essential elements for eye proper functioning including strengthening, brightening and soothing effects. The intraocular fluid (aqueous and vitreous humour) and lens concentration of Fe increased significantly after ocular inflammation (Mc Gahan, 1992). However, Copper is responsible to increase the

sharpness of vision through reducing thing layer of skin in cataract (Siddiqui *et al.,* 2003).

On the basis of detail survey based performence on 100 individuals from Pakistan and Saudi Arabia with the approval of Bioethics Committee. The individuals were under observation for one hour just after application of Asmad. The eye response like itching, watery eye, blurry vision, swelling, redness, burning, drying and other effects was recorded just after 5-10 second till one hour through designed proforma covering individual detail profile history including general information, medical history and lifestyle (Tables 1 and 2).

The most common eye allergic responses produced after the application of Pakistan and Saudi Arabia Asmad brands are itching and watery eyes till within specific time period. Few of the individuals also reported slight blurry vision after application of sample PSH1 and SBS samples but after few seconds it was found satisfactory. This initial blurry vision exists for very short period of time which get recovered in few seconds. This slight blurry vision might be appeared due to the watery eye of individuals as it did not persist for long time. Among all samples subjected for study the worst allergic response was produced by Saudi Arabia sample coded SAS. The only sample produced burning, redness, itching and watery eyes. The chemical composition review of this sample confirmed the presence of unique element i.e. Molybdenum (Mo) which was missing in all other brands of both countries samples. However, the element Molybdenum has several important roles in the body but in eye it can cause major discomfort because of its reported hazardous effect. The element known to cause irritation, redness and

Table 1: Asmad Samples Collected from Pakistan and Saudi Arabia Region, Sample Numbers 1 to 5 from Pakistan While Sample Numbers 6 to 10 are from Saudi Arabia

Sample Origin	Sample No.	Sample Name
Pakistan Asmad brands	1	PHS1
	2	PHS2
	3	PLS
	4	PMS
	5	PSS
Saudi Arabia Asmad brands	6	SBS
	7	SAS
	8	SHS
	9	SMS
	10	STS

Table 2: Application of Asmad to Eye and Their Responses

Individuals Samples	Eye Response							
	Itching	Redness	Watery	Dryness	Swollen	Burning vision	Blurry	Other
PHS1	+	–	+	–	–	–	+	–
PHS2	+	–	+	–	–	–	–	+
PLS	+	–	+	–	–	–	–	–
PMS	+	–	+	–	–	–	–	–
PSS	+	–	+	–	–	–	–	–
SBS	–	–	+	–	–	–	–	+
SAS	+	–	+	–	–	+	–	–
SHS	+	–	+	–	–	–	–	–
SMS	+	–	+	–	–	–	–	–
STS	+	–	+	–	–	–	–	–

Presence (+) or absence (–) of corresponding effects

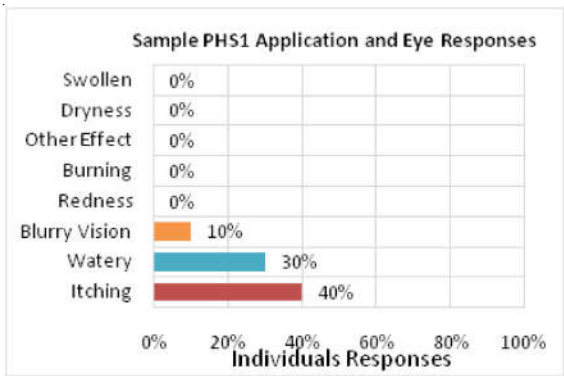

Fig. 1(a)
Sample PHS1 application and responses in (%)

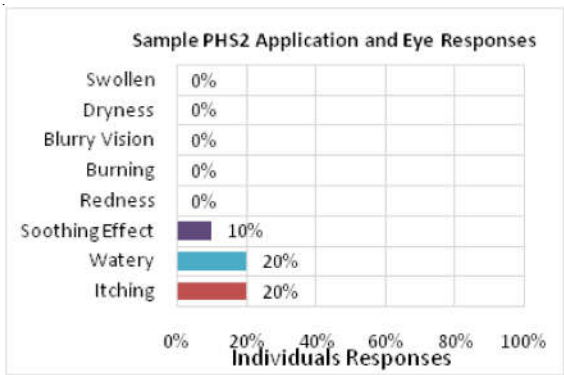

Fig. 1(b)
Sample PHS2 application and responses in (%)

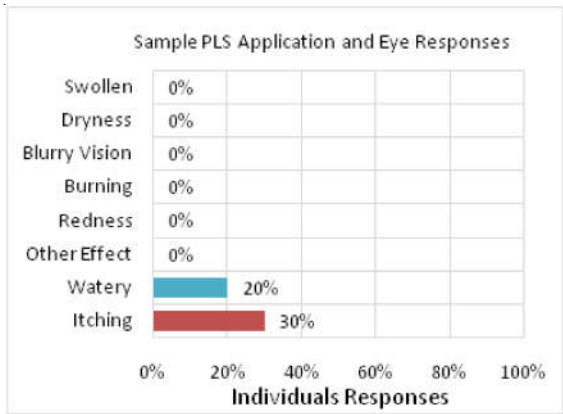

Fig. 1(c)
Sample PLS application and responses in (%)

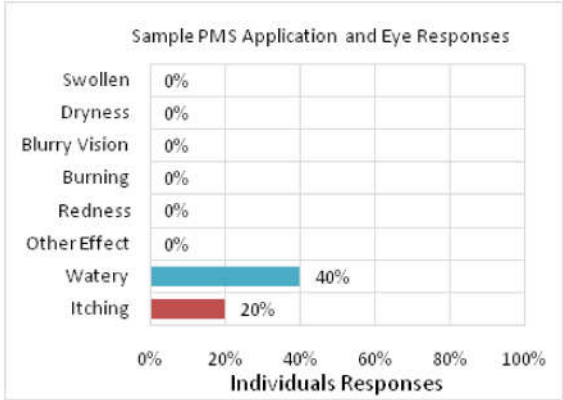

Fig. 1(d)
Sample PMS application and responses in (%)

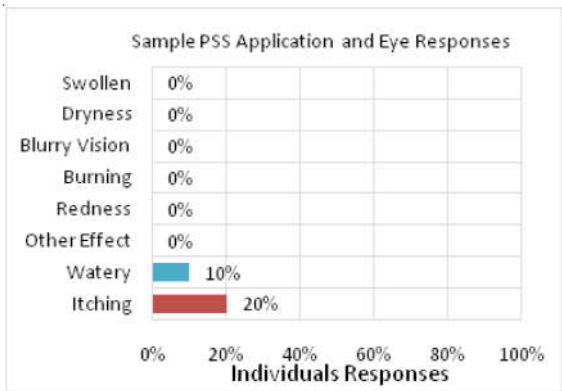

Fig. (e)
Sample PSS application and responses in (%)

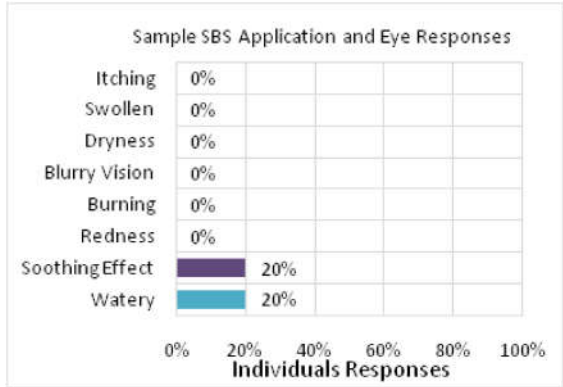

Fig. (f)
Sample SBS application and responses in (%)

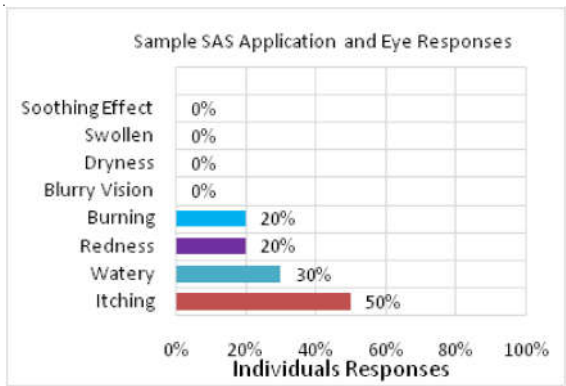

Fig. (g)
Sample SAS application and responses in (%)

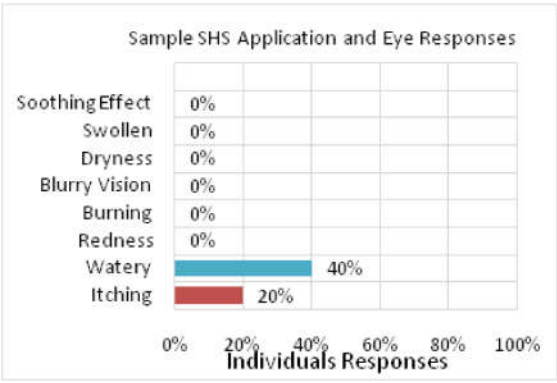

Fig. (h)
Sample SHS application and responses in (%)

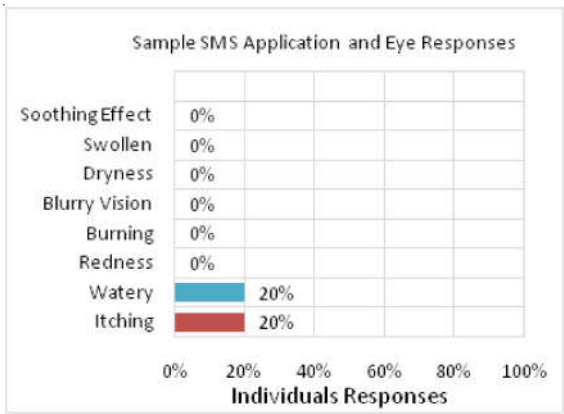

Fig. (i)
Sample SMS application and responses in (%)

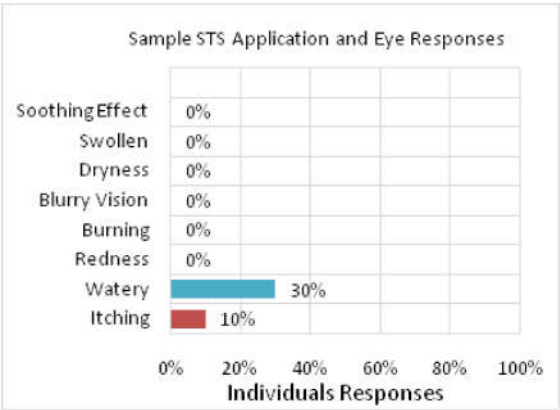

Fig. (j)
Sample STS application and responses in (%)

SBS, SAS, SHS, SMSS, STS (Saudi Arabia)

PHS1, PHS2, PLS, PMS, PSS (Pakistan)

Fig. 2
Market available Pakistan and Saudi Arabia eye products/ Asmad used in Pakistan (PHS1, PHS2, PLS, PMS and PSS) and Saudi Arabia (SBS, SAS, SHS, SMS and STS) used in human eyes

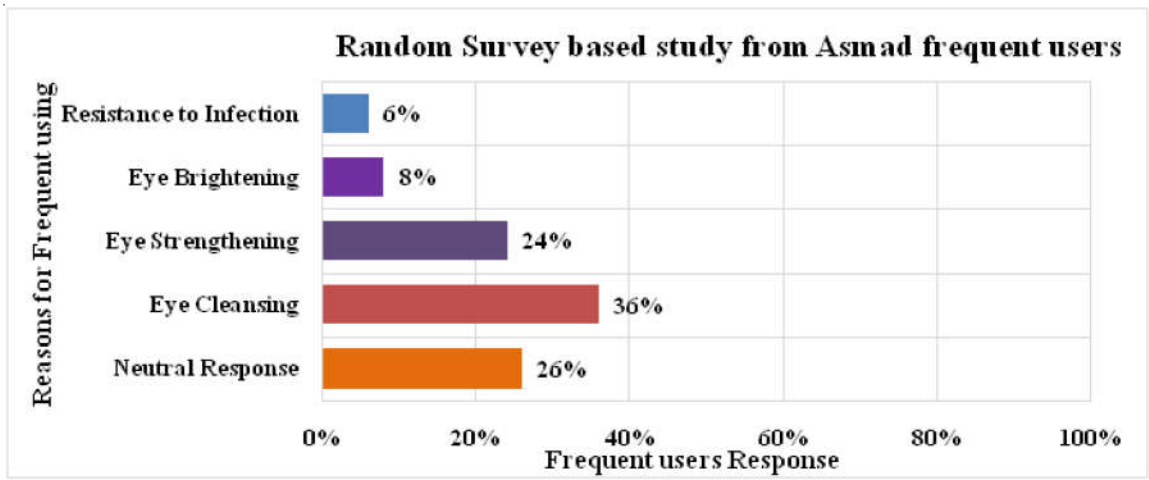

Fig. 3
Random survey study and responses of Asmad frequent users from Pakistan and Saudi Arabia

other effects on eye as mentioned in safety data sheet (MSDS), hazardous identification (Terk, 2015).

However, the overall study found satisfactory. The study also revealed that lead containing products did not produce any unusual response, even sample PHS2 containing lead in significant amount produced soothing effects on eye. The follow up after 15 and 30 days from each individual found satisfactory as there was no unwanted response was reported.

Therefore, Food and Drug Administration (FDA) has recommended safe daily diet Pb (Lead) intakes 75 micrograms (mcg) for adult, 25 mcg for pregnant women, 15 mcg for children age 7 or up and 6 mcg for children under age of 6 years. FDA estimated safety level of Pb amount known to cause health problem is 750 mcg for adult, 250 mcg for pregnant women, 150 mcg for children above 7 years and 60 mcg for children under 6 years. (Food and Drug Administration, 2010). For cosmetic products FDA recommends Pb limit 10ppm or less (FDA Guidance, 2016).

The additional random survey feedback report from frequent users of both countries confirmed the actual cause and benefits associated with Asmad. The study revealed that 26% individuals gave neutral response for the use of Asmad while 74% individuals gave positive feedback i.e. the frequent use of Asmad caused the eye cleaning (reported by 36%), eye brightening (reported by 8%), eye strengthening (reported by 24%) and cause resistance to any possible infection (reported by 6%).

Over the last 20 years, kohl/surma has gained a bad repetition, due to its high content of lead (Pb) (Warley *et al*., 1968; Ali *et al*., 1978). But the later studies confirmed that Lead toxicity is associated while it administered orally. The study (Healy *et al.,* 1982; Aslam *et al.,* 1980) confirmed that the primary route for lead absorption is not trans-corneal transport while the lead absorbed through semi-external route not reach in blood stream because the probability of absorption from trans-corneal or semi-external route is negligible. Even the lead (Pb) at lower

concentration may produce beneficial effects rather than harmful effects. Hence for the toxic elements, the amount must not exceed from the required amount as recommended by the regulatory agencies.

4. CONCLUSION

The current study confirmed that Pakistani samples can be considered safer as compared to Saudi Arabian samples because after application of Asmad brands, the worst response was produced by Saudi Arabian sample coded as SAS, which produced burning, redness, watery and itching. The element chemical study revealed that it was the only brand contain element Molybdenum (Mo) that could be the only cause of burning and redness along with itching and watery eye as mentioned in its characteristic only when applies to eye, the irritation, redness and burning was exist till 2 to 3 minutes then came to satisfactory stage. Furthermore, the manufacturer should follow the defined regulatory limit of each incorporated elements to minimize the toxic effects associated with Asmad. Prior to apply as cosmetic or medical purpose samples these should be analysed according to the standard Pharmacopeial protocol (ultra-fine particle size, homogenized and free from contaminants) for the safeguard. Moreover, the survey based study by frequent users also confirmed that Asmad can produce significant effect including cleansing, brightening, strengthening and resistance from possible infection to eye. The research findings prove the safe use of Asmad as cosmetic and therapeutic agents for healthy human eyes without causing any serious consequences.

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Development of New Isonicotinic Acid Hydrazide Derivatives for the Treatment of Depression

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Abstract

Isonicotinic acid hydrazide (INH) has been widely known for its anti-tubercular effects in the medical world. Previously our group reported the synthesis of its quaternary analogues and their anti-mycobacterium activity. In this research study, the potential of eleven synthetic compounds on different pharmacological activities in rodents has been addressed. These compounds were tested for their antidepressant activity following forced swim test. It was noticed that some of the derivatives possessed antidepressant effects and structure activity relationship (SAR) was also established.

Key words

Pyridine-4-carbohydrazide, CNS stimulants, Depression, SAR, INH.

1. INTRODUCTION

In today's world, study of drugs affecting brain remained an imperative area of research, especially synthesis of new compounds acting at the central nervous system is of main interest for the medicinal chemists.

In 1951, iproniazid (Fig. 1) a derivative of isonicotinic acid hydrazide was synthesized as anti-tubercular compound. It was accidentally found that this clinically established anti-tubercular drug produced euphoria in patients receiving this medication. This side effect of the drug turned out to have significant effect in depression and motivated the researchers for the synthesis of newer antidepressants. In 1951, Iproniazid (Fig. 1), a derivative of INH was synthesized as antitubercular compound. In 1952, this compound reported to inhibit monoamine

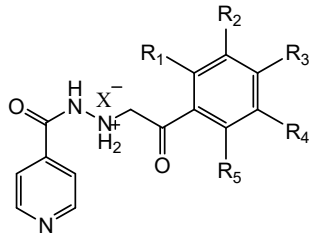

Fig. 1

oxidase (MAO) and also demonstrated psychostimulatory action. Iproniazid was then used as 'antidepressant' but it was withdrawn from the market due to its side effects such as depression. Later, these findings offered the basis for the development of new generations of antidepressant drugs (Ban, 2006; Lopez-Munoz *et al.,* 2007; Ramachandraih *et al.,* 2011) which led to development of new antidepressant (Thomas *et al.,* 2016), antianxiety (Amr *et al.,* 2008) and anticonvulsant (Malhotra *et al.,* 2012; Tripathi *et al.,* 2011) activities. In this study, we also scrutinized newly synthesized INH analogues (Table 1) for their potential on central nervous system (CNS) (Naeem *et al.,* 2016).

Table 1: Synthetic Analogues of Isonicotinic Acid Hydrazide

S.No.	Compound Name	
1.	[2-oxo-2-(4-phenylphenyl)ethyl](pyridin-4-yl formamido) azanium bromide	X = Br, R_1, R_2, R_4, R_5 = H, R_3 = C_6H_5
2.	[2-(3,4-dihydroxyphenyl)-2-oxoethyl](pyridin-4-yl formamido) azanium chloride	X = Cl, R_1, R_4, R_5= H, R_2, R_3 = OH
3.	[2-(4-chlorophenyl)-2-oxoethyl](pyridin-4-yl formamido) azanium bromide	X = Br, R_1, R_2, R_4, R_5 = H, R_3 = Cl
4.	[2-(4-fluorophenyl)-2-oxoethyl](pyridin-4-yl formamido) azanium bromide	X = Br, R_1, R_2, R_4, R_5 = H, R_3 = F
5.	[2-(3-nitrophenyl)-2-oxoethyl](pyridin-4-yl formamido) azanium bromide	X = Br, R_1, R_3, R_4, R_5 = H, R_2 = NO_2
6.	[2-(4-nitrophenyl)-2-oxoethyl](pyridin-4-yl formamido) azanium bromide	X = Br, R_1, R_2, R_4, R_5 = H, R_3 = NO_2
7.	[2-(2,4-difluorophenyl)-2-oxoethyl](pyridin-4-yl formamido) azanium chloride	X = Cl, R_2, R_4, R_5 = H, R_1, R_3 = F
8.	[2-(4-bromophenyl)-2-oxoethyl](pyridin-4-yl formamido) azanium bromide	X = Br, R_1, R_2, R_4, R_5 = H, R_3 = Br
9.	[2-(2,5-dimethoxyphenyl)-2-oxoethyl](pyridin-4-yl formamido) azanium bromide	X = Br, R_2,R_3, R_5= H, R_1, R_4 = OCH_3
10	[2-(4-methoxyphenyl)-2-oxoethyl](pyridin-4-yl formamido) azanium bromide	X = Br, R_1, R_2, R_4, R_5 = H, R_3 = OCH_3
11	(2-oxo-2-phenylethyl) (pyridin-4-yl formamido) azanium bromide	X = Br, R_1, R_2, R_3, R_4, R_5 = H

2. MATERIALS AND METHODS

2.1. *Treatment of Mice*

Albino mice of either sex (weight 20-30 g) were procured from Dow University of Health Sciences Karachi, Pakistan. They were kept in poly-acrylic cages under controlled environmental conditions with free access to water and diet.

INH and its derivatives 1-11 were dissolved in water and injected intraperitoneally at the dose of 50 mg/kg body weight and a control group received water only.

2.2. *Pharmacological Screening*

This qualitative study involved the monitoring of CNS stimulating and depressant activity, cardio-vascular system, eye, ear and other general observations. During which the mice were observed according to the set guidelines(Turner, 1965)and the results were recorded in a Pharmacological Screening Chart (Miranda, 1986).

2.3. *Forced Swim Test*

Forced swim test (FST) remained a useful pre-clinical test in research to assess the antidepressant activity of new compounds (Can *et al.,* 2012; Khisti *et al.,* 2000; Lucki, 1997). In this test, acylindrical tank of 30 cm height, 20 cm diameter with water level of 15 cm was used. Control and treated mice were individually placed in the center of the tank and their response (immobility) was noticed for a time period of 5 minutes (Can *et al.,* 2012).

3. RESULTS AND DISCUSSION

The results of general pharmacological screening and forced swim test are presented in Tables 2 and 3 respectively. As shown in Table 2, INH and its analogues 4, 5 and 9 reduced whereas compounds 6, 7 and 8 increased the motor activity of animals during general pharmacological test.

However, INH derivatives 2, 4, 6, 9 and 11 reduced the immobility phase of the mice during forced swim test (Table 3).

Table 2: General Pharmacological Screening of Isonicotinic Acid Hydrazide and Its Analogues

S.No.	Behavioral parameters	Control	INH	1	2	3	4	5	6	7	8	9	10	11
A)	**CNS depression**													
1.	Motor activity	0	–	0	0	0	–	–	0	0	0	–	0	0
2.	Ataxia/Staggering gate	0	0	0	0	0	0	0	0	0	0	0	0	0
3.	Anesthesia	0	0	0	0	0	0	0	0	0	0	0	0	0
4.	Respiratory rate	0	0	0	0	0	0	0	0	0	0	0	0	0
5.	Paralysis	0	0	0	0	0	0	0	0	0	0	0	0	0
6.	Screen griping	0	–	0	0	0	0	0	0	0	0	0	0	0
7.	Sedation	0	0	0	0	0	0	0	0	0	0	0	0	0
B)	**CNS stimulation**													
1.	Startle response	0	0	0	0	0	0	0	0	0	0	0	0	+
2.	Motor activity	0	0	0	0	0	0	0	+	+	+	0	0	0
3.	Coarse body tremors	0	0	0	0	0	0	0	0	0	0	0	0	0
4.	Convulsions	0	0	0	0	0	0	0	0	0	0	0	0	0
5.	Fasciculation	0	0	0	0	0	0	0	0	0	0	0	0	0

S.No.	Behavioral parameters	Control	INH	1	2	3	4	5	6	7	8	9	10	11
C)	**CVS observations**													
	Heart rate	0	0	0	0	0	0	0	0	0	0	0	0	0
D)	**Eye observation**													
1.	Pupil size	0	0	0	0	0	0	0	0	0	0	0	0	0
2.	Nystagmus	0	0	0	0	0	0	0	0	0	0	0	0	0
3.	Lacrimation	0	0	0	0	0	0	0	0	0	0	0	0	0
4.	Exophthalamus	0	0	0	0	0	0	0	0	0	0	0	0	0
5.	Endophthalamus	0	0	0	0	0	0	0	0	0	0	0	0	0
E)	**Ear observation**													
1.	Blanching	0	0	0	0	0	0	0	0	0	0	0	0	0
2.	Hyperemia	0	0	0	0	0	0	0	0	0	0	0	0	0
3.	Cyanosis	0	0	0	0	0	0	0	0	0	0	0	0	0
F)	**General observation**													
1.	Grooming	0	0	0	0	0	0	0	+	0	0	0	0	0
2.	Irritability	0	0	0	0	0	0	0	0	0	0	0	0	0
3.	Tail erection	0	0	0	0	0	0	0	0	0	0	0	0	0
4.	Spontaneous activity	0	0	0	0	0	–	0	0	0	0	0	0	+
5.	Touch response	0	0	0	0	0	0	0	0	0	0	0	0	0
6.	Sound response	0	0	0	0	0	0	0	0	0	0	0	0	0
7.	Passivity	0	0	+	0	0	0	0	0	0	0	0	0	0
8.	Circling motion	0	0	0	0	0	0	0	0	0	0	0	0	0
9.	Writhing	0	0	0	0	0	0	0	0	0	0	0	0	0
10.	Body tone	0	–	0	0	0	0	0	0	0	0	0	0	0
11.	Climbing	0	0	0	0	0	0	0	0	0	0	0	0	0
12.	Stereotype	0	0	0	0	0	0	0	0	0	0	0	0	0
13.	Tail lashing	0	0	0	0	0	0	0	0	0	0	0	0	0
14.	Vocalisation	0	0	0	0	0	0	+	0	0	0	0	0	0
15.	Salivation	0	0	0	0	0	0	0	0	0	0	0	0	0
16.	Diaarhoea	0	0	0	0	0	0	0	0	0	0	0	0	0

No change in activity (0), Decrease in activity (–) and Increase in activity (+)

Table 3: Forced Swim Activity of Isonicotinic Acid Hydrazide and its Analogues

Compounds	Mean immobility time (sec) ± SEM	
	Control	Test
INH	38.1±3.503	24.8±6.1683
1.	129.3±6.731	158.3±7.121**
2.	122.4±6.70	108.4±5.069
3.	23.8±4.950	56.3±13.015
4.	112.5 ±1.827	35.8±5.721*
5.	82.7±5.283	90.8±5.131
6.	37.8±2.947	29.5±3.804
7.	106.4±5.528	115.6±7.622
8.	52.4±3.851	88.4±3.194**
9.	113.4±1.826	40.2±7.560**
10.	33.8±2.719	47.2±8.149
11.	47.6±3.113	17.6±4.821**

Isonicotinic acid hydrazide (INH)
All the compounds (1-11) were administered intraperitoneally (50 mg/kg)
Significant differences by Student's t-test: P<0.05*, P<0.01** as compared to control,
significant data p 0.5 (*)
highly significant data 0.001 (**)

3.1. *General Pharmacological Screening*

Structural investigation from Table-2 revealed that INH and its phenacyl derivatives 4, 5 and 9 with *para* fluoro, *meta* nitro and *ortho-meta* dimethoxy groups, respectively caused reduction in motor activity of animals. On the other hand, phenacyl derivatives 6, 7 and 8 with *para* nitro, *ortho-para*fluoro and *para*bromo substituents enhanced the motor activity of the animals. It can be said that the attachment of electron-withdrawing group at the *para* position might facilitate the development of CNS stimulant drugs.

3.2. *Forced Swim Test*

Depression is a feeling of sadness and all other negative emotions. Today there is a more demand for new antidepressant molecules because the available medication have been associated with serious side effects. The findings of FST suggest that compound 2,a phenacyl derivative of INH with two hydroxyl groups at*meta* and *para* positions decreased the passivity of mice. Similarly dimethoxy groups at *ortho and meta* positions in compound 9 showed appreciable struggle in treated animals as compared to control. Therefore, it can be inferred that the presence of electron-donating

moiety at *meta* position might help in producing new compounds having antidepressant activity.

It was also observed that *para* fluorine atom in derivative 4 and *para* nitro group in 6 also reduced the latency phase of mice during FST. This feature highlighted the significance of electron-withdrawing groups at *para* position supporting the development of antidepressant molecules. Interestingly, un-substituted aromatic ring in compound 11 also showed significant reductions in immobility time suggesting that that attachment of different substituent's at various positions in the ring can alter their nature and either depressive or antidepressive effects.

4. CONCLUSION

The study concluded that the chemical modification of INH with phenacyl ring improves its action on central nervous system especially the derivatives with electron-withdrawing groups at the *para* position may serve as lead molecule for the development of CNS active drugs.

ACKNOWLEDGEMENT

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Production of *Siderophore* and Its Role as Therapeutic Agent

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Abstract

Siderophores are iron chelators, which are produced by bacteria under iron deficient conditions required for their growth. Therefore, *siderophores* can be used as a carrier to direct drug into the bacteria and kill them. The present study was designed to screen *siderophore* production using different bacterial cultues using iron deficient medium and its synergistic capability to kill drug resistant bacteria. *Siderophore* under iron deprived condition was evaluated by chrome azurol S (CAS) assay. Whereas, broth micro-dilution method and checker board assay were used to determine antimicrobial properties of selected drugs or epigallocatechin gallate (EGCG) individually or in combination with synthetic *siderophore*. Results demonstrated that the entire tested microorganisms produced *siderophore* under iron deprived condition as evident by orange halo zones in CAS agar plates. Gram negative bacteria produced more *siderophore* as reflected by orange colour with bacterial zone inhibition of 17-22mm as compared to Gram positive bacteria (13-15mm). As compared to antibiotics and EGCG, acetohydroxamic acid (aHa; synthetic *siderophore*) showed no antibacterial properties (1500 - 6500 µg/ml). The synergism of aHa with tetracycline, ceftriazone and EGCG (FIC index <0.5) against *S. typhi*, methicillin resistant and sensitive *Staphylococcus aureus* and *E. coli* were evident. In conclusion, *siderophore* may be considered as potential candidate to design different combination therapy against emerging antimicrobial resistant pathogens.

Keywords

Siderophore, Chrome azurol S Assay (CAS), Checkerboard assay, Fraction inhibitory concentration index (FIC), Synergism, Epigallocatechin gallate (EGCG).

1. INTRODUCTION

Iron constituted about 35% of the earth mass and considered as essential nutrient for both human and microbes. In the host, nutritional

immunity restricts the availability of iron to invading pathogens by binding to various proteins (Arnold, 2018; Cassat and Skaar, 2013). Iron due to its oxidation reduction potential holds a key position in cellular function such as aerobic respiration, ATP production, heme formation, DNA synthesis, etc (Abbaspour *et al.,* 2014; Raymond *et al.,* 2003). In normal human serum the levels of free iron is ~10-18-10-24 M which is far below the concentration required by bacterial pathogens to grow and cause infection (Raymond *et al.,* 2003). A concentration of, at least, 1 mM of iron is needed for optimum growth; however, this concentration varies for different organisms (Cassat and Skaar, 2013; Fischbach *et al.,* 2006). In an iron deprived condition, microorganisms produce low molecular weight siderophores which have strong affinity for iron (Behnsen and Raffatellu, 2016; Crosa and Walsh, 2002). Siderophore, a Greek word meaning "iron carrier" is produced by microorganisms having an extremely high affinity for ferric ions (Chu *et al.,* 2010; Schwyn and Neilands, 1987). There are different classes of siderophores like hydroxamates, thiohydroxamates and catecholates (Ahmed and Holmstrom, 2014; Boukhalfa *et al.,* 2003). Many bacteria can synthesize their own siderophores, or utilize other microbial- and plant-siderophores for iron acquisition (D'Onofrio *et al.,* 2010). The mechanism of iron acquisition is known to be a virulence factor for human and animal pathogenic bacteria (Koh and Henderson, 2015).

Siderophores are commonly produced by most aerobic and anaerobic microorganisms (Ahmed and Holmstrom, 2014; Koh and Henderson, 2015). Different types of siderophores have been identified such as: Enterobactin, mycobactin, pyoverdin and pyochelin (Brandel *et al.,* 2012; Raymond *et al.,* 2003; Rodriguez and Smith, 2006). Depending on the participating chelating group by bacteria and fungi siderophores have been further classified into three classes: Catecholates, Hydroxamates and Mixed ligands (Sah and Singh, 2015).

Siderophores can be used for selective delivery of antibiotics in antibiotic resistant bacteria (Gorska *et al.,* 2014; Mollmann *et al.,* 2009). It is a potentially antimicrobial approach to utilize bacterial own iron transport system to overcome drug resistant bacteria. Keeping this in mind present study was designed to investigate siderophore production ability of multidrug resistant clinical isolates especially methicillin resistant *Staphylococcus aureus* (MRSA) and some enteropathogens. Furthermore, synergistic capability of synthetic siderophore (Acetohydroxamate) with different drugs or epigallocatechin gallate (EGCG) to treat MDR infections were also determined.

2. MATERIAL AND METHODS

2.1. *Bacterial Strains and Culture Medium Condition*

Bacterial cultures such as enteropathogenic and enterotoxigenic *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Shigella dysentriae* and different strains of Methicillin Resistent *Staphylococcus aureus* were used for siderophore detection. Purity of the above mentioned cultures were confirmed by Gram staining and biochemical characterization (data not shown). For routine use these cultures were maintained on Tryton Soya agar or broth (TSB; TSA; Oxoid UK) and incubated overnight at 37°C under aerobic conditions. Iron deficient medium containing MM9 was prepared by as described earlier (Payne, 1994). Briefly, solution-1 KH_2PO_4 (0.3 g), NaCl (0.5 g), NH4Cl (1 g), NaOH (6 g)

and PIPES (30.24 g) in 1 L was mixed and autoclaved followed by mixing with solution-2 (30 ml of 10% (m/v) deferrated casamino acids, (Contaminating iron was removed with 8-hydroxyquinoline (3%) in chloroform), fructose (2.0 g), $MgCl_2$ (1M, 1ml) and of $CaCl_2$ (100 mM, 1 ml) in one liter was prepared, filtered and sterilized.

2.2. *Minimum Inhibitory Concentration (MIC) Determination*

The minimal inhibitory concentrations (MIC) of ampicillin, ceftriazone, tetracycline, and EGCG were determined by micro dilution method according to CLSI standards and as mentioned earlier (CLSI, 2012; Khan *et al.,* 2017). Briefly, Mueller Hinton broth (100 µl) was transferred to each well of 96 well plate containing antibiotic (100 µl, 1 mg/ml) to first well and two fold dilutions were prepared starting from well 1-10 to achieve 500-1.0 µg/ml concentration. An inoculum (5 x 10^5) cells were inoculated in each well except 12th well which served as a negative control, whereas, 11th well served as positive control and incubated at 37°C for 18 to 24 hours under aerobic conditions, and MIC of drugs was recorded as the lowest concentration which inhibited the bacterial growth.

2.3. *Detection of Siderophore Production*

Chrome azurol (CAS) assay was performed to detect siderophore production under iron free condition as described before (Payne, 1994). The CAS agar plates were prepared by addition of CAS (60.5 mg in 50 ml deionized water) and 10 ml of iron (III) in a mixture ($FeCl_3$-$6H_2O$, 1 mM and HCl, 10 mM). Under stirring this solution was slowly mixed with HDTMA (72.9 mg) dissolved in water (40 ml). The resultant dark blue solution was autoclaved and mixed with an autoclaved mixture (900 ml) of water containing agarose (10 g) and Tris (1 mM), the pH was adjusted to 6.8. The plates were poured and sealed with polythene bags and stored in refrigerator until use. For CAS assay the plates were punched with holes using a borer. Each hole was properly labeled and was filled with of cell free filtrates (50 µl) of different bacteria grown in iron-deficient medium to validate siderophore production, and incubated at 37°C for 5 hours. Presence of siderophores was indicated as the appearance of orange halos around the well. Siderophore activity was expressed as the square value of the halo diameter.

2.4. *Checkerboard Synergism Assay*

The synergistic capability of synthetic siderophore acetohydroxamate (aHa) with tetracycline, ampicillin, ceftrizone and EGCG was determined by calculating fractional inhibitory concentration (FIC) index using checkerboard assay (Sopirala *et al.,* 2010). The concentration range of each antimicrobial agent in combination ranged from 1/32 times the MIC (1/32 × MIC) to 4 × MIC. Two fold dilutions of drugs A and B were made. The initial inoculum was same as used for MIC. The FIC index of each antibiotic in combination was calculated by following formula:

Fractional inhibitory concentration index (FIC) = of drug A + FIC of drug B

FIC of drug A or B = minimal inhibitory concentrations (MIC) of drug in combination/ MIC of Drug alone.

Results were interpreted based on following scale:

FIC index: ≤0.5 (Synergism), >0.5 to ≤1 (Additive), >1 to ≤4 (Indifference) and >4 (Antagonism).

3. RESULTS AND DISCUSSION

The minimal inhibitory concentration of tetracycline, ampicillin, ceftriazone and green tea was recorded as the lowest concentration of antibiotic producing complete inhibition of visible growth (Table 1). Antibiotics were selected on the basis of their susceptibility pattern against microorganism used (Result not shown). Different microorganisms have different MIC ranges with respect to antibiotics.

Siderophores are commonly produced by most aerobic and anaerobic microorganisms to counter iron deficient environment. Different strains of microorganisms were grown and tested for siderophore production on CAS agar well diffusion method. Results showed that all the tested microorganisms were positive for siderophore production (Table 2). Siderophore produced by microorganisms abstract Fe^{+3} from blue tenary complex of CAS result in changing color from blue to orange (Guan *et al*., 2001). Gram negative bacteria (*E. coli*, *S. typhi*, *K. pneumoniae*, *P. aeruginosa* and *S. dysentery*) produced more siderophore (16-25 mm) as reflected by orange halo zones around the well (Fig. 1) as compared to Gram positive bacteria (MRSA clinical strain, *S. epidermidis, S. pyogenes* (12-16 mm) in the absence of iron as compared to iron sufficient media.

Different studies also verified the production of siderophores by microorganisms in iron deprived conditions and that enteropathogens produced more siderophore in iron limited condition as compared to Gram positive bacteria (Faraldo-Gomez and Sansom, 2003; Furrer *et al.,* 2002; Lankford and Byers, 1973; Palyada *et al.,* 2004). Among various other methods to detect siderophore, Chrome azurol S agar diffusion assay is one of the

Table 1: Minimum Inhibitory Concentration of Antibiotics, EGCG and Acethydroxamic Acid against Different Micro-organisms

S.No.	Micro-organisms	Minimal Inhibitory Concentration (MIC) (µg/ml)				
		Tetracycline	Amplicillin	Ceftriazone acid	EGCG	Aceto-hydoxamic
1.	MRSA	62.5	1000	500	3120.0	1950.0
2.	MSSA	31.25	250	250	190.0	3125.0
3.	*Salmonella typhi*	31.25	62.5	3.9	1560	3125.0
4.	*Staphylococcus epidermidis*	62.5	62.5	31.25	6250	1512.0
5.	*Escherichia coli*	15.62	7.81	1.95	3120	6250.0
6.	*Escherichia coli* (401)	15.62	62.5	7.81	6250	6250.0
7.	*Escherichia coli* (UTI)	0.48	31.25	31.25	3120	6250.0
8.	*Pseudomonas aeruginosa*	15.62	7.81	3.90	1560	1512.0

Methicillin Resistant *Staphylococcus aureus* (MRSA) *and* Methicillin Sensitive *Staphylococcus aureus* (MSSA)

Table 2: Siderophore Production and Detection of Different Groups of Micro-organisms by Chrome Azurol S Agar Diffusion Assay

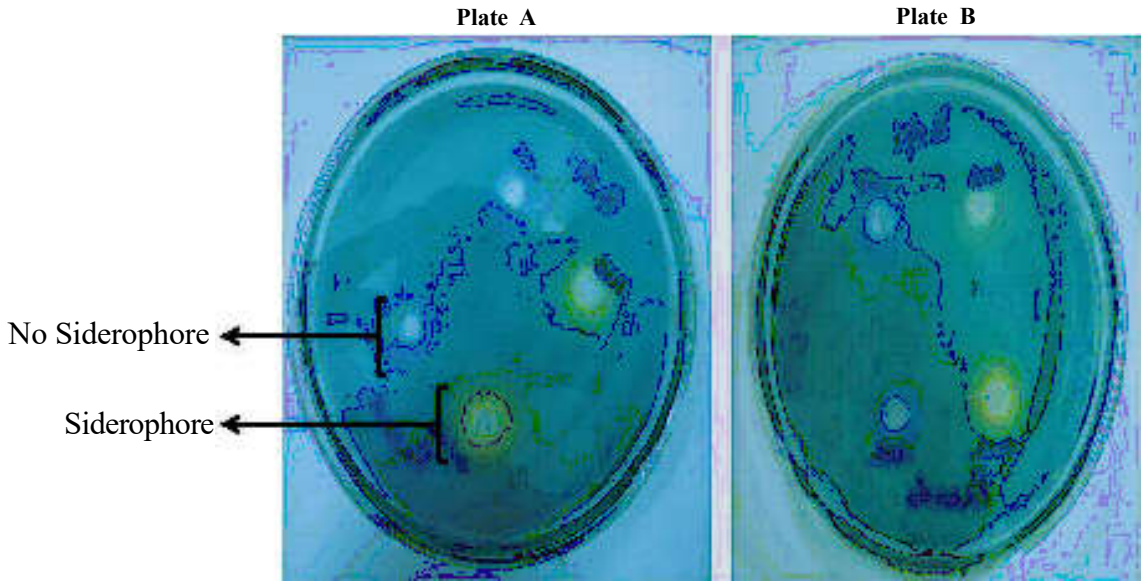
S.No.	Micro-organisms	Zone of Inhibition (mm)	
		Iron Deficient Medium	Iron Containing Medium
1.	*Escherichia coli*	25	0
2.	*Enteropathogenic Escherichia coli*	20	0
3.	*Enterotoxigenic Escherichia coli*	18	0
4.	*Staphylococcus epidermidis*	15	0
5.	*Staphylococcus aureus*	15	00
6.	*Streptococcus pyogenes*	16	0
7.	*Salmonella typhi*	20	0
8.	*Salmonella paratyphi* A	16	0
9.	*Bacillus subtilis*	15	0
10.	*Klebsiella pneumoniae*	14	0
11.	*Pseudomonas aeruginosa*	21	0
12.	*Corynibacterium diphtheriae*	15	0
13.	*Enterobacter aerogenes*	13	0
14.	*Micrococcus luteus*	13	0
15.	*Shigella dysentriae*	18	0
16.	*Proteus mirabilis*	14	0
17.	*MRSA strain 1-10*	14-19	0

Methicillin Resistant *Staphylococcus aureus* (MRSA)

universal and cost effectiveness method used for detecting siderophore production as described previously (Schwyn and Neilands, 1987). Irrespective of chemical nature this assay can detect total siderophores in chemically defined media. Principle involved the extraction of iron from Fe^{+3}-CAS conjugate by siderophore which has great affinity for iron which converts blue color to orange (Guan *et al.,* 2001).

Our results clearly demonstrated that resistant and toxin producing bacteria methicillin resistant *Staphylococcus aureus*, *Escherichia coli*, enteropathogenic and enterotoxigenic *Escherichia coli*, *Pseudomonas aeruginosa* and *Shigella dysentria* produced more siderophores as compared to less virulent strains (*Enterococcus aerogenes, Micrococcus luteus* and *Proteus mirabilis*). A correlation exist that siderophore production is directly related to virulence (Granato *et al.,* 2016). *Pseudomonas* strains positive for siderophore production were more virulent in establishing

Fig. 1: Siderophore production and detection by Chrome Azurol S (CAS) Assay



In both plates (A and B) clear well represents absence of siderophore while yellowish orange colored halo zone represents siderophore production in plate A

infection in mice as compared to non siderophore producers (Little *et al.,* 2018; Takase *et al.,* 2000). Likewise, *Yersinia pestis* siderophore mutant strain was less virulent to cause plague in animal model as compared to non mutant strains (Fetherston *et al.,* 2012; Miller *et al.,* 2010). Thereby suggesting that siderophore are involved in the virulence of the pathogens.

Checkerboard method was used to evaluate synergistic studies of synthetic siderophore (Acetohyroxamic acid; aHa) with other antibiotics. The combination shows pattern of synergism, indifference and antagonism (Table 3). The combination of acetohydroxamic acid and ceftriazone showed synergism (FIC index <0.5) against methicillin sensitive or resistant *S. aureus* and *S. typhi* strains. Besides, ceftriazone, acetohydroxamic acid also showed appreciable synergism with EGCG against methicillin sensitive or resistant *S. aureus* and *E. coli* (Table 3). This might be an important as EGCG itself plays an important role as an iron chelator as well as possesses antimicrobial properties (Hatcher *et al.,* 2009; Reznichenko *et al.,* 2006) indicating that siderophores and their analogues have tremendous therapeutic potential (Brandel *et al.,* 2012; Rodriguez and Smith, 2006). It might be possible that different drugs may be linked with synthetic siderophores which can be manipulated to enter the cell and exert its function and hence overcome the emerging antimicrobial resistance. These conjugates have selective antimicrobial activity because the microbes recognize specific siderophores (Diarra *et al.,* 1996). Although, this study lacks conjugate preparation but provides support that antibiotics can be synergized with siderophore. Other investigator used green tea as an iron chelator with

Table 3: Fractional Inhibitory Concentration of Different Combination of Antibiotics and Acetohydroxamaic Acid

Micro-oraganism	Combination	FIC Index	Relation
MRSA	Tetracycline + Acetohydroxamic acid	0.7	Indifferent
MSSA		0.7	Indifferent
Salmonella typhi		0.18	Synergy
Escherichia coli		3	Antagonism
MRSA	Ampicillin + Acetohydroxamic acid	0.62	Indifferent
MSSA		0.5	Indifferent
Staphylococcus epidermidis		0.76	Indifferent
Escherichia coli		3.0	Antagonism
MRSA	Ceftriazone + Acetohydroxamic acid	0.37	Synergy
MSSA		0.25	Synergy
Salmonella typhi		0.18	Synergy
MRSA	Green tea + Acetohydroxamic acid	0.2	Synergy
MSSA		0.18	Synergy
Salmonella typhi		0.6	Indifferent
Escherichia coli		0.37	Synergy

Methicillin Resistant *Staphylococcus aureus* (MRSA) and Methicillin Sensitive *Staphylococcus aureus* (MSSA)

Fractional Inhibitory Concentration (FIC) Index:
Synergism (≤0.5), Additive (>0.5 to ≤1), Indifference (>1 to ≤4) and Antagonism (>4)

antimicrobial properties which also showed synergism with antibiotics suggesting its importance to utilize it either as siderophore or as antibacterial agents (Hu *et al.,* 2001). The EGCG interacts positively with β-lactam drugs to treat MRSA (Hu *et al.,* 2001). Siderophore drug conjugate such as albomycin inhibited tRNA synthetase and cured bacterial infections in a mouse model (Pramanik *et al.,* 2007). Microcin E422m, an antimicrobial peptide produced by *Klebsiella pneumonia*, containing several catecholate groups that binds Fe^{+3}, internalize through siderophore outer membrane transporters and kill pathogens (Thomas *et al.,* 2004). Such siderophore drugs have been named sideromycins (Pramanik *et al.,* 2007; Wencewicz *et al.,* 2009).

4. CONCLUSION

In conclusion, siderophore can be used as a carrier system to deliver drug and can be used to cure alarming antimicrobial resistant pathogens.

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Treatment of Cardiovascular Diseases (CVDs) in Unani Medicine

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Abstract

Unani Medicine is a traditional system and commonly practiced worldwide. The concept of health in Unani Medicine is based on a combined equilibrium of body, mind and soul. Cardiovascular diseases (CVDs) are also caused by disturbance in this equilibrium. There is no simple, efficacious and cost effective cure for many heart disorders in allopathic medicine. On the other hand, Unani Medicine presents effective, safe and cost effective approach to manage various heart disorders which have no satisfactory treatment in conventional medicine. This review enlist twenty eight Unani classical books in which treatment options for various cardiovascular diseases have been mentioned. Most of them now provides scientific justification for their efficacy, thereby the role of Unani medicine in health care has played a pivotal role and has a bright future which cannot be ignored.

Key words

Unani, *Adwiya Qalbiyya,* Cardiovascular diseases.

1. INTRODUCTION

Cardiovascular diseases (CVDs) are major cause of death globally, as compared to other diseases. An estimated 17.7 million people died in 2015, representing 31% of all global deaths. Of these deaths, an estimated 7.4 million were due to coronary heart disease and 6.7 million were due to stroke (Shanthi Mendis, 2107). There is no simple, efficacious and cost by effective cure for many heart disorders by western medicine. On the other hand, Unani Medicine presents efficacious, safe and cost effective approach to manage various heart disorders which have no satisfactory treatment in conventional medicine.

The equilibrium of body mind and soul is advocated to be maintained through the principles of six essential factors of life, which includes: 1) Air, 2) Food and drinks, 3) Bodily movement and repose, 4) Psychic movement and repose, 5) Sleep and wakefulness and 6) Evacuation and retention. Any disturbance in this equilibrium leads to unhealthy state.

Unani scholars have dealt with CVDs in detail. Renowned Unani scholar Ibn Sina in

research based booklet *Risala Adwiya qalbiyya* focused on the drugs beneficial in heart ailments. In the classical literature of Unani System of Medicine (USM), heart has been described as a body organ, which pumps the blood in the body to keep a person alive and healthy. Being the centre of *Quwwat Haywaniyya*, it performs its functions through this faculty. Ibn Sina, also emphasized about the correlation between emotional disorders and heart ailments.

Cardiovascular diseases are the leading cause of deaths increased from 17.3 million in 2008 expected to be 23.6 million by 2030.

Unani medicine occupies an important place in the rich traditional heritage of indigenous systems of medicine and contributes significantly to the health care. The treatment of CVDs in Unani System of Medicine is based on drugs of herbal, mineral and animal origin. In ancient classical literature of Unani medicine hundreds of drugs have been mentioned possessing cardio-protective and cardio-tonic activity.

Ibn Sina has mentioned in his *Risala Qalbiyya* 63 drugs that are beneficial for different cardiac diseases. The description of different cardiac problems and their management with Unani drugs have been scattered in Unani classical literature in different languages in the books of *Mufaradaat* (Single drugs) as well as *Murakkabat* (Compound drugs). A comprehensive detail in this regard has been found in large number of Unani classical books as shown in Table 1.

Table 1: List of Unani Classical Books Used

S.No.	Unani Classical Books	S.No.	Unani Classical Books
1.	*Kitab al-Hashaish*	15.	*Ghina Muna*
2.	*Al-Hawi fi'l Tibb*	16.	*Tazkira Uli'l Albab*
3.	*Jami Ibn-i-Baitar*	17.	*Minhaj al-Dukkan*
4.	*Al-Qanoon fi'l Tibb*	18.	*Qarabadeen-i-Kabir*
5.	*Kamil al-Sana'a*	19.	*Qarabadeen Qadri*
6.	*Al-Moalajat al-Buqratiya*	20.	*Ilaj al-Amraz*
7.	*Kitab al-Mukhtarat*	21.	*Ajala Nafia*
8.	*Zakhira Khwarizm Shahi*	22.	*Makhzan al-Adwiya*
9.	*Tohafatul Momineen*	23.	*Taleef Shareefi*
10.	*Ikhtiyarat Badi'i*	24.	*Qarabadeen Jalali*
11.	*Yadgar Raza'i*	25.	*Muheet-i-Azam*
12.	*Kitab al-Tasreef*	26.	*Qarabadeen-i-Azam*
13.	*Kitab al-Tayseer*	27.	*Iksir-i-Azam*
14.	*Sharah al-Asbab wa'l Alamat*	28.	*Rumooz-i-Azam*

Management of CVDs

In today's era, various Unani cardiac drugs have been scientifically validated and proved their efficacy globally in the management of different cardiac diseases. Following are some important Unani single drug which has been documented in Unani classics and also their efficacy have been established on the scientific basis:

1) *Aabresham* (Silk cocoon; *Bombyx mori*)

According to Razi and Antaki *Aabresham* is used in the treatment of *Zofe Qalb* (Weakness of heart). Ibn Sina mentioned it as *Mufarreh Qawi* while Ibn Baitar wrote as *Muqawwi Qalb*. The scientific studies showed that: Alcoholic extract of *B. mori* has significant cardioprotective activity against myocardial infarction (Srivastav *et al.,* 2013) and *Aabresham* guards against cardiometabolic syndrome. It attenuates dyslipidemia, and associated hypertension by decreasing oxidative stress (Nepal *et al.,* 2012).

2) *Mushk* (Musk)

According to Razi, Ibn Sina and Ibn Baitar *Mushk* is known as *Muqawwi Qalb*. Both with reference to Ishaq bin Imran advocated that it is *Mufarreh* and used in *Khafaqan*. As per Maseehi and Azam Khan it is good *Mufarreh Qalb.* The *Mushk* (Musk) improved the blood perfusion of the ischemic myocardium; the total effective rate was 70.6% without any detectable adverse reaction (Luo *et al.,* 1996).

3) *Inab* (*Vitis vinifera*)

Razi described that *Inab* is useful in the patients of *Khafqan*. The available data strongly support the recommendation that a diet rich in fruits and vegetables, including grapes, can lower the risk for cardiovascular disease (Mustali and Joseph, 2009).

4) *Badranjboya* (*Nepeta hindostana*)

According to Razi *Badranjboya* is used in *Zofe Qalb*. Ibn Sina and Ibn Baitar documented that it is *Mufarre Qalb* and *Muqawwi Qalb* used in *Khafaqaan*. Ibn Baitar suggested its use as *Munaqqi Qalb* and also as promising in *Waja ul Qalb* and *Zoafe Qalb*. Kirmani indicated its usefulness in *Sue Mizaj Barid Qalb*. A recent study *Badranjboya* demonstrated its encouraging potential in the prevention of atherosclerosis (Azhar *et al.,* 2017).

5) *Baadrooj* (*Ocimum gratissimum* Linn.)

According to Ibn Sina and Ibn Baitar it is *Mufarre Qalb* and *Muqawwi Qalb* used in *Khafaqaan*. Ibn Baitar mentioned it as *Munaqqi Qalb* used in *Waja ul qalb* and *Zoafe Qalb*. Oral feeding of hydroalcoholic extract of *O. sanctum* to male Wistar rats subjected to chronic-resistant stress significantly prevented the chronic-resistant stress/induced rise in plasma level, myocardial superoxide dismutase and catalase activities as well as the microscopic changes in the myocardium (Priyabrata *et al.,* 2010).

6) *Tuffah* (*Malus sylvestris* Mill.)

According to Ibn Sina, Ibn Baitar and Ibn Zuhr *Tuffah* is *Muqawwi Qalb* and *Mufarreh Qalb*. *Antaki* mentioned that it is useful in *Zofe Qalb*. *Malus sylvestris* appeared as a good source of polyphenolic compounds having therapeutic effects on many diseases e.g. cardiovascular and atherosclerosis caused by reactive oxygen species and oxidative stress (Stojiljkovic *et al.,* 2016).

7) *Jadwar* (*Delphinium denudatum* Wall.)

Ibn Baitar with reference to Ibn Sina mentioned that *Jadwar* is *Mufarreh Qawi* and *Muqawwi Qalb*. In a controlled study all the

Table 1: Treatment of Cardiovascular Diseases (CVDs) in Unani Medicine

S.No.	Botanical and Unani name	Image	References
1.	*Abresham* (Silk cocoon; *Bombyx mori*)		Srivastav *et al.,* 2013, Nepal *et al.,* 2012
2.	*Mushk* (Musk)		Luo *et al.,* 1996
3.	*Inab* (*Vitis vinifera*)		Mustali and Joseph, 2009
4.	*Badranjboya* (*Nepeta hindostana*)		Azhar *et al.,* 2017
5.	*Baadrooj* (*Ocimum gratissimum* Linn.)		Priyabrata *et al.,* 2010
6.	*Tuffah* (*Malus sylvestris* Mill.)		Stojiljkovic *et al.,* 2016
7.	*Jadwar* (*Delphinium denudatum* Wall.)		Mohsin *et al.,* 2008
8.	*Daarchini* (*Cinnamomum zeylanicum* Blume)		Priyanga *et al.,* 2013
9.	*Neelofar* (*Nymphaea alba* Linn.)		Jaya *et al.,* 2016
10.	*Ward* (*Rosa damascena* Mill.)		Amir *et al.,* 2015
11.	*Rumman* (*Punica granatum*)		Mahalaxmi *et al.,* 2010
12.	*Sandal* (*Santalum album* Linn.)		Shamim *et al.,* 2010
13.	*Zaranbaad* (*Zingiber zerumbet* Rosc ex Smith)		Hemn *et al.,* 2015
14.	*Zafraan* (*Crocus sativus* Linn.)		Mohammad *et al.,* 2015, Sachdeva *et al.,* 2012

30 patients with coronary chest pain induced on exertion, disappeared in 20 patients after the administration of drug. This improvement may be due to reduction in heart rate reducing the oxygen demand by myocardium and also due to its anti-inflammatory activity (Mohsin *et al.,* 2008).

8) *Daarchini* (*Cinnamomum zeylanicum* Blume)

According to Ibn Sina *Darchini* is *Mufarreh*. The *in-vitro* and *in-vivo* studies demonstrated numerous beneficial health effects of *C. zeylanicum*, including anti-inflammatory and anti-microbial properties, thereby reducing cardiovascular disease (Priyanga *et al.,* 2013).

9) *Neelofar (Nymphaea alba* Linn.)

Ibn Baitar mentioned with reference to Ibn Sina that *Neelofar* is used as *Muqawwi Qalb.* Ibn Zuhr mentioned its usefulness in *Warm Ghisha al Qalb*. *Nymphaea alba* showed a significant decline in the levels of serum cholesterol, phospholipids, triglyceride, low density lipoproteins (LDL), very low density lipoprotein (VLDL) and significant rise in the serum high density lipoprotein (HDL) levels in hyperlipidemic rats (Jaya *et al.,* 2016).

10) *Ward* (*Rosa damascena* Mill.)

Ibn Sina mentioned its use in *Ghashi* and Ibn Baitar with reference to Ibn Sina wrote its use in *Khafaqaan* and *Ghashi Haar*. A hypotensive effect of hydro-alcoholic extract of *R. damascena* suggests its beneficial effect to control blood pressure (Amir *et al.,* 2015).

11) *Rumman* (*Punica granatum*)

According to Razi, *Rumman* is used in the treatment of *Khafaqaan*. Ibn Sina mentioned its use as *Muqawwi Sadr* and *Mujallie Qalb*, however, Antaki used it in *Zofe Qalb*. *P. granatum* reduces cardiotoxic effects of isoproterenol and may be of value in the treatment of myocardial infarction (Mahalaxmi *et al.,* 2010).

12) *Sandal* (*Santalum album* Linn.)

According to Ibn Baitar with reference to Ibn Sina, *Sandal* is used as *Muqawwi wa Mufarreh Qalb*. Razi mentioned with reference to Ibn Masweh and its use in *Khafaqaan*. A significant reduction in serum total cholesterol and triglyceride levels of rabbits treated with water soluble portion of stem powder of *S. album* very evident (Shamim *et al.,* 2010).

13) *Zaranbaad* (*Zingiber zerumbet* Rosc ex Smith)

Razi mentioned that *Zaranbad* is used in the treatment of *Khafaqaan*. *Ibn Sina* wrote it as *Muqawwi Sadr* and *Mujalli-e-Qalb*. *Z. zerumbet* alone act as a prophylactic agent, and as a supplementary treatment with simvastatin, significantly reducing early plague formation, development, and establishment *via* significant reduction in serum lipid profile, accompanied by suppression of oxidative damage, and hence alleviated atherosclerosis lesions (Hemn *et al.,* 2015).

14) *Zafraan* (*Crocus sativus* Linn.)

Ibn Sina mentioned that *Zafraan* is used as *Muqawwi Qalb* and *Mufarreh*. Kirmani and Azam Khan documented its use in *Sur Mizaj Ratab Qalb*. One study on *Zafraan* demonstrated that administration of the aqueous extract of *saffron* petals (500 mg/kg) reduced blood pressure in rats (Mohammad *et al.,* 2015). It also reported to possess signicant cardio-protective effect by preserving hemodynamics and left ventricular functions (Sachdeva *et al.,* 2012).

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Comparative Account of *Calendula officinalis* L. and *Tagetes erecta* L. in the Light of Pharmacognostic, Pharmacological and Traditional Uses

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Abstract

This research work is based on the comparative studies of two wound healing plants i.e. *Calendula officinalis* L. and *Tagetes erecta* L. which belongs to the family (*Asteraceae*). Both colourful species are found throughout the world including Pakistan. These ornamental plants are being utilized as an antiseptic, antifungal, anti-inflammatory, antibacterial and wound healing agents in modern system of medicine. Furthermore *Calendula* has culinary use due to its carotenoid and phenolic contents while *Tagetes* has a poisonous principle, thiophene which proved as good companion plant in agricultural practices. This review focus on comparative account of pharmacological, phytochemical and traditional uses of *C. officinalis* L. and *T. erecta* L. using available authentic data.

Keywords

Calendula officinalis L., *Tagetes erecta* L., Phytochemistry, Asteraceae, Wound healing activity.

1. INTRODUCTION

Calendula officinalis L. (*Asteraceae*) is an aromatic, annual herb with bright orange daisy like flower which are used for medicinal and culinary purpose. It is grown as an ornamental plant in the garden. It is a native of central, eastern and southern Europe and North Africa but it is also found in warmer parts of the world like Pakistan, India, Afghanistan and China. In Pakistan it is known as Gul Ashrafi. Other common names of *Calendula officinallis* includes, Marigold, Gold bloom (English), Butter blume (German), Chin Chan Ts'ao (Chinese), Zergul (Hindi) (Young, 1950; Backer and Van Den, 1965). The plant has reported to possess many diverse pharmacological activities. Plant extract can be used as analgesic, anthelmintic, anti-emetic, anti-pyretic, antiseptic, anti-spasmodic, astringent, cardiotonic, carminative,

cholagogue, dermagenic, diaphoretic, diuretic, hemostatic, immunostimulant, lymphatic, uterotonic, and as vasodilator. Externally it is used for treating skin inflammation, bleeding wound, minor burns. In Homeopathic system of medicine, it is employed for the healing of second and third degree burns and to diminish scar formation. It is also used to stimulate regrowth of skin (Cromack and Smiyh, 1998; Bcerentrup and Robbelen, 1987).

Tagetes erecta L., (*Asteraceae*) it's been used traditionally for the treatment of wounds. It is a common aromatic herb which yields strong aromatic essential oil (Farjana *et al*., 2009). *T. erecta* locally called in Pakistan as Gainda and in different territories named as African marigold, Aztec marigold, big marigold, and saffron marigold. It has larger flowers; Yellow, orange, golden or bicolored with many whorls divided into disc and ray florets are held either well above the fine textured dark green foliage or tucked in with the foliage. The plant grows to the height of 1-3 feet and spreads to 0.5 feet. Leaves are arranged in opposite pattern and leaf types are of odd pinnately compound. The margin is dentate and shape is oblong. The leaf blade length is less than 2 inches and color of leaf is green. Characteristic of flower is showy (Edward and Teresa, 1999).

2. MATERIAL AND METHOD

Fresh flowers of *C. officinalis* L. (1500 gm) and *T. erecta* L. (2000 gm) were purchased from local nursery of area in November 2009, and identified by Prof. Dr. Surraya Khatoon, Department of Botany, Faculty of Science, University of Karachi.

2.1. *Traditional Uses*

Traditionally *C. officinalis* L. has been used for the inflammation of oral and pharyngeal mucosa. It is a detoxifying herb and has been utilized for the treatment of chronic infections in a form of infusion (Yoshikawa *et al*., 2001; Blumenthal *et al*., 2001). Tropically the flower infusion used as an antiseptic for wound, freckles and conjunctivitis (Rehecho *et al*., 2011). While flowers of *Tagetes erecta* L. used traditionally to cure various infectious diseases. Also employed to cure fever and epilepsy. According to Ayurveda it acts as astringent, carminative, stomachic, in liver complaints and eye diseases. They are known to purify blood. Flower juice is given as a remedy for bleeding piles also cures colds, rheumatism and bronchitis. The Cherokee used it as skin wash and for yellow dye (Kadam *et al.,* 2013; Ahito, 2015). In Brazil and Mexico, marigold used for joint pain and for muscular spasm. Other folklore uses of *Tagetes* includes anemia, irregular menstruation, abdominal pain, muscular and bone pain. Internally used for indigestion, colic, cough and dysentery. Externally used for ulcers, eczema, sore eyes and rheumatism (Shetty *et al.,* 2009; Manisha *et al.,* 2013; Ahito, 2015).

2.2. *Microscopic Characterization*

Transverse section of florets of *C. officinalis* showed upper and lower epidermis which consists of single layer of barrel shaped cells. On the upper and lower side numerous multicellular uniseriate large glandular trichomes are present. The cortical region consists of multicellular hexagonal and rectangular cells with lignin and tannin depositions. While Cystolyth consist of continuous layer of conical elongated fragments. Epidermis cell are thin walled with tannin deposition. In *T. erecta*ray florets of upper epidermis consists of single layered barrel shaped cells covered with cuticle but lower epidermis consists of large cystolyth while cortex composed of polygonal cells. Disc florets

Table 1: Similar Diagnostic Features of the Powdered Florets of *C. officinalis L.* and *T. erecta L.*

Type of cells	Figures	Characteristic features
Epidermal trichomes		Glandular trichomes are multicellular, unbranched, uniseriate i.e. globular, ovoid head is present with multicellular stalk
Ca-oxalate crystals		Prismatic type. In *C. officinalis* they are deposited in various cellular fragments. While in *T. erecta* they are found in parenchymatous cells of hypanthium
Oil glands		They are big, isolated and deposited in parenchymatous cells seen in both plants
Fibers		Group of wavy hair like phloem fibers more abundantly seen in *C. officinalis* but in *T. erecta* elongated and conical fibers associated with parenchymatous cells are observed
Pollen grains		Thick walled, spherical, spiny pollen grains are abundant in *C. officinalis* whereas less and more spiny walled i.e. immature and mature pollen grains are observed in *Tagetes erecta*
Epidermis		Cells are slightly rectangular in shape, thin and straight walled. Rectangular segmented thin walled epidermis occasionally seen lignin deposition in case of both species.
Collenchyma		Thick walled, air spaces are tiny or absent. Oval or polygonal.

Table 2: Comparative View of Phytoconstituents in *C. officinalis* and *T. erecta*

Phyto-constituents	*C. officinalis*	References	*T. erecta*	References
Terpenoids	Sitosterols, stigmasterols, diesters of diols 3-monoesters of taraxasterol, _-taraxasterol, lupeol	Adler *et al.,* 1975 Wilkomirski *et al.,* 1979 Zittwel-Eglseer *et al.,* 1997	β-sitosterol, β-daucosterol, 7-hydroxysitosterol, lupeol erythrodiol	Kojima *et al.,* 1990 Zhou *et al.,* 2007 Greco *et al.,* 1990 Xue *et al.,* 2008 Antonio *et al.,* 1981
Quinones	Plastoquinone, phylloquinone, toco-pherolubiquinone, phylloquinone)	Janiszowska *et al.,* 1976	6-ethoxy-2,4-dimethylquinoline	Gallagher and Stahr, 1980
Flavonoids	Quercetin, isorhamnetin isoquercetin, isorhamnetin-3-OD glycoside, narcissin, calendoflaside (calendoflavobioside, rutin, neohesperidoside, isorhamnetin, 3-O-neohesperidoside, isorham-netin-3-O-2G-rhamnosyl rutinoside, isorhamnetin-3-Orutinoside, querce-tin-3-O-glucoside and Quercetin-3-O-rutinoside	Kurkin *et al.,* 2007 Vial Ollivier *et al.,* 1989 Ukiya *et al.,* 2006	Quercetagetin quercetagetin-7-methyl ether quercetagetin-7-O-glucoside kaempferol syringic acid gallic acid	Huang *et al.,* 2006 Vilegas *et al.,* 1999 Nair *et al.,* 1995 Xio *et al.,* 2006 Yang *et al.,* 2003 Huang, *et al.,* 2007
Volatile oil	thujene, pienene, limonene, 1,8-cineol, p-cymene, trans-ocimene, terpenene, carene, sabinene, nonanal, terpene-4-ol, 3cylohexene-1-ol, phellandrene, terpeneol, geraniol, carvacrol, caryophyllene, cadinene, cadinol	Okoh *et al.,* 2007	Limonene, caryophyllene, terpinolene, dihydrotagetone, ocmene and piperitone were also detected. The major bio component of flowers of *Tagetes erecta* is carotenoid; includes all trans and cisisomers of zeaxanthins (5%), all trans and cis isomers of lutein, and lutein esters (88%)	Hethelyi *et al.,* 1987 Hethelyi *et al.,* 1988 Leigh *et al.,* 1999

Table 3: Comparative Pharmacological Activities of *C. officinalis* and *T. erecta*

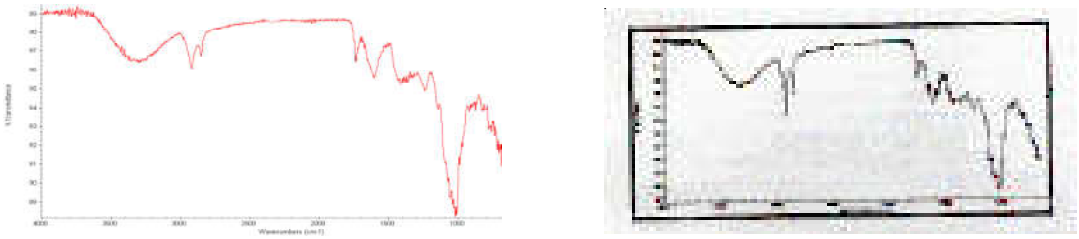
Activities	Plant name	Literature search	References
Anti-inflammatory and analgesic activity	*C.O.*	The extract of *C. officinalis* flowers was evaluated for anti-inflammatory activity using (TPA) induced inflammation in mice. The results revealed that the calendula flower have potent anti-inflammatory activity	Ukiya *et al*., 2006 Della *et al*., 1990 Della *et al*., 1994
		The isolated compounds i.e., faradiol-myristic acid, faradiol-palmitic acid and taraxasterol prevent croton oil induced oedema	Zitterl *et al*., 1997
	T.E.	Chloroform, methanol, ether and hydroalcholic fractions of *T. erecta* claimed to possess analgesic and anti-inflammatory properties. These fractions produced significant effects in acetic acid induced writhing, hot plate method, tail immersion method in mice, and in carrageenan paw edema in rats	Chatterjee *et al.,* 2009 Charaborthy *et al.,* 2009; Shinde *et al.,* 2009)
Wound healing activity	*C.O.*	Various doses of the flower extract were investigated against thermal induced burns in animal model. There was a significant reduction observed	Chandra and Kutton, 2008
	T.E.	The wound healing activity may due to free radical scavenging action and the phyto-constituents (flavonoids) present in it which either due to their individual or additive effect on the process of wound healing	Ibrahim *et al.,* 2011
	C.O.	The petal extract of flower showed more prevailing antioxidant activity as compared to the flower head extract.	Frankic *et al*., 2008 Popovic *et al*., 1999
Antioxidant activity	*T.E.*	An ethanolic extract of flowers and volatile oil of plant were studied for antioxidant activity. Results shown antioxidant activity in all *in vitro* assays – DPPH, reducing power, and superoxide radical scavenging activity, with better reducing power than standard ascorbic acid	Rosa *et al.,* 2006 Basavraj, *et al.,* 2011

Activities	Plant name	Literature search	References
Hepato-protective activity	*C.O.*	Effective hepatoprotective activity was noted in CCl_4-intoxicated liver in albino rats. Histo enzymology indicate the decreased of LDL and HDL level	Rasu *et al.,* 2005 Lin *et al*., 2002
	T.E.	Ethyl acetate fraction of *T. erecta* shown significant hepatoprotective activity in carbontetrachloride induced hepatopathy model	Giri *et al.,* 2011
Antimicrobial activity	*C.O.*	Essential oil of flower reported to have potent inhibitor of gram positive and gram negative bacteria as well as fungal strains	Kubas *et al.,* 1972
	T.E.	Different fractions of flowers shown significant antibacterial, antimicrobial activity; volatile oil of the plant reported to possess fungi toxic activity	Patrick *et al.,* 2011 Rhama and Madhavan, 2011
Anticancer activity	*C.O.*	Compound isolated from the flower possess cytotoxic activity. Calenduloside was active against leukemia, colon cancer and melanoma cell lines. While faradiol and taraxasterol prevent the growth of tumor cell	Ukiya *et al.,* 2006 Medina *et al.,* 2006
	T.E.	Lutein was isolated from petals of *T. erecta* has shown cytotoxic effects against Hep2 cancer cell line. The maximum viability of Hep2 cell line was 8.88% which proved as significant cytotoxic effects	Ayyadurai *et al.,* 2013

(C.O = *Calendula officinalis,* T.E = *Tagetes erecta*)

Fig 1: *Calendula officinalis* L.

Fig 2: *Tagetes erecta* L.



(a) **(b)**

Fig 3: Fourier transform infrared absorption spectrum (a); *C. officinalis* and *T. erecta* (b)

composed of barrel shaped cells having tube like covering. Mesoderm consists of lower and upper layer of hexagonal cells with lignin deposition. Numerous oil glands are also seen. The microscopic characteristics of both species (Rizwani and Zahid, 2014) (Table 1).

2.3. *Phytochemistry*

The Fourier transform infra-red (FTIR) spectra of both Plants showed in Fig. 3. In case of *C. officinalis* the stretching frequency at 2920 and 2847 cm-1 showed the presence of alkanes. Whereas, 850 and 670 cm-1frequencies were due to the alkyl halide respectively. Similarly, in *Tagetes erecta* stretching vibrations at 2920 and 2850 cm^{-1} showed the presence of alkanes. While 1650 and 1250 cm^{-1} frequency was due to the primary amine and aromatic amines respectively (Rizwani and Zahid, 2014). Main groups of constituents that are present in *C. officinalis* and *T. erecta* has depicted in Table 2.

2.4. *Pharmacological Activities*

Literature reported various pharmacological activities on *C. officinalis* and *T. erecta* which are tabulated in Table 3.

2.5. *Toxicology*

Different doses of extract of calendula were administered in animal model to determine the toxic effect of the extract. The results from diverse studies illustrated that there is no sign of toxic effect and no pathological changes occurred after the acute or long term use (Elias *et al*., 1990; Iatsyno *et al*., 1979). Both aqueous and ethanolic fractions of flowers of *T. erecta* found to be safe during acute toxicity study. The study was in accordance to OECD Guidelines 425, Up and down procedure using albino Wistar rats (Shetty *et al.,* 2009; Manisha *et al.,* 2013). The LD_{50} value for chloroform fraction was found to be 8964.8 mg/kg body weight on Long Evan rats (Farjana *et al.,* 2009). The study carried out according to the *Lork D Method* of acute toxicity study. Seed and leaf extract of *T. erecta* shown LD_{50} value 357.43 µg/insect against the strain *Tribolium castaneum* (Islam and Talukder, 2005).

3. RESULTS AND DISCUSSION

Both *Calendula officinalis L.* and *Tagetes L. erecta*are botanically and morphologically different but they look like same at a glance and both of them are sold in the herbal market as adulterants to each other in

their dried states. *Calendula* and *Tagetes* are commonly used for decorative purposes also shares antibacterial, skin regenerative and anti-inflammatory properties. The topical application of the flower of *Calendula* used in conditions that are also indicated for *Tagetes species* leaf and flower preparation i.e. in pain relief and hyperkerotic lesions skin infection, ulceration and inflammatory conditions (Saify, 1988). In the light of above data gathered by microscopic, phytochemical, pharmacological and traditional utilizations both plants are almost identical in all aspects except some specifications i.e., Tagetes plant is used only tropically rather than orally because of the presence of thiophenes which acts as a poison for living creatures whereas, aroma of *Calendula* is more acceptable than *Tagetes* because of toxic compound which is not present in *Calendula.* Pharmacognostic investigation revealed that both plants are almost identical. Along with the apparently botanical diversion of their floral parts calendula has single pattern of petals while Ta Chemical features depicted the existence of similar chemical nature of compounds except thiophenes. From this comparison it emerges that these plants are employed in identical or similar kinds of ailments but topical application of tagetus is more suitable in this regard. Therefore, due to rich cultivation in Pakistan we can use *Tagetes erecta* externally in place of *Calendula officinalis* against various wound healing and other infectious diseases.

4. CONCLUSION

Despite the slight morphological, chemical and pharmacological differences, both *C. officinalis* L. and *T. erecta* L. shares a surprisingly wound healing activity.

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