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Editorial

No doubt Ayurveda is emerging so each one of us should be a part of this by supporting scientific documentation proving facts of power of Indian herbs. Like the theme of the New Year "Har Ghar Ayurveda" is a fact that India has but it should be in abroad too. How proud you will feel when someforeigner discusses power of Indian herbs with you and in reply you tell your experience and your work ona herb and its potentials.

Friends this year the seminar UJPAH board have chosen herbs of Cancer because of the papers reports indicate that during Covid period many patients went undiagnosed consequently Post Covid-19 impact haveincreased the numbers of patients increase in mortality rate in both Lung cancer patients and Non Lung cancer patients. Similarly a recent study report that the Covid - 19 infections of host cells is facilitated by Trans membrane make cancer patient vulnerable.

Good news is that a Global centre for Traditional Medicine of WHO will be setup in Gujrat. Other initiative such as "Heal in India and Heal by India", Heal in India will bring the world to India to seek its knowledge in Ayureveda, a seminar was held On 8th December 2022 in Goa,organised the 9th World Ayurveda Congress and Arogya Expo. In this Expo hundreds of Healthcare professionals and experts in traditional medicines participated from 53 countries and gained first-hand knowledge of Ayurveda.Uttarakhand is also fortunate to have an Ayurvedic University & UCOST to support the cause. This is our mission and I am grateful to the UJPAH board members to make this issue a memorable for science fraternity of the Uttarakhand and to all those scientist, research scholar, students and teachers who contributed for bringing out this issue and the people of science at large. Before concluding, I express my special gratitude to our chief guests, special and other guest present and the management who have provided all the guidance. In the end I thank to Dr. I P Saxena, Dr.Himmat Singh an non agenarian sharing their experience with us. Once again thanks to everyone. May almighty bless you.

Dr. S. Farooq
Chief Editor

Content

Protective role of *Withania somnifera* root extract on lipid peroxidation of Erythrocytes

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Abstract – *Withania Somnifera* (Ashwagandha) is an important medicinal plant that has been used in Ayurvedic and indigenous medicine. It is also known as Indian ginseng. It exerts antioxidant, anti-inflammatory and immune modulatory activities. In the present study, we studied the free radical scavenging activity of *Withania Somnifera* root extract. The radical scavenging effect of the extract was studied by its effect on hydroxy radicals generated by Fe-ascorbate-H_2O_2 system. Our results showed that *Withania Somnifera* root extract (1-10,000 µg/ ml) resulted in a significant dose dependent increase in percent inhibition of hydroxylation. Further, we studied the *in vitro* effect of *Withania Somnifera* root extract on Lipid peroxidation in terms of MDA level of human erythrocytes. Our results showed a significant dose dependent decrease in lipid peroxidation at a concentration of (1-1000 µg/ ml) as evident by decrease in level of MDA. Maximum effect was observed at 100-1000 µg/ ml. *Withania Somnifera* root extract thus can act as a potential antioxidant containing compound and protects erythrocytes from oxidative damage by either directly scavenging the free radical, sparing it or activating other enzymatic processes. At higher concentration (10,000 µg/ ml), *Withania Somnifera* extract increased the oxidative stress as evident from erythrocyte MDA level which became significantly more than the maximum effect shown at 1000 µg/ ml of extract.

Introduction

Changing lifestyle and environment conditions have predisposed common man towards numerous diseases. Nowadays the major threat for the survival among others include chemicals, toxic metals and the stress of modern living which are the major cause of various type of diseases. "One of the paradoxes of life on this planet is that molecules that sustain aerobic life i.e. oxygen, is not only fundamentally essential for energy metabolism and respiration, but it has been implicated as major cause in many disease and degenerative disorders. The importance of ROS and free radicals in pathogenesis of various diseased conditions has attracted increasing attention over the past decades.(Valko et al., 2007) ROS are generated in specific organelles of cells under normal physiological conditions and these can easily initiate the peroxidation of membrane lipid leading to the

accumulation of lipid peroxides (Farmer and Mueller, 2013). Antioxidant helps our body in protecting us from oxidative damage by neutralizing the free radicals directly or scavenging them through a series of reaction coupled with anti-oxidative enzymes (Baek and Lee, 2016). In addition to endogenous there are number of exogenous antioxidants that include lots of natural and synthetic antioxidants.

Overproduction of ROS results in oxidative stress. On a global scale free radical combined induced oxidative damage has claimed more lives than all of the wars and plaques thought-out human history. Of all deaths, nine out of ten people die from cancer, heart attack, rheumatic diseases, skin wrinkling, diabetes, alzheimer's disease, parkinson disease, AIDS and stroke and in these conditions, oxidative damage caused by free radicals has been implicated as one of the major causes (Rizvi and Zaid, 2001).

Oxidative damage generates reactive oxygen species (ROS) and reactive nitrogen species (RNS) which mainly include free radicals which are chemical entities possessing a single electron. Free radical can be formed either by losing an electron or accepting an electron. These free radicals are actually responsible for cellular damage and cause various diseases (ligouri et al., 2018). Reactive species include free radical such as superoxide anion radicals ($O_2^{-}\cdot$), hydrogen peroxide (H_2O_2), hydroxyl radical ($\cdot OH$) and hydroperoxyl radical ($\cdot HO_2$). These free radicals can influence each and every molecule of the body, such as DNA, proteins, lipids and carbohydrates (Rowe, et al., 2008 and Griffiths, et al., 2014).

The most important source of free radical production are the auto-oxidation of molecule such as hydroquinone, catechol and ferredoxin, the activity of enzyme such as xanthine oxidase, aldehyde oxidase and NADPH-cytochrome P_{450} reductase, the electron transfer reaction of the inner mitochondrial membrane and the metabolism of xenobiotics or drugs (Mohora, 2001). Our immune system also produces free radicals to destroy bacteria etc (Knight, 2000). Most often source of free radical is the environment to which our body is exposed such as air, tobacco smoke, radiation, toxic wastes and various chemicals (Aseervatham et al., 2013).

Nature did not leave us defenseless against this onslaught by free radicals. Antioxidants play a crucial role in preventing or delaying the damage caused by these oxidants. Antioxidants trap the lone pair of electron from free radicals and neutralize them. Thus various antioxidants are free radical scavenger and may be of enzymatic and non-enzymatic nature. This antioxidant can be any substance or constituent of molecule, synthetic or natural origin which delays or inhibits propagation of reactive species and prevent the oxidative damage of a target molecule (Zaid et al., 2007)

Our body actually has its own army of antioxidants and antioxidant enzyme which are able to neutralize free radicals and render them harmless. But due to increasing exposure of our body to high levels of these reactive species the administration of exogenous antioxidant as food constituent or therapeutic agents is beneficial. Most exogenous natural antioxidant comes from raw vegetable, fruits, spices, herbs and various medicinal plants and include molecule such as α-tocopherol, vitamin A, vitamin B, vitamin C and various polyphenolic compounds. These are known as natural antioxidant. In

addition to these there are synthetic antioxidants also like dimethyl sulphoxide, BHT, and BHA etc. Natural antioxidants are always appreciated over synthetic one, because they lack toxic side effects and show effect by multiple mechanisms. (Sharma et al., 2017)

Long-established systems of traditional medicine have evolved from systematic recordings of human experience over several millennia. Although not strictly based on concepts of modern science, they nevertheless are founded on a corpus of organized knowledge written in documents, and the evident conclusion is that the alleged "trial and error" methodology has provided useful drugs for humans. Medicinal plants have been gaining increasing importance over the past decades due to their increased use as a source of herbal drugs (Huang. et al., 1992).

Nature is the treasure house of unexplored medicinal plants. In search of effective antioxidant from natural source, we have studied the antioxidant property of *Withania Somnifera* on human erythrocyte *in vitro*. *Withania Somnifera* has been reported to have anti-inflammatory, anti-tumor, anti-stress, immuno modulatory properties, hemopoetic effect, rejuvenating effect on nervous system (Speed et al., 2021 and Dhar et al., 2015)

In the present study, we studied the free radical scavenging activity of Aqueous-alcoholic (50% alcohol and 50% distilled water) *Withania Somnifera* root extract. The radical scavenging effect of the extract was studied by its effect on hydroxyl radical generation by Fe-ascorbate-H_2O_2 system and its effect on lipid peroxidation (MDA levels) in red blood cell.

Material and method

Preparation of extracts

Dry powder of *Withania Somnifera* roots was used for extract formation *Withania Somnifera* extract was prepared in soxhlet assembly (Anand and Zaid 2020). In this extraction process the raw material was placed in a thimble made of cotton and inserted into a wide central tube of the soxhlet extractor. Aqueous-alcoholic (50% distilled water and 50% alcohol) extraction was done for 72 hours at temperature between 60°C to 80°C. After incubation the residual solvent was dried at room temperature in vacuum dedicators under reduced pressure. The dried extract was finally dissolved in DMSO and diluted to appropriate concentration by distilled water.

Effect of *Withania Somnifera* extract on Hydroxyl ($OH^{\cdot}$) radical generation

Hydroxyl radicals were generated by Fe^{3+}-ascorbate-H_2O_2 system (Rowley and Halli well, 1983). Formation of hydroxyl radicals appears to occur by the following reaction:

$$Fe^{3+}\text{- EDTA} + AH^{-} \longrightarrow Fe^{2+}\text{- EDTA} + A^{-}$$
$$Fe^{2+}\text{- EDTA} + H_2O_2 \rightarrow Fe^{3+}\text{-EDTA+ OH} + OH^{-}$$

$$AH + H_2O_2 \longrightarrow O_2 + {}^{\cdot}OH + OH^{-}$$

Hydroxyl radicals so formed were quantitated by their ability to attack aromatic compounds with the formation of hydroxylated product. In this study salicylate (2-hydroxy benzene) at a concentration of 2.5mM is included in the reaction mixture, and the hydroxylated product (2, 3-dihydroxy benzoate) is extracted with ether and assayed spectrophotometrically at 510 nm.

The reaction mixture of 2 ml contains 2.5mM salicylate, 0.3mM EDTA, 0.1mM $FeCl_3$ (fresh solution just before use), 2mM ascorbate (fresh), 150mM KH_2PO_4-KOH buffer (pH 7.4), and 10 µl of *Withania Somnifera* extract ratio (0-10,000 µg/ml final concentration), reaction was started by the addition of 1mM H_2O_2. The reaction mixture was incubated for 90 minutes at 37^0C. At the end of incubation, reaction was stopped by adding 80µl (11.6 N) HCl and 0.5g NaCl.

Hydroxylated product (2, 3-dihydroxy benzoate) was then extracted with 4 ml chilled diethyl ether, 3 ml of upper layer was taken and evaporated to dryness on sand bath. Residue so obtained was dissolved in 0.5 ml cold double distilled water. To this was added in order 0.25 ml 10 % (w/v) TCA dissolved in 0.5M HCl, 0.5 ml 10 % (w/v) sodium tungstate, and 0.5 ml 0.5 % (w/v) sodium nitrite (freshly prepared). Reaction mixture was made to stand at room temperature for 5 minutes and then added 1 ml 0.5M KOH and absorbance read at 510 nm and compared with the extinction coefficient of hydroxylated product (2, 3-dihydroxy benzoate).

Effect of *Withania Somnifera* extract on Lipid peroxidation (MDA) of erythrocytes

MDA is the most abundant individual aldehyde resulting from lipid peroxidation and its determination by TBA is the most common method of estimating lipid peroxidation. (Esterbauer and Cheeseman, 1990)

Isolation and treatment of erythrocytes with *Withania Somnifera* extract

Blood was washed 2-3 times with Krebs ringer phosphate buffer with 5mM glucose, pH-7.4 (KRPG). Packed erythrocytes were suspended in 4 volume of KRPG. Suspended erythrocytes were incubated with different concentrations of *Withania Somnifera* root extract (1-10,000 µg/ml) for 30 mints. After incubation erythrocytes were washed 2-3 times with KRP buffer and then packed RBC were obtained. Packed RBC was used for MDA estimation (Rizvi et al., 2007)

Estimation of MDA content of erythrocytes

Packed erythrocytes 0.2 ml was suspended in 3 ml of KRP buffer. To 1 ml lysate was added 1 ml of 10 % TCA (for ppt. of protein) and centrifuged for 5 minute at 1000g.1 ml of supernatant was added to 1 ml of 0.6 % TBA in 0.05 M/l of NaOH and boil for 20 minute at temperature greater than 90^0C. Cooled and absorbance taken at 532 nm and 630 nm as OD1 and OD2. For MDA level, final OD was calculated as OD2 – OD1. Results were calculated from the reading of known MDA standards (Rizvi et al., 2007)

Results and Discussion

Effect of *Withania Somnifera* root extract on Hydroxylation

The effect of different concentration (1-10,000 µg/ ml) of *Withania Somnifera* root extract on Hydroxyl radicals generated by Fe^{3+}-ascorbate-H_2O_2 system is shown in table-1. *Withania Somnifera* root extract(1-10,000 µg/ ml) showed a dose dependent decrease in hydroxyl radical generation as evident by a decrease generation of hydroxylated product. *Withania Somnifera* extract significantly inhibited formation of hydroxylated products generated by Fe-ascorbate-H_2O_2 system (Table-1) which may be due to blocked generation of OH radical primarily either by chelating Fe^{3+}

or by decomposing H_2O_2 in the reaction system and/ or secondary due to the scavenging of OH radicals (Gulcin, 2020).

Table-1 Effect of *Withania Somnifera* root extract on hydroxy radical generation by Fe^{3+}-ascorbate-H_2O_2 system. Each value is the mean of at least 5-6 independent experiment. Values are expressed as mean ± S.D.

Conc. of *Withania Somnifera* root extract. (µg/ml)	n mole of hydroxylated product	% inhibition of hydroxylation
0	92.3 ± 4.2	00
1	61.6 ± 3.8	33.26
10	55.2 ± 2.2	40.19
100	51.5 ± 2.9	44.74
1000	43.3 ± 3.0	50.08
10,000	40.4 ± 3.2	56.29

Effect of *Withania Somnifera* extract on Malondialdehyde (MDA) content of erythrocytes

The effect of different concentration (1-10,000 µg/ ml) of *Withania Somnifera* root extract on Malondialdehyde (MDA) content of erythrocytes is shown in figure-1.MDA is one of the end products in lipid peroxidation. *Withania Somnifera* root extract (10-1000 µg/ ml) showed a significant dose dependent decrease in MDA level of RBC as compared to normal control. Maximum effect was observed at a concentration of 1000 µg/ ml) of *Withania Somnifera* root extract.

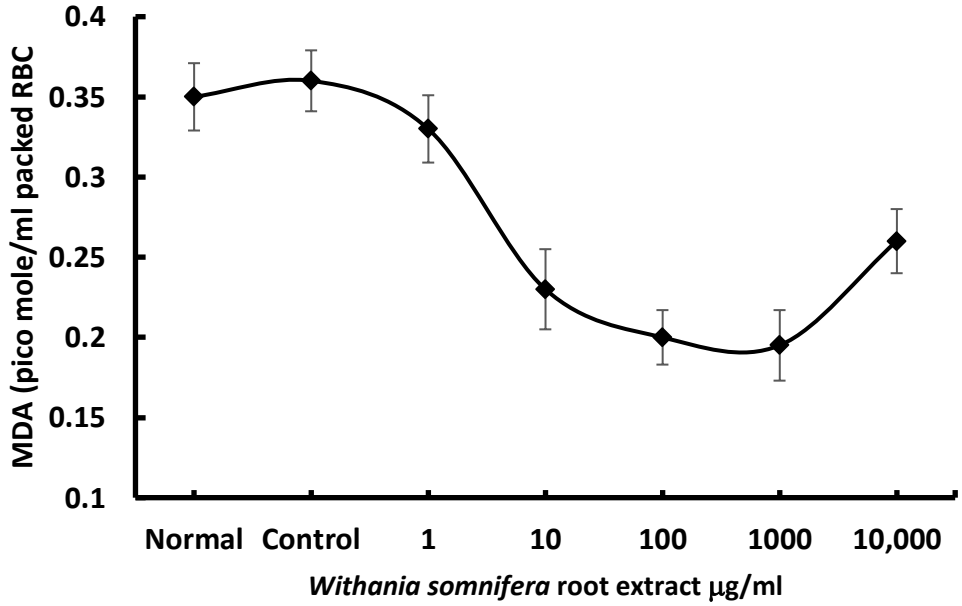

Figure-1 Effect of *Withania Somnifera* extract on MDA level of erythrocyte results are expressed in pico mole/ml of packed RBC. Each value is the mean of at least 5-6 independent experiment. Values are expressed as mean ± S.D.

Our results showed that erythrocyte MDA level decreases with increasing concentration of *Withania Somnifera* extract upto concentration of 1000 µg/ml but further increase in extract concentration 10,000 µg/ ml resulted in a significant increase in the MDA level as compared to the maximum effect shown at 1000 µg/ ml.

An increased MDA concentration of erythrocytes is indicator for prooxidant status in erythrocytes that can result in oxidative stress. Our results are similar with previous reports on MDA (Rizvi et

al., 2005). Erythrocytes can be highly susceptible to oxidative damage due to their high content of Polyunsaturated fatty acids present in their membranes along with high content of oxygen due to hemoglobin makes them prone to oxidative processes. An increase in MDA is known to cause a decrease in fluidity of membrane and can make erythrocytes fragile (Ozturk et al., 2003). Thus the oxidative process and accumulation of MDA can result in clinical conditions. Thus treatment of erythrocytes with *Withania Somnifera* extract may thus protect erythrocytes from stress induced oxidative damages by either directly scavenging the free radical or activating other processes. *Withania Somnifera* contains many antioxidant compounds including (Somniferine, Somnine, Withanaine, Somniferinine, Withanolides etc.) which are known antioxidants. (Khan et al., 2021). Our results also show that at higher *Withania Somnifera* concentration 10000 µg/ ml the extract may itself can cause generation of free radicals as high levels of antioxidants can itself cause generation of free radical (Iwasaki et al., 2014) Further studies are needed to elucidate its mechanism of action and its cytotoxic properties.

Conclusion

The present study was undertaken to evaluate the antioxidant effect of Withania Somnifera. Antioxidant and free radicals scavenging activities in extract of *Withania Somnifera* on RBC was studied *in-vitro*.

Our study shows:

1. *Withania Somnifera* extract shows dose dependent inhibition of hydroxylation (OH formation). This indicates that *Withania Somnifera* extract posses an effective antioxidant and hydroxyl radical scavenging properties.
2. A decrease in lipid peroxidation (MDA content) of erythrocyte was observed at *Withania Somnifera* extract concentration of 10-1000 µg/ml as compared to normal control.
3. Our result also showed that, much higher concentration of *Withania Somnifera* extract (10,000 µg/ml) started to show adverse effect on erythrocytes lipid peroxidation as evident from increase in MDA levels as compared to maximum effect shown at 1000 µg/ml.

From our study, we can conclude that the *Withania Somnifera* extract can act as a potential antioxidant and protect the erythrocytes from oxidative damage by either directly scavenging the free radical or activating other processes. This property of *Withania Somnifera* may be due to its antioxidant features because *Withania Somnifera* contains many antioxidant compounds including (Somniferine, Somnine, Withanaine, Somniferinine, Withanolides etc.) which are known antioxidants. But its indiscriminate use especially at higher concentrations should be avoided. As *Withania Somnifera* at higher concentration itself can cause generation of free radicals. Further studies are needed to elucidate its mechanism of action and its cytotoxic properties.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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Algal population growth and dynamics of Asan Wetland

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Abstract- A one year study of the phytoplankton community composition was carried out in the Asan wetland, a reservoir fed by River Yamuna in Uttarakhand India. In terms of bio-volume, phytoplankton community was generally dominated by Bacillariophyceae. Mean phytoplankton standing crops were highest in the wetland. The frequency and severity of algal blooms was increased significantly. To control their expansion, it was essential to identify the factors responsible for blooming of waters. Nutrient enrichment (mainly due to anthropogenic activities) and environmental factors (including the climate change) were considered the major catalyst for onset, proliferation and development of blooms. The phytoplankton of the Asan wetland was studied for one year with physical and chemical variables in relation to a pollution gradient. Analysis of the physical and chemical variables and phytoplankton density indicated that the wetland is experiencing heavy pressure of pollution due to anthropogenic activities. The dominant phytoplankton community mainly comprises of family, Chlorophyceae, Bacillariophyceae and Myxophyceae. Physical factors, though vital, had an indirect effect in facilitating the interaction among various available nutrients. In terms of phytoplankton density and diversity common genera observed include *Chlorella, Chlaymydomonas, Spirogyra, Ulothrix, Hydrodictyon, Cladophora, Cosmarium, Chlorococcum, Oedogonium, Microspora, Desmidium, Chara, Zygenema, Syndesmus, Volvox, Ceratoneis, Amphora, Caloneis, Fragilaria, Navicula, Synedra, Diatoms, Gomphonema, Pinnularia, Melosira, Tabellaria, Denticula, Cymbella, Cyclotella, Nostoc, Anabaena, Oscillatoria, Rivularia, Coccochloris and Phormidium.* Several genera were found most prominent during the study period having no seasonal impact on their abundance and variation. The spatial and temporal patterns observed in some of these dominant species were attributable to patterns in key environmental variables including temperature, flow, pH, dissolved oxygen and nutrient concentrations.

Keywords: Asan wetland, Algal blooms, Bacillariophyceae, Phytoplankton and Nutrient enrichment.

Introduction

Since last few decades, phytoplankton blooms and factors regulating their development have been a central theme in limnological research. In the last 20 years, the incidence of harmful algal blooms in surface waters due to increased nutrient loading mainly arising out of human activities (Anderson et al. 2002) has increased dramatically all over the world (Onderka 2007). Due to eutrophication, water become turbid, phytoplankton biomass increases, diversity of the community decreases and finally single or a few species dominate (Oliver and Gnaf 2000). The overwhelming growth of organisms known as 'blooms' harm ecosystems, fishery resources, human health, and al sore creational use of water through smothering of benthic habitats (Fouzia, *et al*. 2013). Cyanobacteria (blue-green algae), a group of oxygen producing photosynthetic prokaryotes are natural component of phytoplankton community, especially in tropical zones. They play a detrimental role in aquatic food web and are the most notorious bloom formers (Paerl et al. 2001). Under favorable growth conditions, cyanobacteria dominate the aquatic biota and cause blooming of water bodies, which adversely affect the domestic, industrial, and recreational use of water. In addition to producing odor and degrading the quality of water, cyanobacterial blooms produce a variety of toxins that cause hepatotoxicity, asthma, dermatitis, and eye irritation in humans and pose a serious risk to public health (Codd et al. 2005).

Factors responsible for algal bloom formation are yet to be established. A multitude of factors such as nutrients, light, temperature, pH, dissolved oxygen, buoyancy regulation, and stratification of algal species, selective grazing, viral infection, parasitism, and antibiosis interact in structuring phytoplankton community of water bodies (Paerl et al. 2001). In addition to the two well-known growth limiting nutrients, nitrogen and phosphorous, carbon plays a vital role in regulating the algal growth. During the course of bloom formation, a continuous release and biodegradation of organic matter takes place (Paerl et al. 2001).Carbon dioxide produced by bacterial oxidation is utilized by algae during photosynthesis, and O_2 is released. This way the cycle of nutrients operates and leads to eutrophication of water bodies. Competition for limiting nutrients is an important factor controlling the dynamics of phytoplankton community (diversity, abundance, and species succession; Chapra 1997). Species with high affinity for nutrients generally occupy oligo trophic waters, while low-affinity forms harbor eutrophics. The question, to what extent nutrients are involved in phytoplankton dominance of eutrophic water bodies, remains unresolved. In tropical regions, eutrophication of water bodies has become a menace due to conditions favorable for microbial growth. There is a need to identify the factors and processes involved in blooming of water bodies. The present study based on2 years data on the water chemistry and phytoplankton composition of Asan wetland (reservoir) attempts to evaluate the environmental factors that are responsible and favoured the algal growth. The present work also describes the dominant physical–chemical variables and their relation with phytoplankton density and community.

Material and Method

Study area

The Asan wetland is a small man-made wetland of ca. 4 sq km area, located 40 km west of Dehradun, in Doon valley on Dehradun-Paonta road. Geographically it is situated between latitude 30º 24'-30º 28' N and longitude 77º 40'-77º 44' E, near the confluence of the two perennial rivers, River Asan and Yamuna. The Asan wetland has a barrage which is 287.5 m long, the river bed being 389.4 m above sea level, with minimum and maximum water levels respectively at 402.4 m and 403.3 m asl. The other name of Asan wetland in Dehradun is Asan barrage or Asan lake or Dhalipur lake. It was created in 1967 as a result of the construction of Asan barrage at the confluence of the river Yamuna and Asan through Dhalipur power house. Asan wetland has all type of facilities and services that can entertain the visitors to their hearts content like water skiing, boating, rowing, kayaking, canoeing etc. It is the hub of numerous migratory birds and chooses this water resort as their home and dwell there for the whole winter season. The Asan wetland attracts 53 species of water birds of which 19 are winter migrants from Eurasia.

Figure- 1 Satellite view of Asan wetland

Figure-2 Asan wetland

Sampling strategy

The present study was carried out monthly during the year August 2011 to July 2012. Physico-chemical parameters viz. temperature, velocity, conductivity, total dissolved solids (TDS), pH, total alkalinity, total hardness, chloride, dissolved oxygen (DO), biochemical oxygen demand (BOD), phosphate, nitrate, sodium and potassium were analyzed by following the standard methodology of APHA (1998). For analysis and enumeration of phytoplankton, samples were collected with the help of plankton net of bolting silk no. 25 with a mesh size of 55µm attached with a collection tube at the base of net. For this a known volume (10 L) of water was filtered through the planktonic net and sample was collected inside the collection tube. The sample was then transferred in sterilized tubes of 250 ml capacity and preserved in 4%formaldehyde solution (APHA, 1998; Trivedi and Goel, 1986). The phytoplanktons were made identified following Alfred et al. (1973); Randhawa (1959); Vollen winds (1969) and Peat (1974). Phytoplankton data was also analyzed by statistical approaches like standard deviation (SD) and Pearson correlation coefficient (r).

Results and Discussion

Physico-chemical parameters

Monthly fluctuations in the values of different physico-chemical parameters in Asan wetland have been given in table -1.

Table1: Monthly variation in physico-chemical parameters of Asan wetland from 2011-2012

Month / Parameters	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	April	May	June	July	Avg.±S.D
Temperature (ºC)	21.0	20.0	19.0	18.0	17.0	15.0	17.0	19.0	21.0	22.0	22.0	20.0	**19.25±2.17**
Velocity (m/s)	0.57	0.53	0.49	0.38	0.35	0.33	0.36	0.34	0.36	0.43	0.55	0.48	**0.43±0.08**
Conductivity ($\mu mhocm^{-1}$)	0.125	0.137	0.132	0.128	0.138	0.141	0.137	0.139	0.155	0.162	0.149	0.135	**0.139±0.01**
TDS (mg/l)	500	300	200	200	200	100	200	200	300	300	200	400	**258.33±108.36**
pH	8.2	8.3	8.1	8.4	8.2	8.0	8.2	8.1	8.3	8.1	8.2	8.3	**8.2±0.11**
Total alkalinity (mg/l)	196.0	165.0	173.0	192.0	166.0	153.0	162.0	159.0	147.0	158.0	173.0	182.0	**168.33±15.05**
Total Hardness (mg/l)	92.0	95.0	110.0	105.0	94.0	85.0	92.0	84.0	93.0	105.0	98.0	115.0	**97.33±9.59**
Chloride (mg/l)	34.25	32.44	28.68	35.21	29.87	27.55	31.47	34.21	37.38	35.77	48.29	42.65	**34.81±5.90**
D.O (mg/l)	10.67	10.72	10.92	11.25	11.43	11.56	10.74	10.33	11.23	10.67	10.36	10.47	**10.86±0.41**
B.O.D (mg/l)	2.87	2.68	2.64	2.36	2.41	2.25	2.64	2.57	3.29	3.38	3.57	3.71	**2.86±0.49**
Phosphates (mg/l)	0.922	0.734	0.582	0.563	0.572	0.563	0.628	0.657	0.735	0.752	0.648	0.629	**0.66±0.10**
Nitrates (mg/l)	0.75	0.83	0.92	1.21	1.33	1.25	1.32	1.29	1.38	1.44	1.63	1.57	**1.243±0.277**

± S.D = Standard Deviation

During the present investigation temperature was recorded maximum in the months of May and June and minimum in the month of January. The average

temperature recorded in Asan wetland was 19.25±2.17 ^{0}C.The velocity was low throughout the year with average value of 0.43±0.08 m/s. Temperature, flow and standing water levels are the important environmental factors of marked variations that affect all the other environmental characteristics of freshwater ecosystems. Conductivity is an important physical quality parameter, which explains the ionic status of all waters and its measurement. This is mostly influenced by dissolved salts such as sodium chloride and potassium chloride. Relatively lower values of conductivity were recorded throughout the study period with an average value of 0.139±0.01 (µmhocm^{-1}). This was because of dilution effect of freshwater which the wetland received from river Yamuna. TDS of the wetland followed the similar trend as that of conductivity. These were maximum in months of summer and minimum in winter with an average value of 258.33±108.36 mg/l. This pattern of fluctuations in TDS is in conformity with those of Gurumayum et al. (2002). However, Rajurkar et al. (2003) have reported minimum values of TDS during post monsoon. However appreciable TDS values were observed during all the months indicating the mixing of pollutants from anthropogenic activities in and around the lake. The pH of water in Asan Lake in general showed an alkaline tendency during all the months with an average value of 8.2±0.11.Talling and Talling (1965) have reported that the pH value in lake water increased with increasing alkalinity. Alkalinity is a measure of buffering capacity of water and is important for aquatic life in a freshwater system because it equilibrates the pH changes that occur naturally as a result of photosynthetic activity of phytoplankton (Kaushik and Saksena, 1989).The total alkalinity of the Asan wetland was high throughout the study period. Maximum alkalinity was reported in the month of August and minimum total alkalinity was recorded in the month of April with an annual average of 168.33±15.05 mg/l. Monthly variations were quite significant. The increase in total alkalinity was the sign of anthropogenic impact on the Asan Lake. The higher total alkalinity may be also due to the decomposition of organic matter settled at the bottom.

The total hardness is defined as the sum of Ca and Mg concentrations, both expressed as $CaCO_3$ in mgL^{-1}. Carbonates and bicarbonates of Ca and Mg cause temporary hardness. Sulphates and chlorides cause permanent hardness. Hardness has got no adverse effect on human health. Water with hardness above 200 mg/l may cause scale deposition in the water distribution system and more soap consumption. During the present study total hardness of Asan wetland was low with an average value of 97.33±9.59 mg/l. Chloride in the natural waters occurs due to the pollution from sewage. The value of chloride in the present study indicated that the Asan wetland free from sewage as the concentration of chloride was low with an annual average value of 34.81±5.90 mg/l. The relatively low concentration of chloride in Asan wetland may be due to dilution effect of River Yamuna. The values of chloride observed in this study are not significant when compared to tolerable values posted by USEPA though chloride contents in water are not harmful.

Dissolved Oxygen (DO) is essential to all forms of aquatic life including the organisms that break down man-made

pollutants. Oxygen is soluble in water and the oxygen that is dissolved in water will equilibrate with the oxygen in atmosphere. Oxygen tends to be less soluble as temperature increases (Kannel et al. 2007). DO of Asan wetland was recorded maximum in the month of January and minimum in the month of March with mean value of 10.86±0.41 mg/l. There is no defined trend in variation of maximum value of DO in all the months. This indicated that the turbulences of water in Asan wetland, which may be beneficial for dissolved solid breakdown through self-pollution regulating mechanisms of fresh water system was found here (Hassan et al. 2009). The quality of the water in terms of DO content is always of primary importance, because at the waste discharge points, the DO is required for aerobic oxidation of the wastes. Also, Zeb et al., (2011) explained that DO levels are important in the natural self-purification capacity of fresh water ecosystems. A good level of DO indicated a high re-aeration rate and rapid aerobic oxidation of biological substances. The values obtained are well above the values recommended by USEPA, which indicate low level of anthropogenic activities within the study area. Other indirect laboratory test for assessing the DO is the Biological Oxygen Demand (BOD) which is the amount of oxygen required to biologically break down a contaminant. It is often used as a measurement of pollutants in natural and waste waters and to assess the strength of waste, such as sewage and industrial effluent waters (Zeb et al., 2011). BOD therefore is an important parameter of water indicating the health scenario of fresh water bodies (Bhatti and Latif, 2011). In the present study, BOD varied from 2.25 to 3.71 mg/l with the mean value of 2.86±0.49 mg/l. The lower BOD concentration confirmed non existence of point source pollution. Phosphate is the nutrient considered to be the critical limiting nutrient, causing eutrophication of fresh water systems (Rabalais, 2002). It is major nutrients that triggers eutrophication and required by algae in small quantities (Bandela, et al, 1999).P additions to landscape enter water via waste water effluents and soil erosions, and also from detergents. Therefore, Phosphate in large quantities in water is an indication of pollution through sewage and industrial waste. Higher Phosphate in bottom water may result from decomposition of organic matter and its release from sediments under the anoxic conditions. It also limits the growth of all the algal forms most often and hence, the Phosphate nutrient assessment of waters is crucial to the monitoring investigations of natural freshwater bodies. In instances where phosphate is a growth limiting nutrient, the change in its concentration can cause the stimulation or inhibition in the growth of photosynthetic aquatic micro and macro organisms such as phytoplankton and green bacteria (Droop, 1983 and APHA, 1998). In the present study the concentration of phosphate was consistently low with an average value of 0.66±0.10 mg/l. Nitrate levels over 10 mg/l in natural waters normally indicate man made pollution, but the measured values in this study were within the limit range. Man made sources of include, fertilizers, livestock, urban runoff, septic tanks and waste water discharges. As more land is converted into agricultural land and as urban areas expand, nitrate monitoring is an important tool in accessing locating and mitigating manmade sources of nitrate. Man made sources of phosphate in

the environment include domestic and industrial discharges, agricultural runoff where fertilizers are used and changes in land use in areas where phosphorous is naturally abundant in the soil. In general, phosphates are not very toxic to people or other living organisms. The values measured in this study were under limits with an average value of 1.243±0.277 mg/l. Nitrate and phosphate are important parameters of showing the pollution status and anthropogenic load in river water (Khan and Khan,1997).

Correlation between physico-chemical parameters

The Pearson correlation coefficient (r) calculated between physico-chemical parameters of Asan wetland are presented in table-2.

Table -2 Pearson correlation coefficient (r) between physico-chemical parameters of Asan wetland

	Temperature	Velocity	Conductivity	T.D.S	pH	Total Alkalinity	Total Hardness	Chloride	D.O	B.O.D	Phosphate	Nitrate
Temperature (°C)	1											
Velocity (m/s)	0.656*	1										
Conductivity (µmhocm^{-1})	0.402**	-0.203**	1									
TDS (mg/l)	0.626*	0.608*	-0.160**	1								
pH	0.259**	0.190**	-0.240**	0.372**	1							
Total alkalinity (mg/l)	0.165**	0.569**	-0.720*	0.486**	0.439**	1						
Total Hardness (mg/l)	0.348**	0.398**	-0.050**	0.286**	0.370**	0.449**	1					
Chloride (mg/l)	0.693*	0.440**	0.308**	0.318**	0.398**	0.233**	0.336**	1				
D.O (mg/l)	-0.640*	-0.545**	-0.050**	-0.410**	-0.020**	-0.222**	-0.183**	-0.593**	1			
B.O.D (mg/l)	0.798*	0.476**	0.500**	0.520**	0.212**	0.035**	0.484**	0.836*	-0.579**	1		
Phosphates (mg/l)	0.658*	0.535**	0.082**	0.814*	0.082**	0.183**	-0.159**	0.181**	-0.388**	0.358**	1	
Nitrates (mg/l)	0.095*	-0.339**	0.591**	-0.280**	0.015**	-0.315**	0.163**	0.598**	-0.133**	0.525**	-0.399**	1

Significant at P <0.001* and P <0.05**

The temperature showed positive correlation with all parameters whereas showed negative significant relation with dissolved oxygen (*r = -0.640, p < 0.001*). Velocity showed a negative correlation with conductivity, D.O and nitrates and positive correlation with all other parameters. Conductivity showed negative correlation with total alkalinity (*r = -0.720, p < 0.001*). TDS showed significant positive relation with phosphate (*r = 0.814, p > 0.001*). pH was positively correlated with all the parameters except D.O (*r = -0.020, p < 0.05*). Chloride showed positive correlation with BOD (*r = 0.836, p > 0.001*). DO was negatively

correlated with BOD, phosphate and nitrate where as BOD showed positive correlation with phosphate and nitrate.

Phytoplankton growth and dynamics

The most important and remarkable aspect of the present study was the phytoplankton growth and dynamics of Asan wetland and the results are presented in table-3.

Table-3 Monthly spatial qualitative and quantitative distribution of phytoplankton (Unit/l) in Asan wetland from 2011- 2012

Month / Phytoplankton	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July	Avg.± S.D
Chlorophyceae													
Volvox	8	10	14	17	21	17	12	8	11	15	13	10	**13.00±3.97**
Chlaymydomonas	12	16	13	16	22	18	12	6	14	24	18	15	**15.50±4.77**
Spirogyra	12	14	21	25	33	47	27	13	26	13	11	16	**21.50±10.77**
Ulothrix	5	15	24	27	35	52	33	27	34	42	28	17	**28.25±12.49**
Hydrodictyon	11	8	6	13	17	25	18	10	16	19	14	11	**14.00±5.30**
Cladophore	2	8	12	14	11	18	23	17	25	18	13	10	**14.25±6.41**
Cosmarium	7	5	13	11	16	23	11	8	6	12	7	3	**10.16±5.49**
Chlorococcum	4	7	13	16	12	27	18	4	12	18	13	8	**12.66±6.59**
Oedogonium	9	11	10	13	15	24	15	9	23	19	22	16	**15.50±5.43**
Microspora	10	13	17	21	22	25	13	11	16	23	15	9	**16.25±5.39**
Desmidium	18	21	25	19	20	38	32	21	35	27	16	12	**23.66±7.93**
Chara	11	9	12	15	21	18	13	6	14	11	10	7	**12.25±4.33**
Zygenema	9	13	16	22	25	32	25	12	26	18	11	10	**18.25±7.58**
Syndesmus	10	16	21	16	24	38	22	14	27	15	22	14	**19.91±7.54**
Chlorella	23	16	13	11	10	17	12	7	12	19	21	13	**14.50±4.75**
Total	**151**	**182**	**230**	**256**	**304**	**419**	**286**	**173**	**297**	**293**	**234**	**171**	**249.66±76.34**
Bacillariophyceae													
Ceratoneis	13	15	12	17	22	28	18	12	19	21	16	13	**17.16±4.80**
Amphora	13	10	8	21	26	33	23	15	24	17	22	16	**19.00±7.17**
Caloneis	8	12	15	18	23	38	24	12	27	18	21	12	**19.00±8.29**
Fragilaria	96	120	156	234	252	266	231	173	156	183	144	125	**178.0±55.78**
Navicula	76	125	138	153	115	184	132	126	135	127	114	85	**125.83±28.28**
Synedra	14	17	23	26	29	45	23	19	24	33	17	10	**23.33±9.37**
Diatoms	75	58	84	123	154	172	94	75	69	57	44	39	**87.00±42.18**
Gomphonema	24	33	37	45	51	66	43	28	36	47	32	17	**38.25±13.17**
Pinnularia	10	13	17	23	27	35	24	10	15	22	19	17	**19.33±7.32**
Melosira	9	15	22	14	26	22	16	8	14	18	12	7	**15.25±5.94**
Tabellaria	14	25	34	37	32	45	28	14	24	17	22	10	**25.16±10.54**
Denticula	13	17	24	26	31	37	23	8	19	15	25	16	**21.16±8.11**
Cymbella	14	21	25	32	26	38	26	15	11	16	16	12	**21.00±8.48**
Cyclotella	16	24	26	21	24	28	14	6	8	13	25	11	**18.00±7.57**
Total	**395**	**505**	**621**	**790**	**838**	**1037**	**719**	**521**	**581**	**604**	**529**	**390**	**627.50±189.74**
Myxophyceae													
Nostoc	8	13	24	22	27	33	23	14	24	16	11	10	**18.75±7.80**
Anabaena	9	11	16	24	28	35	24	9	0	13	16	0	**15.41±10.75**
Oscillatoria	10	13	16	21	11	17	11	7	13	19	23	12	**14.41±4.81**
Rivularia	16	23	22	19	20	27	15	10	26	17	14	12	**18.41±5.38**
Coccochloris	7	13	15	19	23	26	12	5	18	23	11	15	**15.58±6.48**
Phormidium	6	10	21	26	24	32	16	7	6	17	13	10	**15.66±8.56**
Total	**56**	**83**	**114**	**131**	**133**	**170**	**101**	**52**	**87**	**105**	**88**	**59**	**98.25±35.23**

± S.D = Standard Deviation

Phytoplankton community structure and species type indicate some of the crucial environmental developments in aquatic systems. Pollution may be measured by either chemical or biological means but the role of a bio-indicator has been well established in aquatic ecosystems. The phytoplankton on which whole of aquatic life depends directly or indirectly are governed by a number of physical, chemical and biological conditions, their interactions, and tolerance of organisms to variations of these conditions (Cronberg, 1999). During the present study monthly results, annual average of the total density of different families of phytoplankton and density of all the species of phytoplankton in Asan wetland was derived. The fluctuations in phytoplankton density were significant during the pre-monsoon, monsoon and post monsoon. The phytoplankton density and diversity was recorded maximum in winter, moderate in summer and minimum in monsoon period. Venkateshwarlu and Menon, (1979) recorded maximum values of phytoplankton in winter and minimum during rainy season. Three families of phytoplankton which include *Chlorophyceae, Bacillariophyceae* and *Myxophyceae*were observed during the study period. Among three families the number of individuals were found maximum in family Bacillariophyceae (627.50±189.74 Unit/L) followed by Chlorophyceae (249.66±76.34 Unit/L) and minimum in Myxophyceae (98.25±35.23 Unit/L).The family Bacillariophyceae was reported with maximum density at and was dominating among the three different families following the trend *Bacillariophyceae>Chlorophyceae>Myxophyceae*.The population dynamics of the phytoplankton is influenced by the climatic conditions as well as the physico-chemical characteristics. A marked difference in the composition and in the abundance of various algal groups was observed during the present study.

The Phytoplankton diversity observed in Asan wetland comprised of 35 genera out of which Chlorophyceae constitute (15 genera), Bacillariophyceae (14 genera) and Myxophyceae (6 genera) (table 3). The qualitative study of phytoplankton revealed that the family Chlorophyceae was represented by *Chlorella, Chlaymydomonas, Spirogyra, Ulothrix, Hydrodictyon, Cladophora, Cosmarium, Chlorococcum, Oedogonium, Microspora, Desmidium, Chara, Zygenema, Syndesmus, and Volvox.* The family Bacillariophyceae was represented by *Ceratoneis, Amphora, Caloneis, Fragilaria, Navicula, Synedra, Diatoms, Gomphonema, Pinnularia, Melosira, Tabellaria, Denticula, Cymbella, and Cyclotella.* The family Myxophyceae was represented by *Nostoc, Anabaena, Oscillatoria, Rivularia, Coccochloris and Phormidium.* Monthly and seasonal were quite evident in phytoplankton species during the study period. The fluctuations in diversity of species may be attributed to climatic and seasonal changes and month wise fluctuations give much more explanation of the ecological tendencies of phytoplankton community in the wetland. The results also revealed that in case of family Chlorophyceae *Ulothrix* was found with maximum number(28.25±12.49 Unit/L)and Cosmarium was found minimum in number (10.16±5.49 Unit/L).Whereas in case of family Bacillariophyceae *Fragilaria* was dominating with maximum number (178.0±55.78 Unit/L) and *Ceratoneis*was

recorded with the minimum number (17.16±4.80 Unit/L). The family Myxophyceae was mostly dominated by *Nostoc* (18.75±7.80 Unit/L) and *Oscillatoria* was reported with lowest number (14.41±4.81 Unit/L). It is well known that a combination of physical, chemical and biological factors determine the distribution of the Bacillariophyceae in Rivers (Fabricus, et al., 2003, Fouzia Ishaq and Amir Khan, 2013). Diversity of phytoplankton is an indication of purity and the use of community structure to assess pollution is conditioned by four assumptions: the natural community will evolve towards greater species complexity which eventually stabilizes; this process increases the functional complexity of the system; complex communities are more stable than simple communities, and pollution stress simplifies a complex community by eliminating the more sensitive species (Cairns, 1974).

Physico-chemical parameters played a major significant role in phytoplankton density. During the present study (table 4) temperature, velocity, TDS, pH, total alkalinity, total hardness, chloride, BOD and phosphate registered negative correlation with total phytoplankton density and conductivity DO and nitrate showed highly significant positive correlation with phytoplankton density.

Table -4 Pearson correlation coefficient (r) between physico-chemical parameters and phytoplankton density of Asan wetland

	Temperature	Velocity	Conductivity	T.D.S	pH	Total Alkalinity	Total Hardness	Chloride	D.O	B.O.D	Phosphate	Nitrate
Chlorophyceae	-0.563	-0.645	0.411	-0.659	-0.393	-0.577	-0.277	-0.383	0.754	-0.326	-0.473	0.305
Bacillariophyceae	-0.811	-0.692	0.038	-0.785	-0.324	-0.351	-0.318	-0.573	0.814	-0.672	-0.65	0.101
Myxophyceae	-0.671	-0.48	0.068	-0.707	-0.274	-0.249	-0.077	-0.51	0.827	-0.55	-0.622	0.051

Development and persistence of dense algal blooms in a wide range of water bodies pose serious problems for water quality management. For the restoration and management of water bodies, it is crucial to identify the factors that ultimately control the algal growth (On derka 2007). Seasonal growth of phytoplankton is strongly influenced by environmental factors and nutritional status of water bodies where these factors show distinct variation with seasons (Paerl et al., 2001). In this study, a close analysis of the physico-chemical parameters indicated relatively decreased availability of phosphate which might be the reason for less algal blooms in the wetland affecting the process of cell division resulting in reduced population. Small amount of nitrate recorded throughout the sampling period indicated that the water body was less influenced by pollution. High concentration of nitrate is usually considered a criterion of pollution (Fernández-Argüelles et al., 2004). It is produced predominantly by bacterial

reduction and partly by oxidation of ammonium. Chloride is an indicator of poor water quality. Its concentration in water body increases with imperviousness of surrounding surfaces (surfaces that prohibit the movement of water from the land surface into the underlying soil). During urbanization, a lot of porous natural lands are being converted into buildings, pavements, roads, parking lots, drive ways, sidewalks, etc. Hindrance in the water's ability to penetrate into the underlying ground increases surface run off, which carry road salts into the receiving water body. Inorganic chloride salts are toxic, and high levels adversely affect the quality of receiving waters as well as stress aquatic organisms (Amirsalari and Li 2007). Almost nothing is known about the impact of chloride on phytoplankton growth and development.

Physical factors such as water temperature and pH indirectly affect the availability of various nutrient sand dynamics of phytoplankton composition (Paerl et al., 2001). Fluctuations in pH affect the availability of carbon. An increase in pH raises carbonate concentration in water, while molecular CO_2 and bicarbonate decrease. At high pH, CO_2 becomes limiting, and species tolerant to low CO_2 thrive (Goldman and Shapiro 1973; Chen and Durbin 1994). High temperature (>20°C) and alkalinity favors algal blooms, whereas low temperature and acidic pH supports the growth of eukaryotic algae (Paerl et al.2001).Temperature of the wetland ranged between 15 to 22 °C. Seasonal fluctuation in water temperature affects N2 fixation, nitrification, solubility of oxygen and carbonate, and availability of light, as a consequence productivity and community dynamics. In the present study, water temperature appeared to be relatively less important. This is probably due to the climatic location of the sampling area where variation in water temperature is small compared to that of temperate freshwater bodies. Algal blooms, generally absent during winter months in temperate regions, are dominant in tropical water bodies and persist throughout the year (Oliver and Gnaf 2000).In eutrophic water bodies, besides nutrients, factors such as irradiance, water column stability, structure of phycosphere, and interaction with macro flora and fauna regulate the development, proliferation, and maintenance of the blooms. Microbial associations occur during all stages of the bloom development. Intensity and specificity of microbial epiphytism may vary dramatically during a seasonal bloom cycle (Oliver and Gnaf 2000; Paerl et al. 2001). With less nutrient concentration and high DO concentration Asan wetland is an oligo trophic fresh water wetland. In such systems, interaction among nutrients and environmental factors is key feature in regulating the growth and development of algal blooms (Fouzia Ishaq and Amir Khan, 2013). In oligo trophic waters, availability of inorganic nutrients in utilizable form (PO_4, NH_4^+, NO_3^-*etc.*) is more important than that of aged water systems, where dissolved organic forms and their recycling by microbes hold the key. Cabrican and Valiela (1999) reported that timing and magnitude of bloom varied between close locations and even within the same location.

Conclusion

Several genera with no seasonal impact on their abundance and variation were most prevalent during the research period. The

temporal and spatial patterns shown in some of these dominant species were linked to variations in important environmental factors like temperature, flow, pH, dissolved oxygen, and nutrient concentrations.

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Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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Solubility enhancement of antiprotozoal agent by solid dispersion and herbal tablet method to improve its rate of dissolution

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Abstract-Drug dissolution is the rate limiting step for bioavailability of poor aqueous soluble drug that consequently affects the in vivo drug absorption. In the present study solid dispersion based drug delivery system of Atovaquone were successfully developed in the form of tablets with improved dissolution characteristic by forming solid dispersion with PEG 4000. For the Atovaquone formulation, F1 was chosen as it has % drug release about 46 % in 180 min. FTIR spectra of drug with other excipients have not shown any interaction and also selected formulation was stable after stability studies. The in-vitro dissolution studies revealed a considerable boost in dissolution rate of Solid dispersions of Atovaquone in contrast to pure drug. From FTIR spectroscopy, it was concluded that there was no well-defined chemical interaction between Atovaquone and PEG 4000 in Solid dispersions, as no important new peaks could be observed. The Atovaquone solid dispersion based tablet (F1) showed 30.33% drug release within first 20 min. and 46.45% drug release within 180 min.Thus from studies, it could be concluded that solid dispersion of poor aqueous soluble Atovaquone by solvent evaporation technique were effectively formulate during PEG-4000 and PVP-K 30 hydrophilic polymers. Thus, the statement can be given that the rate of dissolution and solubility of poor aqueous soluble Atovaquon can be appreciably improved by solid dispersion by use of water soluble carriers by solvent evaporation technique.

Keywords: Bioavailability; Dissolution Enhancement; In-Vitro Evaluation; Solid Dispersion; Atovaquone.

Introduction

Solid dispersion refers to a group of solid products consisting at least two components, generally a hydrophilic matrix and a hydrophobic drug. The matrix can be either crystalline amorphous. The drug can be dispersed molecularly in amorphous particles (cluster) or in crystalline particles (Chion and Riegelman, 1971). Chiou and Rigelman in their classic review, defined these system as the dispersion of one or more active ingredients in an inert carrier matrix at solid state prepared by the melting (fusion), solvent or melting-solvent method (Craig, 2002).

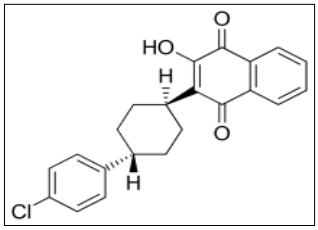
The concept of solid dispersion was originally proposed by Sekiguchi and Obi, who investigated the generation and dissolution performance of eutectic melts of sulfonamide drug and a water soluble carrier in early 1960s (Sekiguichi and Obi, 1961). Solid dispersion represents a useful pharmaceutical technique for increasing the dissolution, absorption and therapeutic efficacy of drugs in dosages forms. They may be also called as solid state dispersions as first used by Mayersohn and Gibaldi or as co-precipitates.

Oral bioavailability of drugs depends on its solubility and/or dissolution rate, therefore major problems associated with these drugs was its very low solubility in biological fluids, which results into poor bioavailability after oral administration (Chawla & Bansal, 2008; Pathak *et al.*, 2008; Chiou & Riegel man, 1971). A drug with poor aqueous solubility will typically exhibit dissolution rate limited absorption, and a drug with poor membrane permeability will typically exhibit permeation rate limited absorption (Vasconcelos *et al.*, 2007). Therefore, pharmaceutical researchers, focuses on two areas for improving the oral bioavailability of drugs include: (i) enhancing solubility and dissolution rate of poorly water-soluble drugs and (ii) enhancing permeability of poorly permeable drugs (Serajuddin *et al.*, 1990). It has been estimated that 40% of new chemical entities currently being discovered are poorly water soluble (Hao *et al.*, 2008; Liu, 2008). Unfortunately, many of these potential drugs are abandoned in the early stages of development due to the solubility problems. It is therefore important to realize the solubility problems of these drugs and methods for overcoming the solubility limitations are identified and applied commercially so that potential therapeutic benefits of these active Solid dispersion (SD) is one of such methods and involves a dispersion of one or more active ingredients in an inert carrier or matrix in solid state prepared by melting, dissolution in solvent or melting-solvent method (Dabbagh & Taghipour 2007; Sekiguchi & Obi 1961). The formulation of drugs having low aqueous solubility using solid dispersion technology has been an active area of research since 1960 (Goldberg *et al.*, 1966). Among the various approaches to improve solubility, the solid dispersion (SD) technique has often proved to be the most successful in improving the dissolution and bioavailability of poorly soluble drugs because it is simple, economic, and advantageous (Kaur & Grant 1980). Solid dispersion means a group of solid products consisting of at least two different components, generally a hydrophilic inert carrier or matrix and a hydrophobic drug. The carrier can be either crystalline or amorphous in nature. Most commonly used carriers for the preparation of SDs are different grade of polyethylene glycols (PEGs) and polyvinyl pyrollidine (PVPs), sugar etc.

Material and Method

Drug Profile

Selected Drug: Atovaquone



Molecular formula: $C_{22}H_{19}ClO_3$

Molecular weight: 366.8 g/mol

Chemical name: 3-[4-(4-chlorophenyl) cyclohexyl]-4-hydroxynaphthalene-1, 2-dione
Synonym: Mepron
Melting point: 216-219 °C
Solubility: Practically insoluble

Material and Chemical

Chemicals required for this research work were Atovaquone purchased from Matrix Lab Hyderabad India, Polyethylene glycol (PEG 4000), Polyvunyl Pyrrolidone, Talc, Magnesium stearate, Ethyl cellulose, Micro crystalline cellulose and Hydrochloric acid were purchased from S D fine Chemical Mumbai.

Preformulaion study

Preformulation may be described as a phase of the research and development process where the formulation scientist characterizes the physical, chemical and mechanical properties of new drug substances, in order to develop stable, safe and effective dosage forms.

Organoleptic Properties

Appearance

Transferred approximately 2 gm of the sample on a white paper spreaded uniformly and examined visually.

Colour

A small quantity of pure drug powder was taken in a butter paper and viewed in well illuminated place.

Solubility

Aqueous solubility is an important physicochemical property of drug substance, which determines its systemic absorption and in turns its therapeutic efficacy. Solubility of Atovaquone was determined in water and methanol, ethanol, chloroform and ethyl acetate

Table -1 Solubility Specifications

Descriptive terms	Approximate volume of solvent in millilitres per gram of solute
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly soluble	From 30 to 100
Slightly soluble	From 100 to 1000
Very slightly soluble	From 1000 to 10,000
Practically insoluble	More than 10,000

Melting point determination

Melting point of Atovaquone was determined by Open capillary method.

Determination of partition coefficient

25 mg of Atovaquone with aqueous phase and n-octanol was taken in three separating funnels. The separating funnels were shaken for 2 hrs in a wrist action shaker for equilibration. Two phases were separated and the amount of the drug in aqueous phase was analyzed spectrophotometrically.

Determination of λ max

A solution of Atovaquone containing the concentration 10 µg/ ml was prepared in 0.1 N HCL and UV spectrum was taken using Shimadzu (UV-1800) double beam spectrophotometer. The solution was scanned in the range of 200 – 400 nm.

Drug – Excipient Interaction Studies by FTIR

Infra-red spectra matching approach was used for the detection of any possible chemical reaction between the drug and

the excipients. A physical mixture (1:1) of drug and excipients was prepared and mixed with suitable quantity of potassium bromide. About 100 mg of this mixture was compressed to form a transparent pellet using a hydraulic press at 10 tones pressure. It was scanned from 4000 to 150 cm-1 in a shimadzu FTIR spectro-photometer. The IR spectrum of the physical mixture was compared with those of pure drug and excipients and matching was done to detect any appearance or disappearance of peaks.

Preparation of standard calibration curve of Atovaquone in 0.1N HCl

100 mg of Atovaquone was accurately weighted into 100 ml volumetric flask, dissolved in 0.1N HCL and volume was made up with 0.1N HCL. Pipette 1ml of this solution into another 10 ml volumetric flask and the volume was made with 0.1N HCl and marked as Stock. From this Atovaquone standard stock solution (1000µg/ml), 1ml solution was diluted to 10 ml using 0.1N HCl solution to get concentrations of 100 µg/ml. from this solution, aliquots of, 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1.0 ml, 1.2 ml and 1.4 ml from standard drug solution were diluted to 10 ml with 0.1M. The absorbance of these solutions was measured at 286 nm 0.1N HCL as a blank.

Preparation of Solid Dispersion

Atovaquone solid dispersion were prepared by using hydrophilic carriers like polyethylene glycol (PEG6000) and Polyvinyl Pyrrolidone (PVK 90) in proportions viz 1:1 (drug: carrier) (50mg:50mg), 1:2 (50mg: 100mg) and 1:1:1 (drug: carrier 1:carrier 2) (50mg: 50mg: 50mg) and (50mg: 100mg: 100 mg) were prepared by solvent evaporation method. Atovaquone and carriers were dissolved in methanol and mixed with magnetic stirring. Solvent was evaporated at reduced pressure at 40^{o}C in a rotatory evaporation apparatus. Subsequently solid dispersion was stored under vacuum over silica gel for 12 hrs. at room temperature. After drying the solid dispersion was passed through a 250µm sieve. Sample was stored in a desiccator and used for further investigation (Leuner & Dressman 2000).

Table-2 Composition of Different Solid Dispersion Preparations

Solid Dispersion (Code)	Atovaquone(mg)	PEG-4000 (mg)	PVP K90 (mg)	Ethanol (ml)
SDF1	50	50	--	30
SDF2	50	100	--	30
SDF3	50	--	50	30
SDF4	50	--	100	30
SDF5	50	50	50	30
SDF6	50	100	100	30

PVP – Poly Vinyl Pyrrolidine (K 90), PEG – Polyethylene Glycol

Evaluation of Solid Dispersion

The prepared formulations of solid dispersions were evaluated for the following Physico-chemical character-ization, *In-vitro* dissolution studies and

Compatibility study

Fourier transform infrared spectroscopy was employed to characterize the possible interactions between the Atovaquone and carriers. In this study pure drug, solid dispersions were studied by FTIR

spectrophotometer.

Drug content estimation

Solid dispersions equivalent to 10 mg of Atovaquone were weighed accurately and dissolved in the 10 ml of methanol. The solution was filtered, diluted suitably and drug content was analyzed by UV spectrophotometer. The Actual Drug Content was calculated using the following equation as follows

$$\% \textbf{ Drug Content} = \left(\frac{\textbf{Mact.}}{\textbf{Mss}}\right) \times \textbf{100}$$

Percentage Practical Yield

Percentage practical yield was calculated to know about percent yield or efficiency of any method, thus its help in selection of appropriate method of production. Solid dispersions were collected and weighed to determine practical yield (PY) from the following equation.

$$\textbf{Practical Yield } (\%) = \left(\frac{\textbf{Practical Mass (Solid Dispersion)}}{\textbf{Theoratical Mass (Drug + Carrier)}}\right) \times \textbf{100}$$

Determination of Solubility of Atovaquone Solid Dispersion

Drug solubility studies were performed in triplicate by adding excess amount of Atovaquone and solid dispersion to methanol and 0.1 N HCl pH (1.2). Solutions containing flasks were kept on a rotary Shaking Incubator for 24 hrs. After 24hrs, solutions were analyzed using UV spectrophotometer.

Preparation of Solid Dispersion Based Formulation

Compressed tablets containing selected solid dispersed product with surfactants were prepared separately by direct compression method. Diluent lactose, dry binder polyvinyl pyrrolidone, and lubricants talc, magnesium stearate were used as excipients. All ingredients were sieved through 40 meshes and blended with lubricants and compressed in single station machine with flat punches.

Table- 3 Formulation of Solid Dispersion Based Tablets

Formulation Code	F1	F2	F3
SD - Formulation	--	SDF2	SDF6
Atovaquone	60	--	--
Poloxamer 407	30	--	--
Lactose	132	34.5	
PVP	5	5	5
Mg stearate	1	1	1
Talc	2	2	2
Total weight	230	230	230

Evaluation of Solid Dispersion Based Tablet Formulation (Pre-Compression)

Bulk density

It is a ratio of mass of powder to bulk volume. The bulk density depends on particle size distribution, shape and cohesiveness of particles. It is expressed in gm/ml and is given by the formula:

Bulk density=M/Vo

Where,

M = mass of the powder

Vo = bulk volume of the powder

Angle of repose (θ)

It is defined as the maximum angle possible between the surface of the pile of the powder and the horizontal plane. Fixed

funnel method was used. The angle of repose was then calculated using following equation:

Angle of repose Ø = tan-1(h/r)

Where, h=height of the pile

r = radius of the pile

Table-4 Flow properties and corresponding angle of repose

Flow property	Angle of repose
Excellent	25-30
Good	31-35
Fair	36-40
Passaple	41-45
Poor	46-55
Very poor	56-65
Very very poor	>66

Tapped density

Ten gram of powder was introduced into a clean, dry 100 ml measuring cylinder. The cylinder was then tapped 100 times from a constant height and the tapped volume was read. It is expressed in gm/ml and is given by:

Tapped density=M/Vt

Where, M = mass of the powder ,

Vt = final tapping volume of the powder

Compressibility index (Carr's index)

Compressibility index is used as an important parameter to determine the flow behavior of the powder. Carr's index can be represented by Equation:

Comprassibility Index

$$= \left(\frac{\mathbf{TD - BD}}{\mathbf{TD}}\right) \times \mathbf{100}$$

Hausner's ratio

Hausner's ratio is used to predict the flow ability of the powders. This method is similar tocompressibility index. Hausner's ratio can be represented by Equation:

$$\text{Hausner'Ratio} = \frac{\text{Tapped Density}}{\text{Bulk Density}}$$

Table-5 Scale of flow ability

Flow character	Compressibility index (%)	Hausner's ratio
Excellent	<10	1.00 – 1.11
Good	11 – 15	1.12 – 1.18
Fair	16 – 20	1.19 – 1.25
Passable	21 – 25	1.26 – 1.34
Poor	26 – 31	1.35 – 1.45
Very poor	32 – 37	1.46 – 1.59
Extremely poor	>38	>1.60

Evaluation of Solid Dispersion Based Tablet Formulation (Post-Compression)

The formulated tablets were evaluated for the following physicochemical characteristics:

General Appearance

The formulated tablets were assessed for its general appearance and observations were made for shape, color, texture and odor.

Weight variation

Randomly selected twenty tablets were weighed individually and together in a single pan balance. The average weight was noted and standard deviation calculated. The tablets pass the test if not more than two tablets fall outside the percentage limit and none of the tablet differs by more than double percentage limit.

PD = [(W_{avg} – $W_{initial}$) / (W_{avg})] x 100
Where,
PD = Percentage deviation
W_{avg} = Average weight of tablet
$W_{initial}$ = Individual weight of tablet

Thickness

The thickness and diameter of tablets was determined using Vernier Caliper. Twenty tablets from each batch were used and average values were calculated.

Hardness

The Monsanto hardness tester was used to determine the tablet hardness. The tablet was held between affixed and moving jaw. Scale was adjusted to zero; load was gradually increased until the tablet fractured. The value of the load at that point gives a measure of the hardness of the tablet. It is expressed in kg/cm2.

Drug content

Tablets were crushed and the powder equivalent to 100mg of drug were accurately weighed and transferred to 50 ml volumetric flask. To this flask, sufficient amount of distilled water was added to dissolve the tablets completely. Then, the volume of flask was made up to the mark with same solvent. From this solution, 1ml of the sample was pipette out and transferred to 10 ml volumetric flask. The volume in the second flask was made up to the mark with distilled water. From this 0.6ml, 0.8ml and 1ml samples were withdrawn and volume was made up to 10ml to maintain concentration within the beer's range. This final diluted solution was estimated UV spectrophotometrically (Goldberg, 1966).

Friability

Twenty tablets samples were weighed accurately and placed in friabilitor (Roche Friabilitor). After the given specification (4 min at 25 rpm), loose dust was removed from the tablets. Finally tablets were weighed. The loss in weight indicates the ability of the tablets to withstand this type of wear. The % friability was then calculated by:

% Friability = (Loss in weight / Initial weight) × 100

In-vitro Dissolution study

In-vitro dissolution studies of the prepared formulations were performed using USP type II (Paddle) apparatus with paddle rotating at 50 rpm in 900ml of 0.1 N HCl at 37 ± 0.5ºC. At fixed time intervals, 5ml samples were withdrawn, filtered and replaced with fresh dissolution media. Concentration of Atovaquone in each sample was determined by UV spectrophotometer.

Result and Discussion

Description

The colour, odour, nature and taste of the API were evaluated and were found to be as per the monograph.

Description of Atovaquone

Atovaquone was dark yellow color and order less.

Solubility study

Solubility study of Atovaquone is reported in table-6.

Table-6 Solubility Study of Atovaquone

S.No.	Solvent	Solubility
1.	Water	+ - - -
2.	0.1N HCl	+ - - -
3.	Mathanol	++++
4.	Ethanol	++++

Melting point determination

The melting point of Atovaquone was found to be 220.5° C.

Determination of λ max

Solution was scanned under UV-Vis Spectrophotometer and λ max was determined. It was found to be as per the monograph.

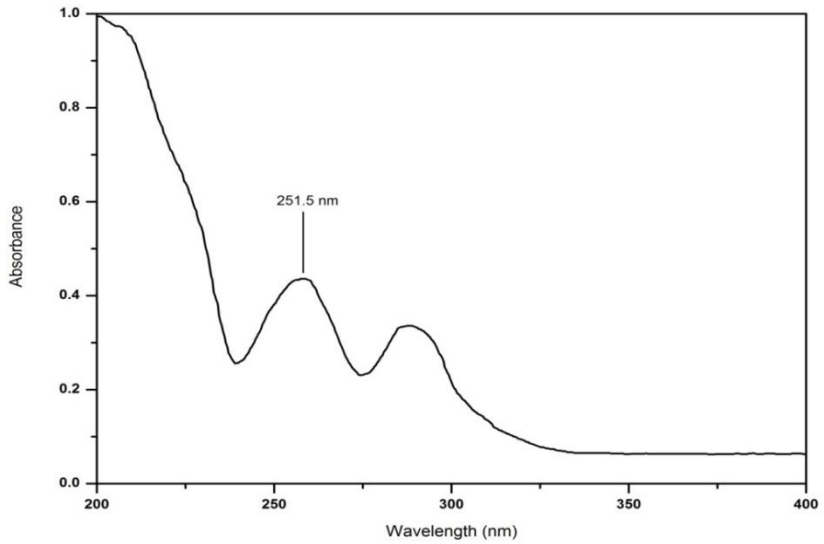


Figure-1 UV spectra of Atovaquone

Wavelength

Wavelength of maximum absorption of Atovaquone in 0.1N HCL was found to be 251.5 nm

Partition coefficient

Partition coefficient of Atovaquone inn-octanol was found to be 0.23

Table-7 Standard calibration Curve data of Atovaquone in 0.1N HCL

S. No	Concentration(µg/ml)	Absorbance at λ max 251.5 nm
1	0	0
2	2	0.184
3	4	0.425
4	6	0.678
5	8	0.896
6	10	1.163

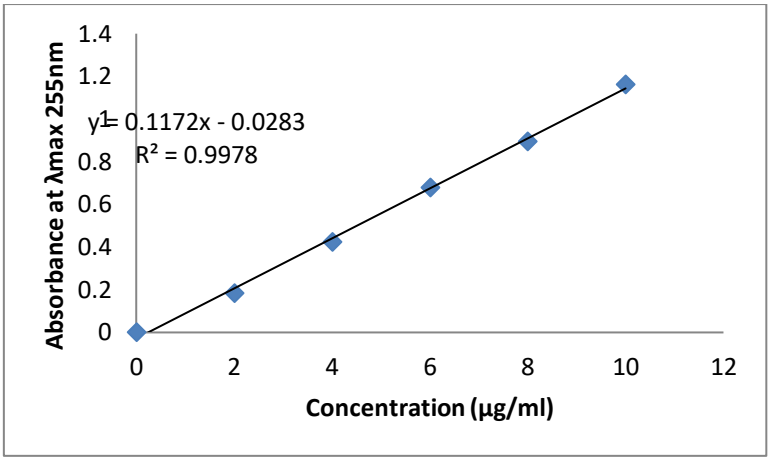


Figure-2 Standard calibration curve of Atovaquone in 0.1N HCL

Evaluation of Solid Dispersion

FTIR study

The FTIR spectra of pure Atovaquone, carriers and solid dispersion of drug with carrier are shown in Tables and Figures. (Figure 3-4 and Table 8)

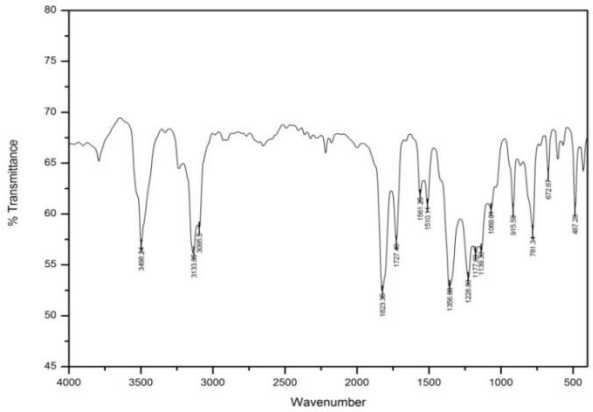


Figure-3 Standard FTIR spectra of Atovaquone

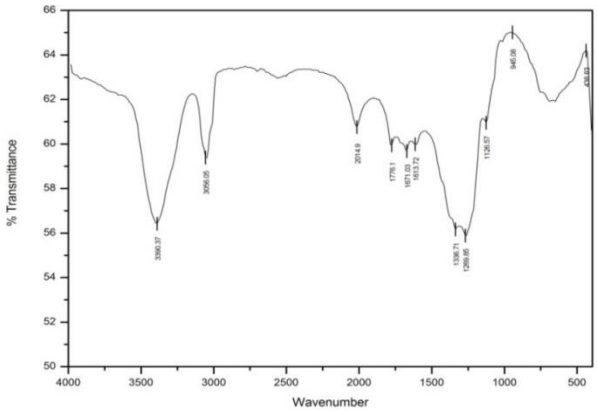


Figure-4 Standard FTIR spectra of PVP K 90

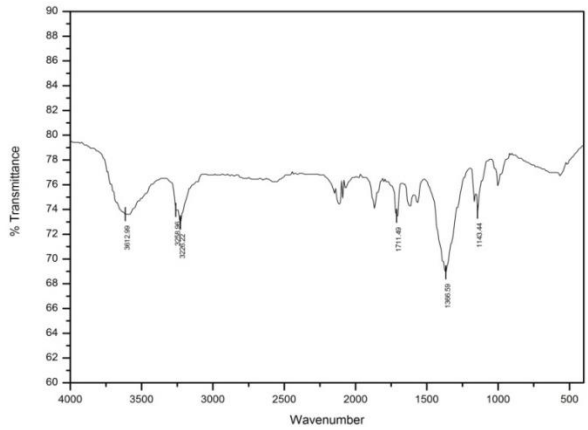


Figure-5 Standard FTIR spectra of PEG 4000

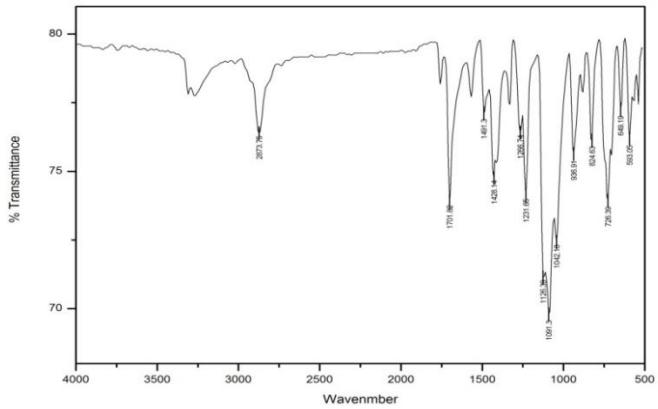


Figure6: Standard FTIR spectra of Solid Dispersion (SD-2)

Table-8 FTIR Spectral Data of Atovaquone, PV K90, PEG 4000 and Solid Dispersion Formulation.

S.No.	IR Spectrum	Peaks(cm-1)	Groups	Stretching /Deformation
1	**Atovaquone**	3483.99	O-H (Alcohol)	Stretching
		1823.24	C=O (Aromatic)	Stretching
		781.36	C-Cl (Alkyl halide)	Stretching
		3095.42	C-H (Aromatic)	Stretching
2	**PVP K90**	3463.99	O-H(Alcohol)	Stretching
		1674.24	C=C(Alkene)	Stretching
		1062.36	C-F(Alkyl halide)	Stretching
		573.42	C-Br(Alkyl halide)	Stretching
3	**PEG4000**	3446.54	O-H(Alcohol)	Stretching
		2889.37	C-H(Alkane)	Stretching
		1640.10	C=O(Amide)	Stretching
		542.79	C-BR (Alkyl halide)	Stretching
4	**Solid Dispersion ()**	3270.38	O-H (Alcohol)	Stretching
		1711.50	C=o (Carbonyl)	Stretching
		1138.58	C-F(Alkyl halide)	Stretching
		744.83	C-Cl(Alkyl halide)	Stretching

Drug Content

Drug content uniformity of Atovaquone solid dispersion in all the formulations (SD1 to SD 6) was shown from 92.56±1.25 to 98.16±1.53 respectively. As shown in Table-9 and Figure-7.

Table-9 Drug content, % Practical Yield and Solubility of Atovaquone in Solid Dispersion formulations

Formulation code	Drug content (in %)	% Practical Yield	Solubility (µg/ml)
SD-(Drug)	-	-	4.84±1.15
SD-1	95.83±2.14	82.17±2.53	22.45±2.09
SD-2	96.28±2.42	84.56±1.19	45.86±2.53
SD-3	92.56±1.25	75.86±1.23	8.53±1.21
SD-4	94.72±1.43	78.54±1.52	12.34±2.34
SD-5	98.16±1.53	81.92±2.53	19.34±3.09
SD-6	96.27±2.20	80.50±1.19	33.95±1.83

* All the values are expressed as mean ± SD; n=3

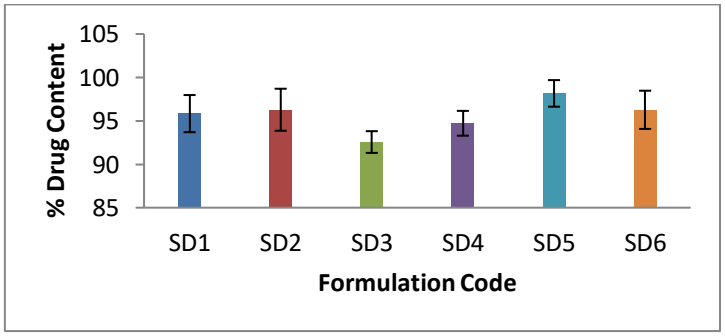


Figure-7 % Drug content of Atovaquone in Solid Dispersion formulations

% Practical Yield and Solidity Estimation

% Practical Yield of Atovaquone solid dispersion in all the formulations (SD1 to SD 6) was shown n Table 6.7 and Figure 6.8. Higher practical yield was found for formulation SD-2 and lowest for SD-3. Solidity study was also performed and results show good improvement in solubility enhancement and was found higher for formulation SD-2. Table 9 and Figure 8 & 9.

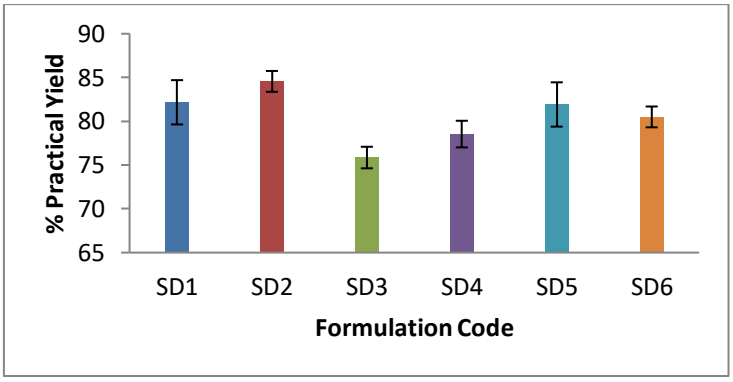


Figure-8 % Practical Yield of Atovaquone in Solid Dispersion formulations

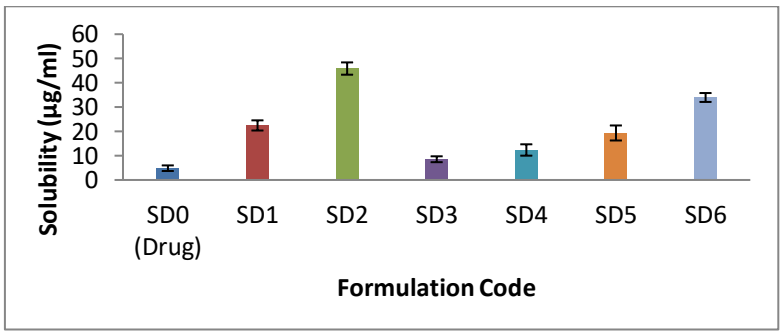


Figure-9 Solubility of Atovaquone in all Solid Dispersion formulations

Pre-Compression Study of Atovaquone Solid Dispersion Formulations

Precompression studies of powdered blend were performed on parameters like bulk density, tapped density, Carr's index, Hausner's ratio and angle of repose as shown in the table below. Angle of repose was found to be 26.09±2.17, 26.51±2.65, and 26.71±3.09. Bulk density 0.53±0.10, 0.52±0.11 and 0.53±0.07g/cm3, tapped density 0.63±0.08, 0.59±0.07 and 0.59±0.05g/cm3, Hausner's ratio 1.07±0.11, 1.09±0.15 and 1.09±0.10,Carrs index 12.58±1.64, 12.32±1.35 and 12.51±1.47were found for F1, F2 and F3 solid dispersion formulations respectively and reported in Table 10 and Figure 10-11.

Table-10 Pre Compression Study of Atovaquone Solid Dispersion Formulations

Formulation Code	Angle of repose (o)	Bulk density (g/ml)	Tapped bulk density (g/ml)	Hausner ratio	Carr's index (%)
F1	26.09±2.17	0.53±0.10	0.63±0.08	1.07±0.11	12.58±1.64
F2	26.51±2.65	0.52±0.11	0.59±0.07	1.09±0.15	12.32±1.35
F3	26.71±3.09	0.53±0.07	0.59±0.05	1.09±0.10	12.51±1.47

* All the values are expressed as mean ± SD; n=3

Post-Compression Study of Atovaquone Solid Dispersion Formulations

The formulated tablets were evaluated for their organoleptic characters. The tablets are round in shape and white in colour. All the tablets showed elegance in appearance. The hardness of the tablets was measured by Monsonto hardness tester. The hardness of all the formulations was found to be in the range of 7.12 to 7.45 kg/cm2. It indicates all the tablets have adequate mechanical strength. Twenty tablets of each formulation were selected for weight variation test. The accepted percentage deviation was ±7.5 for 130-324mg weight tablets. It was within the I.P. limit and all the tablets passed the weight variation test. Friability test was carried out by Roche friabilitor. The maximum weight loss should be not more than 1%. All thetablets passed the friability test. All formulations were exhibited good drug content. Table11 and Figure 12-15.

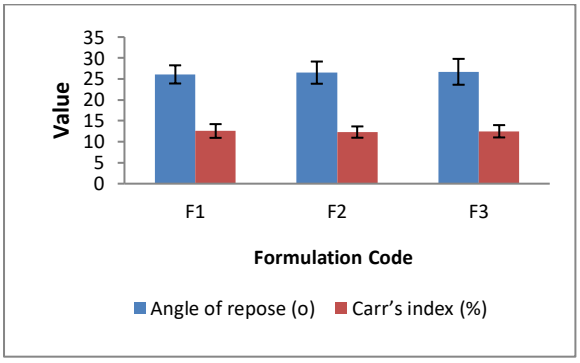


Figure-10 Pre Compression Study of Atovaquone Solid Dispersion Formulation (Angle of repose (⁰) and Carr's index (%))

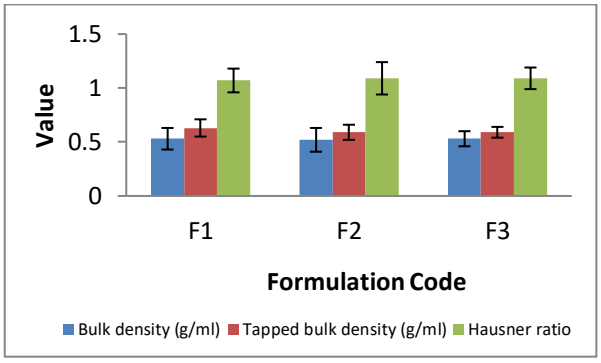


Figure-11 Pre Compression Study of Atovaquone Solid Dispersion Formulations (Bulk density (gm/mL), Tapped density (gm/mL) and Hausner's ratio)

Table-11 Post Compression Study of Atovaquone Solid Dispersion Formulations

Formulation Code	Average Weight	Hardness (kg/cm2)	Friability	% Drug content
F1	351.77±1.26	7.12±0.22	0.53±0.014	99.64±1.27
F2	348.65±1.69	7.35±0.28	0.55±0.018	99.25±1.45
F3	352.12±1.38	7.45±0.14	0.54±0.012	98.76±1.36

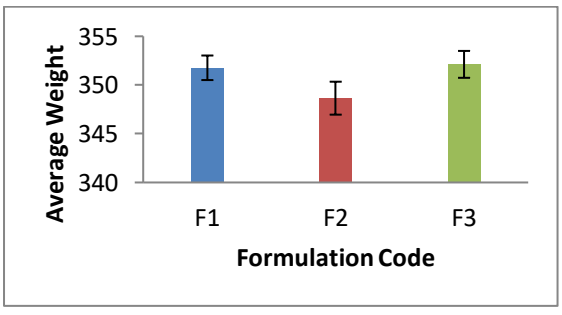


Figure-12 Average Weight of Designed Solid Dispersion Formulations

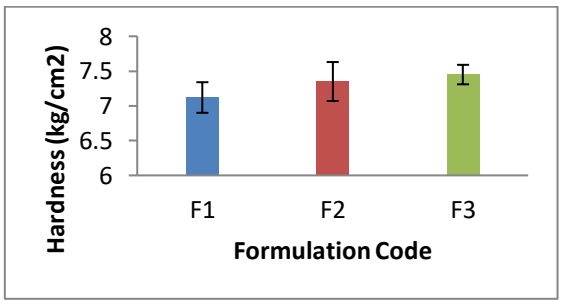


Figure-13 Hardness of Solid Dispersion Formulations

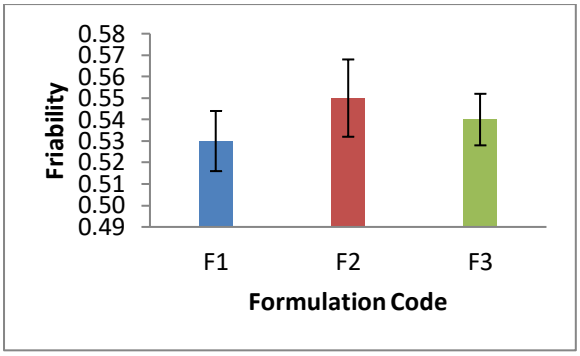


Figure-14 Friability of Solid Dispersion Formulations

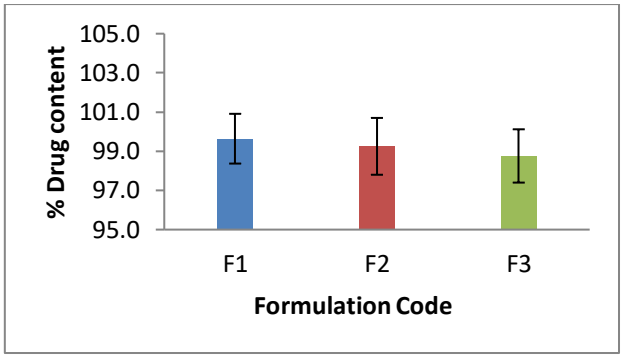


Figure-15 % Drug Content of Solid Dispersion Formulations

In- Vitro Drug Release Study of Atovaquone Solid Dispersion Formulations

In-vitro drug release studies were done for optimized formulations. The drug release was found to show maximum in case of F1 with 46.45% in 180min..as shown in table and figure.

Table-12 % Drug Release Studies of Solid Dispersion Formulations and Marketed Preparations of Atovaquone (Marketed Suspension-Mepron, Tablet-Malarone)

S.No.	Time (Min.)	F1	F2	F3	Marketed Suspension (Mepron)	Marketed Tablet (Malarone)
1.	0	--	--	--	--	--
2.	10	12.04±0.11	7.46±1.54	4.27±2.55	4.11±2.15	4.17±0.11
3.	20	15.95±1.55	12.68±3.55	11.36±2.23	6.25±3.52	8.66±2.23
4.	30	21.67±1.45	14.95±2.23	13.81±1.25	8.98±2.23	10.81±1.25
5.	40	30.33±2.78	16.67±1.45	14.09±1.56	10.47±1.56	12.16±3.55
6.	50	36.19±1.65	18.32±2.53	17.46±3.45	12.42±0.11	13.49±1.54
7.	80	38.49±3.51	20.43±1.22	19.64±1.21	14.96±1.53	15.66±3.55
8.	120	39.47±2.25	23.12±2.55	22.26±1.54	15.81±2.07	17.28±1.61
9.	150	42.13±1.55	26.97±1.45	23.23±2.61	17.43±3.49	19.25±1.52
10.	180	46.45±1.23	28.85±1.56	24.71±2.52	19.13±1.23	21.74±1.47

* All the values are expressed as mean ± SD; n=3

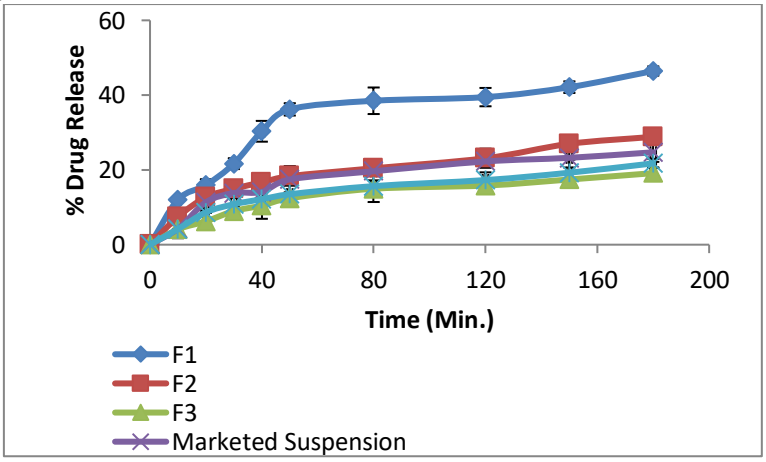


Figure-16 % Drug Release Studies of Solid Dispersion Formulations and Marketed Preparations of Atovaquone(Marketed Suspension-Mepron, Tablet-Malarone)

Conclusion

Thus from studies, it could be concluded that solid dispersion of poor aqueous soluble Atovaquone by solvent evaporation technique were effectively formulated using PEG-4000 and PVP-K 30 hydrophilic polymers. thus, the statement can be given that the rate of dissolution and solubility of poor aqueous soluble Atovaquone can be appreciably improved by solid

dispersion by use of water soluble carriers by solvent evaporation technique. The solubility and dissolution rate of Atovaquone can be enhanced by formulating Solid dispersions of Atovaquone with PEG 4000. The solubilisation effect of PEG 4000, reduction of particle aggregation of the drug, formation of microcrystalline or amorphous drug, increased wet ability and dispersibility, and alteration of the surface properties of the drug particles might be responsible for the enhanced solubility and dissolution rate of Atovaquone from its Solid dispersion. The results showed that the formulation satisfied the objective of enhancement of dissolution, ease of administration and safety. Success of the present study recommends a detailed investigation in to in-vivo studies for its effective use in clinical practice.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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Determination of carbohydrate levels in fruits by UV-Visible Spectrophotometer

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Abstract- Fruits are very nutrient-dense and incredibly healthy. A rapid method was developed for the quantitative estimation of carbohydrates present in the different natural green leafy vegetables by UV-Visible Spectrophotometer. A study was carried out to determine the soluble carbohydrate content of some selected fruit which include apple, banana, custard apple, guava, papaya, grapes, pineapple, orange, pomegranate, kiwi fruit, mango, strawberries. Soluble carbohydrate was determined by Anthrone method Spectrophotometry at wavelength of 750 nm[1]. For most people, between 40% and 60% of total calories should come from carbohydrates, preferably from complex carbohydrates (starches) and naturally occurring sugars[2].

This study was aimed at evaluating the soluble carbohydrate content of some selected fruits consumed locally with a view to determine whether they meet the dietary requirement of consumers. It is envisaged that the findings of the investigation would provide additional information on the nutritional status of the fruits.

Key words: Carbohydrates, UV-Visible Spectrophotometer, Wavelength, Anthrone

Introduction

Carbohydrates are compounds made up of carbon hydrogen and oxygen, thus they are regarded as hydrates of carbon represented as C (H_2O). Carbohydrates act as the primary source of energy which is converted into glucose to generate energy essential for metabolism in every cell of the body[1]andare of special importance as they constitute more than 50% of the dry weight of most fruits. Though there is no absolute requirement of carbohydrates, they are essential to ensure that energy is available to the body to perform its normal functions. Carbohydrates perform numerous roles in living things. Polysaccharides serve for the storage of energy (e.g., starch and glycogen), and as structural components (e.g., cellulose in plants and chitin in arthropods)[3-7]. The 5-carbon monosaccharide ribose is an important component of coenzymes (e.g., ATP, FAD, and NAD) and the backbone of the genetic molecule known as RNA. The related deoxyribose is a component of DNA[2]. Saccharides and their derivatives include many other impor-

tant biomolecules that play key roles in the immune system, fertilization, preventing pathogenesis, blood clotting, and development.

In food science and in many informal contexts, the term carbohydrate often means any food that is particularly rich in the complex carbohydrate starch (such as cereals, bread, and pasta) or simple carbohydrates, such as sugar (found in candy, jams, and desserts) For most people, between 40% and 60% of total calories should come from carbohydrates, preferably from complex carbohydrates (starches) and naturally occurring sugars[6]. Complex carbohydrates provide calories, vitamins, minerals, and fiber. Carbohydrates formula is $C_{12}H_{22}O_{11}$.

Material and Methods

Instrumentation- Ultraviolet visible spectroscopy refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions. Ultraviolet-visible spectroscopy was recorded on ELICO SL-160, India.

Sample preparation- Apple, banana, custard apple, guava, papaya, grapes, pineapple, orange, pomegranate, kiwi fruit, mango, strawberries were taken as samples. 5 gm of each sample was extracted separately in 25ml of distilled water[5].

Chemicals and Reagents- Anthrone, Sulphuric acid were purchased from Merck Specialties pvt. Ltd.

Reagent Preparation- 0.2gm of anthrone was weighed accurately and dissolved in conc^{n} sulphuric acid and make up the volume up to 100ml and finally transferred it in to a 100ml of reagent bottle[7].

Procedure- Pipette out the 1ml of each extracted sample in to a 25ml of volumetric flask and add 2ml of freshly prepared anthrone reagent in each volumetric flask and finally make up the volume up to the mark with distilled water. Reference was prepared by taking 2ml of anthrone reagent in a 25ml of volumetric flask and make up the volume up to the mark with distilled water. To the above prepared samples wavelength was check in UV visible spectrophotometry and wavelength was set at 750nm at that wavelength the developed colour absorbances were noted for the above mentioned samples[7].

Results and Discussion

From the experiment in apple 1.255%, banana 1.35%, custard apple 1.26%, guava 0.55%, papaya 0.685%, grapes 0.79%, pineapple 1.015, orange 0.77%, pomegranate 1.3%, kiwi fruit 0.6%, mango 1.75%, strawberries 0.4% of carbohydrates are evaluated. The recommendation for the general population is that carbohydrate should supply 50 to 55 percent of total calories, and 130 grams per day (520 calories per day) for male and female adults and for athletes is between 55 and 65 percent of total calories.

Table -1

S.No	Name of Fruit	Carbohydrate content (%)
1	Apple	1.386
2	Custard apple	1.33
3	Banana	1.48
4	Guava	0.45
5	Papaya	0.745
6	Grapes	0.889
7	Pineapple	1.095
8	Orange	0.86
9	Pomegranate	1.64
10	Kiwi fruit	0.65
11	Mango	1.88
12	Strawberries	0.48

Conclusion

From the Mango we gain high calories of carbohydrates of total calories to increase the activity levels in the body.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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Ayurveda and its concept of dietetics (*Pathya ahaar kalpana*) – a potential approach to healthy gut Microbiota

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Abstract - The present article deals with the general history of Ayurveda and its concept of dietetics for healthy microbiota. Ayurveda is an ancient science of life which has been practiced in India for the past thousands of years. In addition to the treatment, it provides knowledge about healthy lifestyles through yoga and diet regimens. In Ayurveda, the essence to live a healthy life is through strengthening the tripods of life which are *aahar, nidra* and *brahmacharya*. The *aahar* on the diet not only provides the material needed by our body to grow and maintain its day-to- day function but also individual conditions *mana*. Therefore, there is a need to consider food types and food regimens according to *dosha* and *prakriti* to enrich healthy microbiota which further will enrich a healthy body and mind.

Keywords: Ayurveda, Gut microbiota, Aahar, Lifestyle, Yoga, Balanced diet

Introduction

AYUSH is an acronym for Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homeopathy and are the six Indian systems of medicine prevalent and practiced in India and some of the neighbouring Asian countries with very few exceptions in some of the developed countries. Ayurveda or the science of life is more than 5000 years old (Pandey et al., 2013). Ayurveda had its origin right from the day of creation as it was remembered by Lord Brahma. He is considered the first preceptor of Ayurveda who later on passed the knowledge to his son Daksha Prajapati and in his turn to Lord Ashwini Kumar as who were considered eternal physicians. They in turn passed on the knowledge to Lord Indra the king of Gods. It was Lord Indra who is believed to have taught the same to Sage Bharadwaja who in turn spread the science of Ayurveda on earth by preaching and teaching it to many other sages. This period can be again bifurcated into two periods namely the Vedic and the Samhita Period (Mahesh, 2019).

The Vedic Period was the time when the Vedic thoughts, concepts and practices were composed. The *Samhita* period dedicated to Ayurveda texts namely the *Charaka Samhita*, *Sushruta Samhita*, *Astanga Hridaya*, *Astanga Sangraha* and *Bhela Samhita* got composed and was put into practice. The Aṣṭang Hridaya was originally written in Sanskrit by the ancient Indian scholar Vagbhata. It is a part of the Brhat Trayi and is one of the principal texts of Ayurveda (Vagbhata, 1939). Ashtang

Ayurveda refers to the main eight sections of Ayurveda (Fig. 1). Sushruta, Charaka, and Vagbhata are considered to be "The Trinity" of Ayurvedic knowledge.

Figure- 1 Eight major branches of Ayurveda (Ashtang Ayurveda)

Teaching, learning and practice of Ayurveda mainly take place through sages, Gurukul and father to son. Later there were seats of higher institutionalized learning like the Nalanda, Banaras and Taxashila. The fall of the ancient period marked great influence with the destruction of the universities of Nalanda by Islamic invaders resulting in the migration of scholars. The period from 500 AD to 1500 AD is known for the destruction by the Persian-Afghan invasion followed by the Islamic invasion**..** Initially during the British rule in India, the modern education system that dealt more with western philosophy resulted in the opening of the Modern system of medicine in India as well. But the indigenous system of education was still in vogue and supported by the British initially which was suspended in 1835. The British era saw a rise in the modern education system along with the opening of the people to international levels of interaction and understanding.

The revival of Ayurveda started from the year 1885 to 1947 for its legitimate right and position and more intensely from 1920 to 1930. People like G. Srinivasa Murti, M. M. Gananath Sen of Bengal, Jivaram Kalidas Shastri of Gondal, A Lakshmipathi of Madras, etc were the proponents of this revival who got engaged in a reasoned analysis of the pros and cons contained in different systems of medicine (Ganesan, 2010).

Madan Mohan Malaviya knows that Ayurveda is an ancient medical science based on fundamental natural principles of living a healthy long life. To make it

practically useful in the present era, the integration of current scientific inputs is essential**.** He created an environment, eminent graduates and licentiates, in European medicine and Surgery were employed to give instruction and training to the students of Ayurveda and to help the Vaidyas in preparing works in Sanskrit and Indian vernaculars, on Anatomy, Physiology, Surgery, and Hygiene and other sciences auxiliary to the Ayurveda (Dar and Somaskhandan,1966).

Ayurvedic concept of dietetics

In today's scenario, malnutrition is one of the leading causes of the development of diseases in man. The word malnutrition not only means deficiency of some nutrients but also the excess of them. Today we might have curbed the effect of communicable diseases by the use of antibiotics but we have also passed the way for lifestyle disorders in our society. Marasmus, kwashiorkor, diabetes, obesity, hypertension, cardiovascular disorder, and many more have set up roots in our society all of which have strong concordance with improper diet and lifestyle. Ayurveda set a strong emphasis on the diet and its effect on the body. In Ayurveda, the food in our diet possesses its action through five routes namely *rasa, guna, veerya, vipaka* and *prabhava* (Payyappallimana and Venkatasubramanian, 2016).

Rasa (taste) - All the food materials in the world possess 6 basic tastes through which they exert their action in the body as soon as we eat them (Fig. 2). These are *madhur* (sweet), *amla* (sour), *lavana* (salty), *katu* (pungent), *tikta* (bitter) and *kasay* (astrigent); for example giloy has *tikta ras* (bitter taste). On the basis of their physical properties, the food materials are categorized into *khadya, peet*, *leh* and *leedha*. *Khadya* is the food items that we chew, *peet* are those that we drink, *leedha* and *leh* are paste-like that we suck.

Figure- 2 Selected examples of six *rasa* (taste)

Guna (quality) - *Guna* is an important property of food because of its pharmacological action. Each food item has its own *Guna* like some food items are having anti-inflammatory action such as haldi, some are cardio-protective such as arjuna, some are expectorant such as mulethi and many more.

Veerya (potency) - *Veerya* is the potency of food, for example, black pepper has *ushna veerya* (hot potency). It absorbs after the action of digestive juices.

Vipaka (active principle) - *Vipaka* is the active principle which reaches the blood after getting metabolized in the liver. The taste conversion after digestion is also called *vipaka* for example Jaggery has *madhur vipaka* after digestion.

Prabhava (effect) - Prabhava is the effect of food item. A food shows specific prabhava for example Rudraksha is
an anti-hypertensive, Brahmi is a brain tonic (Medhya). Few examples of herbs (foods) and their properties are shown in Table 1.

Table-1 Different foods and their properties in terms of Ayurveda

Herb/ food	Rasa	Veerya	Vipaka	Prabhava
Guggulu	Astringent, pungent and sweet	Heating	Pungent	Sciatica, neuro-muscular problems and chronic vata dosha
Tagar	Astringent	Heating	Pungent	Insomnia and CNS disorders
Yasthimadhu	Bitter and sweet	Cooling	Sweet	Sore throat and hoarseness
Vidari	Bitter and sweet	Cooling	Sweet	Emaciation and infertility
Vacha	Bitter and pungent	Heating	Pungent	Kapha depression and epilepsy
Vidanga	Pungent	Heating	Pungent	Krumi
Shilajit	Astringent, bitter, pungent and salty	Heating	Pungent	Diabetes, urinary problems and infertility

Food and nutrition in modern science

The balanced diet in today's scenario comprises carbohydrates, protein, fats, nutrients and minerals in such a quantity and proportion which are necessary for growth and proper function of the body and also has provision for the reserve to withstand a brief period of starvation. In today's fast-moving world, people are reliant on fast food which does not cater to our need for nutrients. This practice eventually leads to deficiency and finally malnutrition. This initiates a vicious cycle of malnutrition and disease which complement each other until and unless one breaks them. In one only consume a carbohydrate-rich diet he/ she would eventually land into kwashiorkor which is due to a deficiency of protein in the body. There is stunted growth and wasting in the body is oedematous, the person is irritable, and has altered bowel movements leading to diarrhoea. Another condition called marasmus is a deficiency of carbohydrates in the diet, these two are categorized under protein energy malnutrition which affects children throughout the world. Due to this deficiency, there is a delay in achieving the goals of a child. There is hampered growth and development, which also results in decreased immunity. So to tackle this crisis, one must be cautious about a balanced diet in children.

For infants up to 6 months, the mother's milk is sufficient to meet the needs but milk is not whole until and unless the mother is taking a properly balanced diet (UNICEF, 2019). Any deficiency in the mother would reflect over the infant as the milk would be deficient in it. On average, lactating women require 550 kcal more than a normal woman per day and 29 grams of extra protein.

The most adversely affected age groups by malnutrition comprise children and pregnant or lactating women. Pregnancy is a stressful condition for the mother as there is an exponential increase in the demand for nutrients and other materials by the growing foetus. This usually leads to depletion in the reserves in the body of the mother which makes her weak and vulnerable to various diseases. So any deficiency in this state can adversely affect the mother and the children in her womb, e.g. deficiency of folic acid leads to anaemia in the mother and neural tube defects in the child. Moreover, a deficiency of iodine in the mother leads to affect the brain development of the baby. According to the World Health Organization (WHO, 2020), a healthy diet helps to protect against malnutrition in all its forms, as well as non-communicable diseases, including diabetes, heart disease, stroke and cancer.

Ayurvedic Balanced Diet

The food is also composed of the *panchamahabootas* i.e. *prithvi, jal, agni (tej), vayu* and *akash*. The *mana* in the food is provided by the *prithvi* component, the binding strength by the *jal*, the taste of the food is provided by the *agni (tej)* and *vayu*, and all these are waist in the *akash* (space) (Madan et al., 2021). One cannot tell the nutritive values of a food item just by looking so to make things easier the concept of *shada rasa* and *sapta varna* is coined in Ayurveda for maintaining a balanced diet. It emphasizes that a diet is said to be balanced when the items in it have all six tastes i.e. *madhur* (carbohydrates), *amla* (citrus), *lavana* (sodium chloride) *katu* (pungent), *tikta* (bitter) and *kasay* (astrigent) and posses seven colours in them. Chewing food is an important step in digestion. By chewing properly, one breaks down the food into smaller particles and mixes the saliva thoroughly which acts as a lubricant for the food so that it can be easily swallowed and also helps the enzymes in the saliva to act effectively over it. Improper chewing may result in the swallowing of dry, rough food material which may damage the pharynx, oesophagus and stomach. Improper chewing also decreases the surface area for the action of the digestive enzymes. Saliva is the section which is poured into the oral cavity by the salivary glands. On average, one person produces over 1000 to 1500 ml of saliva per day. The saliva possesses various enzymes like salivary amylase, maltase, and lingual lipase which act on carbohydrates and lipids and initiate their breakdown process. The presence of lysozyme in the saliva inhibits the growth of bacteria also. After chewing, the food is swallowed & pushed into the oesophagus which opens into the stomach. The stomach is a muscular hollow bag of 1-1.5 litter capacity which produces pepsinogen which under the influence of Hydrochloric acid gets activated to break proteins into amino acids. The food here stays for 3-4 hours and is slowly released into the intestine.

Gastric secretion is controlled by neuronal and hormonal mechanisms. The vagus nerve innervating the stomach increases the gastric secretions when stimulated and so does the gastrin hormone secreted by G cells in the mucosa of the stomach. Whereas secretion of CCK-PZ, somatisation, VIP and GIP will have an inhibiting effect on gastric secretion. The food bolus in the stomach gets converted

into chyme which is released slowly into the intestine for the remaining digestion. In the intestine, it is acted upon by pancreatic lipase, protease and amylase and converts fats, and protein glucose into fatty acids, amino acids and glucose respectively. All these digested materials are finally absorbed in the distal part of the intestine the ileum. The undigested food is then pushed into the large intestine in which the water and electrolytes are absorbed. Thus by selecting the food in a particular quantity and proportion one can direct the effect of the food efficiently into the body.

Gut Microbiota and Ayurvedic Diet

The gut microbiota can be correlated with the Ayurvedic concept of *Sahaj krimi* (nonpathogenic microbes). Mainly the function of gut microbiota depends upon the status of Agni (digestive fire). The digestive system has long been an area of critical importance in individuals' physical and mental health (Fulzele and Nagdeve, 2022). In these days, research on the microbiome has started to help us to explore the Ayurveda theories of *Agni* (digestive fire) and *Ama* (metabolic toxins) from a modern perspective. As per the theory of Ayurveda, no disease ever arises without the derangement of Agni. Therefore, the whole preventive and treatment methodology in Ayurveda focuses on the modulation and management of *Agni*. Hence gut microbiota. It is already established that if there is derangement of *Agni*, *Ama* (metabolic toxin) is produced and further it vitiates the doshas which spread throughout the body and manifest as varied diseases. Similarly, from a biomedical perspective, dysbiosis of microbial flora causes a leaky gut by which the toxins of deranged digestive metabolism enter the bloodstream. Consequently, an inflammatory response occurs within the body which expresses as diseases opportunistically (Deepthi et al., 2021). A recent study has shown those gut microbiomes are *prakriti* and physiological characteristics specific in healthy individuals. However, needed to be explored in a broad population of healthy individuals over time and account for all factors that can influence the microbial diversity patterns such as age, gender, geography, food habits and cultural traditions, in order to discover features of the gut microbiome that are unique to different geographical areas/ lifestyles and aid discovery of statistically enriched biomarkers for each *prakriti* (Shalin et al., 2021).

It is now quite evident that the changes in the composition, diversity and abundance of the gut microbiome are affected by several variables including medication (consumption of antibiotics), blood parameters such as RBC count and haemoglobin concentration, bowel habits, dietary composition, health status, anthropometric features, lifestyle and gender (Lopez-Siles et al., 2018). Therefore there are variations in gut microbiota throughout the world. Even in the Indian population, the gut microbiome of healthy adults varies with various factors such as geography, age, gender, diet and/ or *prakriti*. (Dhakan et al., 2019) found that the gut microbiome of the northern Indian population was significantly associated with Prevotella, while the southern Indian was associated with Bacteroides, Faecali-bacterium and Ruminococcus. They further observed the enrichment of metabolic pathways involved in the degradation of complex polysaccharides in Indian plant-based foods. As, a diet provides macronutrients (carbohydrates, proteins, and fats), micronutrients (vitamins and minerals), and phytochemicals (non-nutrient bioactive compounds) which influence the metabolic activity of the mammalian gut microbiota.

Food in western countries is poor in complex carbohydrates; fibre etc. which results in progressive loss of beneficial bacteria and microbial diversity and which further causes chronic disease.

Conclusion and Perspectives

As per Ayurvedic principles eating according to your dosha, especially your dominant dosha, will help to create more balance in the body and it will not only improve gut health but overall health and well-being. The seven *Pathya* of herbal and animal origin mentioned earlier have *Agnivardhaka* (digestive/metabolic stimulating) properties due to their *Laghu* (easy to digest), *Ushna* (hot potency) and *Tikshna Guna* (helping for easy assimilation) and due to these *Guna* (attribute/property) they possess therapeutic activities such as clearing of *Ama*, pacify *Kapha* and *Vata Dosha*. Thus, they stimulate *Agni*, digest *Ama*, remove excessive Kledaka Kapha (subtypes of *Kapha* situated in the *Amashaya*), prevent further production of *Ama* (clear obstruction in channels of circulation) and transport *Pakwa Dosha* from *Shakha* (circulation) to *Koshtha* (cavities of body/hollow organs) for removal from the body. Thus, microbiota in the host acts as a host-microbiota co-metabolic structure, which carries out various metabolic processes in the human body (Ranade et al., 2019). Therefore Indian thali or Ayurvedic thali is an ancient approach to diet that provides both fibre and different phytochemicals by incorporating a variety of plant foods in different colours. This variety helps to restore diversity in the gut bacteria and may potentially prevent or reverse chronic disease (Shondelmyer et al., 2018). Therefore, there is a need to design thali (food) based on *Dosha* and *Prakriti* to enrich good microbiota which further will enrich a good and healthy body and mind.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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Phytochemical analysis of chloroform extract of leaves of *Skimmia laureola*

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Abstract- The air-dried and powdered leaves (3 kg) of *Skimmia laureola* were exhaustively defatted with light petroleum ether (60-80^0). The petroleum free mass extracted with 90% ethanol. The ethanol extract was concentrated under reduced pressure and a suspension of the residue was made with water, which was washed with diethyl ether for several times and then partitioned with $CHCl_3$:H_2O:MeOH (6:4:4) in a separatory funnel. The chloroform layer was separated out and concentrated under reduced pressure to give $CHCl_3$ extract (15g). The chloroform extract (10g) was subjected to repeated CC over Si-gel eluted with different proportions of n-hexane- $CHCl_3$ and $CHCl_3$:MeOH afforded β-stosterol (1), (E)-3'-(4-Hydroxyphenyl)-2'-propenoic methyl ester (2), (E)-3'-(4-Hydroxy-3-methoxyphenyl)-2'-propenoic methyl ester (3), Identified as bergapten (4), Identification of these compounds were made by the analysis of their chemical and spectral data.

Key words: *Skimmia laureola*, chloroform extract, coumarin, furanocoumarin.

Introduction

The genus *Skimmia* belongs to the family Rutaceae is a large genus of strongly scented unarmed shrubs, distributed chiefly in the shady moist localities of temperate and alpine region, up to 1500-3000m. It is distributed throughout the temperate Himalayas from Kashmir in the north to Mishmi and Khasia mountains in the south east[1,2]. The flowers are sweetly and leaves are strongly aromatic[3]. The leaves are often used as incense and burnt near small-pox patients for their supposed curative effects. The smoke produced by burning them is said to purify the air[4] and are used in preparation of dhup and agarbatties. Skimmia species have been reported to possess antifungal, anti fertility, antiplatelete, and spasmolytic, activity[5-7]. Phytochemical studies on *Skimia* species resulted in the isolation of flavonods, terpenoids, iridoids, coumarins, alkaloids, and some fatty esters[8-10].

S. laureola is a strongly scented, evergreen, glabrous shrub, distributed in Northern India and China[1]. From *S.* laureola fatty ester, terpenoids and quinoline alkaloids have been isolated[11-13]. In the present study the phytochemical analysis of chloroform extract of air dried leaves of *S. laureola* was carried out using CC over Si-gel using various solvents afforded β-stosterol (1), (E)-3'-(4-Hydroxyphenyl)-2'-propenoic methyl ester

(2), (E)-3'-(4-Hydroxy-3-methoxyphenyl)-2'-propenoic methyl ester (3), and bergapten (4). Identification of these compounds was made by the analysis of their chemical and spectral data.

Material and Method

CC was carried out over silica gel (60-120 mesh, BDH) with gradient elution method using different solvent systems in the order of increasing polarity. TLC was conducted on SI-gel (E-Merck and BDH) coated on a thin glass plate (0.25 mm thickness containing 13% $CaSO_4$ as binder). Spots on TLC were detected by spraying with 5% H_2SO_4 followed by heating at 100^0C, 5% methanolic KOH, Benedict's reagent, iodine vapours, UV and alcoholic $FeCl_3$ solution. Preparative TLC was carried out on pre-coated reverse-phase TLC on Si-gel 60 HPTLC (Merck, 20 x 20 cm. and 0.25 mm thickness) developed with different proportion of $CHCl_3$:MeOH. PC was carried out on Whatman filter paper No. 1 using descending technique with n-BuOH-pyridine-H_2O (6:4:3) as solvent system and spots were detected by spraying with aniline hydrogen phthalate (AHP) followed by heating. M.Ps. were recorded in BOETIUS microscopic m.p. apparatus. UV-spectra (λ_{max}, nm) were recorded in MeOH on a SYSTRONIC spectrophotometer. IR-spectra (ν_{max}, cm^{-1}) were carried out on FT-IR-8100 Shimadzu spectrophotometer as KBr palettes. NMR spectra were recorded in BRUKER DRX-300 (300 MHz for ^{1}H and 75 MHz for ^{13}C), BRUKER DRX-400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrophotometer with $CDCl_3$ and Aetone-d_6 solvents. Chemical shifts are given in ppm scale with TMS as an internal standard.

Plant Material

The leaves of *S. laureola* were collected from Nachiketa Tal (at an altitude of 2450-2500m), District Uttarkashi, Uttarakhand, India (Garhwal Himalaya), in September 2017. The plant species was identified by Dr. Jai Laxmi Rawat Department of Botany, RCU Govt. PG College Uttarkashi, Uttarakhand. A voucher specimen (DOC 12/2009) was deposited in the Department of Botany, Govt. P.G. College, Uttarkashi and Uttarakhand, India.

Extraction and Isolation

The air-dried and powdered leaves (3 kg) of *S. laureola* were exhaustively defatted with light petroleum ether ($60\text{-}80^0$). The petroleum free mass extracted with 90% ethanol. The ethanol extract was concentrated under reduced pressure and a suspension of the residue was made with water, which was washed with diethyl ether for several times and then partitioned with $CHCl_3$:H_2O:MeOH (6:4:4) in a separatory funnel. The $CHCl_3$ layer was separated out and concentrated under reduced pressure to give $CHCl_3$ extract (15 g). The $CHCl_3$ extract (10g) was subjected to CC over Si-gel eluted with n-hexane- $CHCl_3$ (100:0→1:1) and then with $CHCl_3$:MeOH. The like fractions obtained on elution with n-hexane: $CHCl_3$ (6:4), (5:5) and (3:7) were mixed together and after evaporation of solvent afforded three fraction A, B, & C. Fraction A on CC over Si-gel eluted with n-hexane: $CHCl_3$ (5:5), provide β-stosterol (1), and several other fractions. First few fractions were subjected to preparative TLC using n-hexane: $CHCl_3$ (3:7) solvent afforded (2) (31 mg) and (3) (23 mg). Fraction B was subjected to CC over Si-gel eluted with n-hexane: $CHCl_3$ (3:7), afforded 4 (17 mg).

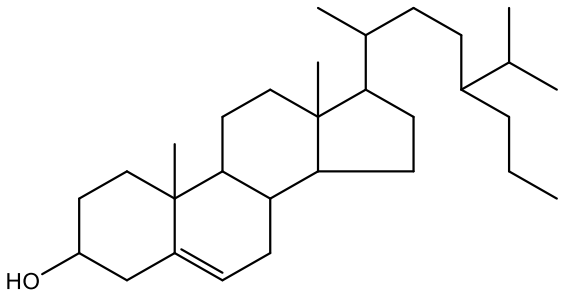
Results and Discussion

β-sitosterol: White amorphous solid
M.P. :135-137^0C
$[\alpha]_D^{25}$**:** -36^0 (c=0.1, $CHCl_3$)

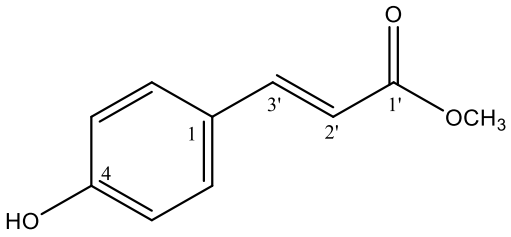
(E)-3'-(4-Hydroxyphenyl)-2'-propenoic methyl ester (2): White amorphous solid
M.P. :191-196^{0}C
IR (ν_{max}^{KBr}): cm^{-1} 3405, 2858, 1705, 1608, 1326, 810 etc.
UV (λ_{max}MeOH): nm 210, 311
^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.74 (2H, brd, *J* = 8.6 Hz, H-2, 6), 6.80 (2H, brd, *J* = 8.6 Hz, H-3, 5), 6.21 (1H, d, *J* = 15.4 Hz, H-2'), 7.48 (1H, d, *J* = 15.4 Hz, H-3'), 3.77 (3H, *s*, OCH_3) and 5.09 (1H, br*s*, OH);
^{13}C-NMR (100 MHz, $CDCl_3$): δ 126.93 (C-1), 130.09 (C-2, 6), 157.97 (C-4), 115.91 (C-3, 5), 168.14 (C-1'), 115.15 (C-2'), 145.23 (C-3'), 51.86 (-OCH_3).

(E)-3'-(4-Hydroxy-3-methoxyphenyl)-2'-propenoic methyl ester (3): Amorphous solid,
M.P. : 191-196^{0}C
IR (ν_{max}^{KBr}): cm^{-1} 3718, 2915, 1709, 1608, 1597 etc.
UV (λ_{max}MeOH): nm 217, 325
^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.01 (1H, d, *J* = 1.9 Hz, H-2), 6.89 (1H, d, *J* = 8.0 Hz, H-5), 7.06 (1H, dd, *J* = 1.9, 8.0 Hz H-6), 6.28 (1H, d, *J* = 15.4 Hz H-2') 7.62 (1H, d, *J* = 15.4 Hz H-3'), 5.87 (1H, brs, -OH), 3.91 (3H, s, -OCH_3) and 3.78 (3H, s, -OCH_3)
^{13}C-NMR (100 MHz, $CDCl_3$): δ 177.02 (C-1'), 147.03 (C-3), 148.21 (C-4), 145.02 (C-3'), 126.98 (C-1), 122.87 (C-2), 115.23 (C-2'), 114.68 (C-5), 109.26 (C-6), 56.17(-OCH_3) and 52.13(-OCH_3).

Bergapten (4): White crystalline solid
M.P.: 189-191^{0}C
IR (ν_{max}^{KBr}): cm^{-1} 2923, 1715, 1612, 1590 etc.
UV (λ_{max}MeOH): 210, 260 and 310 nm
^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.27 (1H, d, *J*=9.8 Hz, H-3), 8.14 (1H, d, *J*=9.8 Hz, H-4), 7.15 (1H, s, H-8), 7.59 (1H, d, *J*=2.5 Hz, H-2'), 7.02 (1H, d, *J*=2.5 Hz, H-3') and 4.27 (3H, s, OCH_3)
^{13}C-NMR (100 MHz, $CDCl_3$): δ 160.33 (C-2), 112.59 (C-3), 139.56 (C-4), 149.74 (C-5), 113.96 (C-6), 158.02 (C-7), 93.78 (C-8), 152.66 (C-9), 106.44 (C-10), 145.02 (C-2'), 105.16 (C-3') and 60.24 (-OCH_3).



1



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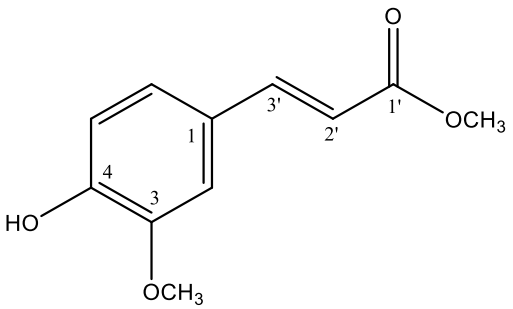
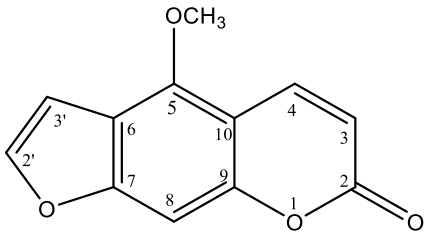


3



4

Figure-Isolated Compounds from *S. laureola*

β-stosterol (1), was identified by Co-TLC and Co-MP with authentic sample[14]. Compound 2 showed strong absorption bands at 3405 for OH, 2858 for CH, 1705 for -C=O, and 1608 cm^{-1} for –C=C- in IR spectrum and two absorption band at 210, and 311 nm for α, β-unsaturated carbonyl group in UV spectrum. The ^{1}H-NMR spectrum displayed three proton singlet at δ 3.77 corroborated with methoxy protons ester group. The two A_2B_2-type *brd* doublets (*J* = 8.6 Hz) in the aromatic region at δ 7.74 and 6.80, indicated presence of di-substituted phenyl ring and was further confirmed to be p-hydroxyphenyl by the ^{13}C chemical shifts of carbon atom at δ 130.09 (C-2, 6), 115.91 (C-3, 5), which was fairly corresponded with hydrogen carrying carbon of p-cresol[15]. Two olifinic protons were observed at δ 6.21 (1H, d, *J* = 15.4 Hz, H-2'), and 7.48 (1H, d, *J* = 15.4 Hz, H-3') were assigned for H-2'and H-3' protons. The value of coupling constant (*J* = 15.4 Hz) of olifinic protons indicated the *trans*-orientation of H-2' and H-3' protons. A broad singlet at δ 5.09 indicated presence of OH group in the molecule. The ^{13}C-NMR spectrum displayed eight carbon resonances (two of double intensity) indicated presence of nine carbon atoms in the molecule while DEPT spectrum displayed presence of one methyl, six methane (two of double intensity), and three quaternary carbon atoms. The presence of methoxy carbon at δ 51.86, an ester carbon at δ 168.14, olifinic carbons at 115.15 and 145.23 and other signals due benzene ring was displayed by ^{13}C-NMR spectrum. On the basis of tese spectral data compound 2 was identified as (E)-3'-(4-Hydroxyphenyl)-2'-propenoic methyl[16].

The spectral data of 3 are similar to those of 2 except with additional methoxy group attached with benzene ring. The presence of three doublets, each for one proton at δ 7.01 (1H, d, *J* = 1.9 Hz, H-2), 6.89 (1H, d, *J* = 8.0 Hz, H-5) and 7.06 (1H, dd, *J* = 1.9, 8.0 Hz H-6) was assigned for H-2, H-5 and H-6 of benzene ring, respectively, indicated tri-substituted catechol type phenyl ring in the molecule[17]. The position of methoxy and OH group at C-3 and C-4 was determined by ^{13}C-chemical shifts of C-3 at δ 147.03 and C-4 at δ 148.21. On the basis of these spectral evidences 3 was identified as (E)-3'-(4-Hydroxy-3-methoxyphenyl)-2'-propenoic methyl ester[16].

The IR spectrum of 4 exhibited strong absorption bands at 2923, for –CH, 1715 for carbonyl carbon and 1612 cm^{-1} for double bond. The UV spectrum displayed characteristic absorption band for coumarin nucleus at 210, 260 and 310 nm[18]. The ^{1}H-NMR spectrum displayed characteristic doublets at δ 6.27 (1H, d, *J*=9.8 Hz, H-3) and 8.14 (1H, d, *J*=9.8 Hz, H-4), assignable for H-3 and H-4 protons of furanocoumarin nucleus[18] which was confirmed by the ^{13}C-chemical shift of C-3 at δ 112.59 (C-3) and 139.56 (C-4). The two doublets (*J* = 2.5), for one proton each at δ 7.59 and 7.02 were assigned for H-2' and H-3' protons of furan ring. ^{1}H-NMR spectrum also displayed presence of methoxyl protons at δ 4.27, which was confirmed by ^{13}C-chemical shift of methoxy C-atom at δ 60.24. The ^{13}C-NMR spectrum also displayed presence of carbonyl carbon at δ 160.33 and olifinic carbons at δ 112.59 (C-3), 139.56 (C-4), and the carbon atoms of furan rings at δ 145.02 (C-2') and 105.16 (C-3'). The position of methoxy group was determined at C-5 position by the chemical shift of H-4 proton, which appeared downfield at δ 8.14 and presence of one proton singlet at δ 7.15, which was assigned for H-8 proton of phenyl ring of C-5 substituted furanocoumarins[19,20]. On the basis of above discussed spectral data 4 was identified as bergapten[21,22].

Conclusion

The phytochemical studies on leaves of *S. laureola* led to the conclusion that the traditional claims for this plants in curing various diseases, may be due to the presence of β-sitosterol, (E)-3'-(4-Hydroxyphenyl)-2'-propenoic methyl ester, (E)-3'-(4-Hydroxy-3-methoxyphenyl)-2'-propenoic methyl ester, bergapten, and other metabolites which I am unable to isolate and identify**.** The extensive pharmacological work on isolated compounds may help in establishing mechanism of action, toxicity and side effects associated with the compounds as well as for generating new lead therapeutic molecules for treating various diseases.

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Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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Development of biochar from crofton weed & relationship between biochar properties and its applicability as a heavy metal removal activity

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Abstract - Biochar is a pyrogenic black carbon produced from thermal degradation of carbon-rich biomass (<700°C) in an oxygen-limited environment, and usually has a porous structure, a surface rich in oxygenated functional groups, strong adsorption capacity, and a certain degree of surface area and stability. Biochar has multiple uses, including agricultural applications for soil remediation and pollution control in water and soil. Biochar has several significant socioeconomic and environmental benefits such as carbon sequestration, pollutant removal, and soil improvement. Pyrolysis temperature affects biochar properties, which in turn determines its application potential. The collected Crofton weed (except for the roots) was washed, air-dried at room temperature, and crushed for passage through a 10-mesh sieve. Here, we examined the properties of Crofton weed biochar (C-BC) produced at different pyrolysis temperatures of 300°C, 400°C, 500°C, and 600°C. We measured the yield, ash content, pH, iodine sorption value (ISV), and elemental composition of C-BC. We also characterized C-BC using scanning electron microscopy (SEM), as well as its ability to remove $Pb^{2+}$and Cd^{2+}contaminantsfrom an aqueous solution. C- BC yield decreased with increasing pyrolysis temperature, whereas ash content and pH increased. ISV first increased at 300–400°C and decreased at 500–600°C. For C-BC produced at pyrolysis temperatures 300–600°C (C-BC300 to C-BC600, respectively), H, N, and O content decreased, but C, Ca, Mg, P, and K content in- creased with increasing temperature. All C-BCs had a certain number of pore structures. Increasing pyrolysis temperatures decreased the amount of -OH, -COOH, aliphatic C-H, and polar C-O on the C-BC surface. The percentage of $Pb^{2+}$and Cd^{2+}removed increased with increasing pyrolysis temperatures. Overall, for C-BC, a low pyrolysis temperature was beneficial for producing a more porous biochar and increased content of water-soluble calcium, magnesium, nitrogen, and phosphorus, whereas high pyrolysis temperatures yield biochar that had high alkalinity, aromaticity, and stability, as well as heavy metal removal activity

Keywords: Pyrolysis, Eupatoriumadeno-phorum Spreng, Iodine Sorption Value (ISV), Surface Morphology, b^{2+} and Cd^{2+}contaminants.

Introduction

Biochar is a pyrogenic black carbon produced from thermal degradation of carbon-rich biomass (<700°C) in an oxygen-limited environment, and usually has a porous structure, a surface rich in oxygenated functional groups, strong adsorption capacity, and a certain degree of surface area and stability (In yang et al., 2016). Biochar has multiple uses, including agricultural applications, particularly for soil remediation, and pollution control in water and soil (Alwabel et al., 2013; Ahmad et al., 2014). Application of biochar It has several significant socioeconomic and environmental benefits such as carbon sequestration, pollutant removal, and soil improvement. Methods for development and utilization of biochar have been a focus of biochar research. Inexpensive and readily available waste biomasses from agriculture, livestock, and industry (e.g., crop residues, livestock manure, wood pellets, sewage sludge) are the main materials used to prepare biochar. The source materials affect the composition and prop- erties of the resulting biochars, which can have varying yield, pH, ash content, surface morphology, and adsorption prop-erties, which in turn determine their application potential (Chan and Xu, 2009). Therefore, studies on the properties of biochar are important for maximizing its effective use. Pyrolysis temperature is one of the most important factors that affect biochar properties (Yuan et al., 2015). Several recent studies have assessed the properties of biochar materials prepared from different feedstocks using different pyrolysis temperatures and provided data that support the development of methods to optimize production of biochars having de- sirable properties (Cao and Harris, 2010; Alwabel et al., 2013; Meng et al., 2013; Wang et al., 2015b; Yuan et al., 2015; In yang et al., 2016).

Eupatorium adenophorum -Spreng (comm-only known as Crofton weed) is a perennial herb or semi shrubby plant that grows between 0.8 and 2.5 m tall. Crofton weed is native to Central America, but is an invasive plant in America, New Zealand, China, India and many other countries (Liu et al., 2006; Li and Feng, 2009; Wang et al., 2017). Several methods have been developed to control crofton weed, including those that involve chemical control, biological control, and manual control. However, these methods have not produced notable results (Guo et al., 2009). Compared with these control methods, resource utilization of Crofton weed has been explored in recent years (Guo et al., 2009; Sahoo et al., 2011; Zheng et al., 2014). As an invasive plant, Crofton weed is easily obtained at low cost. Based on these properties, crofton weed would be a suitable feedstock for preparation of biochar, but there are few reports describing the properties of Crofton weed biochar (C-BC). The aim of this study was to explore the effect of pyrolysis temperature on the properties of C-BC. Here, C-BC was prepared at different temperatures (300°C, 400°C, 500°C, and 600°C) under oxygen-limited conditions, and the yield, ash content, pH, iodine sorption value (ISV), elemental composition, surface morphology, mineral phase, and surface functional group of the resulting C-BC were characterized and analyzed. The ability of C-BC to remove Pb^{2+} and Cd^{2+} from aqueous solution was also determined in a batch experiment. Results from this study can guide the preparation and utilization of C-BC to maximize its potential applications.

Material and Methods

Preparation of C-BC - Sample collected randomly from Jantanwala, Ghanghora, Dehradun (N $30^0$23' 18.9" E 078^0 01'40.2"), which was later shade dried for a week. The dried leaf and stem part was first segregated manually from the aerial part and then crumbled and ground to pass through a 10-mesh sieve. The filtered sample was then passed to quartz-covered crucibles which were then pyrolyzed in a Muffle furnace (Muffle Furnace manufacturing unit name) at pyrolyzing temperatures 300^0C, 400^0C, 500^0C, and 600^0C with the heating rate of 10^0C per min for 4 hr. residence time. The charred product was then placed in a desiccator, once the crucibles attain room temperature, removed and weighed immediately, thereafter the charred samples of different temperatures are further crushed to pass through a 60-mesh sieve and were termed as C-BC300, C-BC400, C-BC500, and C-BC600 respectively.

Yield - Yield of C-BC was studied by weighing the samples and C-BC yield % was calculated using the following Equation (1)

Yield (%) = (m_a/ m_b) x 100%…… (1)

Where m_a and m_b represent the weight of C-BC and Crofton weed, respectively.

Ash content of the C-BC - The ash content was detected by heating C-BC at 1000°C for 2 h and was calculated using Equation (2)

Ash Content (%) = (N_a /N_b) x 100,.....(2)

Where, N_a and N_b refer to the weight of C-BC after and before heating, respectively.

pH of the C-BCs - The pH of C-BC was measured with a pH meter (Eutech Instrument) in a mixture of 1.00g C-BC and 20mL demonized water following a 1hr intermittent equilibrium.

Iodine Sorption Value (ISV mg/g) of the C-BCs - The ISV of C-BC was determined according to Chinese National Standards (GB/T12496.8-2015). In brief, a C-BC sample (0.5g) was mixed with 50mL 0.1M I2-KI solution in a 250mL conical flask, which was placed in an oscillator (240 rpm/min) for 15 min. The solution was then filtered, and 10mL of the filtrate was titrated with 0.1M Na2S2O3. A starch solution (5 g/L) was used as an indicator in the titration process. The consumption volume of Na2S2O3 was used to calculate the ISV of C-BC.

Elemental analysis of the C-BCs – Element analysis of C-BC (carbon [C], hydrogen [H], and nitrogen [N]) was performed using an elemental analyzer (Vario EL III; Elementar Corp., Hanau, Germany). The oxygen (O) content was calculated by subtracting the C, H, N, and ash content from the total quantity (Mimmo et al., 2014). For Ca, Mg, K, and P, 0.10 g C-BC was digested with HNO3 using a microwave digestion method, and the concentrations in C-BC were detected and calculated using a ICP-OES (iCAP 6000 series; Thermo Fisher Scientific, Inc., Waltham, MA).

Surface morphology of the C-BCs - The surface morphology of C-BC was analyzed by scanning electron microscopy (SEM) with a field-emission electron microscope and an operating voltage of EHT 15kV (Zeiss EVO 40- EP).

Removal of Pb^{2+} and Cd^{2+} from aqueous solution - Pb^{2+} and Cd^{2+}solutions (50 mg/L) were prepared with Pb(NO3)2 and Cd(NO3)2 4H2O. The initial pH of the solution was adjusted to 5.0 by adding 0.1 M HNO3 or NaOH solutions. A C-BC sample (0.10 g) was added to 50 mL Pb^{2+} 50 mg/L)

or Cd^{2+} (50 mg/L) solution, and the mixture was shaken for 24 h at 120 rpm/min at room temperature before filtration through a 0.45-micrometer filter membrane. The final pH of the filtrate was measured with a pH meter, and ICP-OES was used to determine the final concentration of Pb^{2+} and Cd^{2+}. In this experiment, the percentage of Pb^{2+} and Cd^{2+} removed was used to evaluate the removal activity of C-BC. The percentage of Pb^{2+} and Cd^{2+} removed was calculated using the following Equation (3)

Percentage removed (%) = [(C0 – C1) / Co] x 100,……. (3)

where C0 represents the original concentration of Pb^{2+}or Cd^{2+} (i.e., 50 mg/L), and C1 refers to the remaining concentration of Pb^{2+} or Cd^{2+}.

Data processing each experiment was repeated three times, and the average value of the three replicates was taken as the experimental result. Excel 2007, Minitab 11, and Origin 8 were used for data management and processing.

Results and Discussion

General properties of C-BC - The yield of C-BC decreased with increasing pyrolysis temperature (Table 1), and this decreasing trend was consistent with other reports concerning biochar produced from herbaceous biomass (Peng et al., 2011; Ronsse et al., 2013; Wang et al., 2015b). The decrease in C-BC yield was related to the further pyrolysis of raw materials upon increases in the pyrolysis temperature (Onay, 2007; Angin, 2013). An obvious decrease ($p<0.05$) occurred as the pyrolysis temperature increased from 300°C to 400°C, which could be due to the decomposition of hemicellulose and cellulose (Cao and Harris, 2010). The yield of C-BC at different pyrolysis temperatures ranged from 28.69% to 54.69%, and was approximately the same as that seen for other biochars, such as rice straw (Peng et al., 2011), canola straw, corn straw, soybean straw, peanut straw (Yuan et al., 2011), and pig manure (Zhao et al., 2013). The ash content of C-BC ranged from 9.7% to 14.87% (Table 1), and increased with increasing pyrolysis temperature, which was also consistent with previous studies (Yuan et al., 2011; Wang et al., 2015b). The increase in ash content was due to increases in the amount of minerals and com- bustion of residual organic matter at high temperature (Cao and Harris, 2010). Upon increasing the pyrolysis temperature from 300°C to 400°C, the ash content increased by 3.80% ($p < 0.05$) after the decomposition of hemicellulose and cellulose in this temperature range (Table 1).

Table - 1 Yield (%), Ash Content, pH and ISV (mg/g) of the C-BC300, C-BC400, C-BC500 and C-BC600

S.No.	Pyrolysis Temperature	Part of the *Eupatorium adenophorum*	Yield (%)	Ash Content (Aerial Part)	Intermittence pH (Aerial part)	ISV (mg/g)
1.	300^0C	Stem part Leaf part Aerial Part	54.69 43.25 49.75	9.74	7.238	234.75 228.73 230.73
2.	400^0C	Stem part Leaf part Aerial Part	37.73 34.10 30.40	14.36	8.721	301.87 264.54 292.84

3.	500^0C	Stem part Leaf part Aerial Part	34.73 30.45 29.73	14.51	10.187	245.35 240.46 242.53
4.	600^0C	Stem part Leaf part Aerial Part	31.78 27.34 28.69	14.87	10.324	220.61 215.15 218.73

The pH of C-BC ranged from 7.238 to 10.324, with only C- BC300 falling outside the alkaline range (pH=7.238; Table 1). Generally, the pH of C-BC increased with increasing pyrolysis temperature. A previous study indicated that biochar pH is significantly and positively correlated with ash content (Wang et al., 2015b). Indeed, the high ash content seen for C- BC prepared at high pyrolysis temperatures was associated with a higher pH, and a significant change in pH occurred between C-BC300 and C-BC400 ($p< 0.05$). Furthermore, organic nitrogen present in amine functional groups was transformed into pyridine-like compounds, and the amount of acidic surface functional groups decreased (De Filippis et al., 2013; Chen et al., 2014), both of which contributed to the increased alkalinity of C-BC with increasing pyrolysis temperature. The surface functional group changes of C-BC are discussed in additional detail in the Fourier transform infrared section.

The ISV of C-BC (Table 1) increased from 230.75 (C- BC300 Aerial Part) to 292.84mg/g (C-BC400 Aerial Part), before gradually decreasing to 218.73 mg/g (C-BC600). The ISV of C-BC400 (299.54 mg/g) was significantly higher than that of the other C-BCs ($p< 0.05$), whereas the ISVs of C-BC300, C-BC500, and C-BC600 were all approximately the same ($p>0.05$). Mianowski et al. (2007) showed a positive correlation between ISV and surface area. Thus, our results indicate that the C-BC surface area increased first, and then decreased with increasing pyrolysis temperature. This is consistent with studies performed by Tsai et al. (2012), Angın (2013), and Lu et al. (2013), who also showed decreased surface area for biochar produced at pyrolysis temperatures >500°C. The general consistency of C-BC properties with those of other biomass-biochars suggests that crofton weed is suitable for conversion into biochar. The C-BC prepared using pyrolysis temperatures >400°C is alkaline, and thus could be used to reduce soil acidity for agricultural planting. All of the C-BCs in this study had a certain degree of ISV, and thus addition of C-BC to soil could be a practical method to reduce nutrient loss and capture pollutants.

Biochar elemental analysis

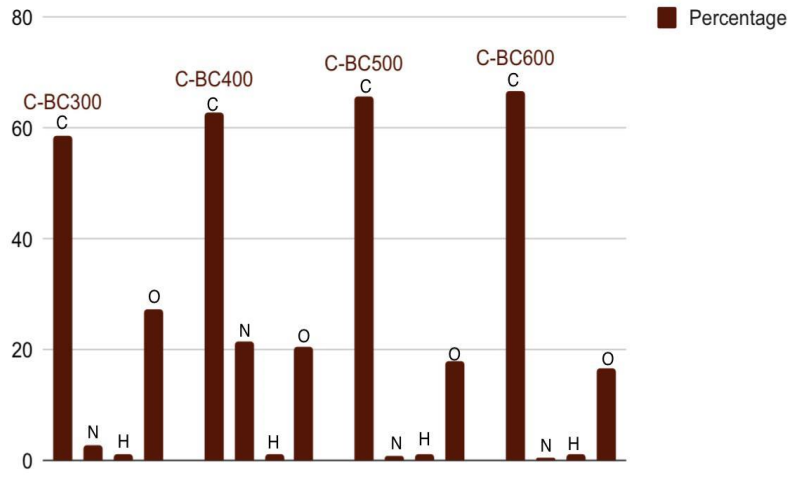
C content of C-BC increased by 26.1% between C- BC300 and C-BC600 (Table 2), likely due to the concentration effect of pyrolysis (Kloss et al., 2012; Sun et al., 2014). Meanwhile, the H, N, and O content decreased with increasing pyrolysis temperature. Specifically, the H, N, and O content decreased from 4.37%, 1.75%, and 25.07% (C-BC300) to 0.85%, 1.25%, and 16.43% (C-BC600), respectively (Table 2). During pyrolysis, the loss of increasing amounts of volatile agents, hemicellulose, and cellulose with increases in pyrolysis temperatures decreases the overall amount of H, O, and N elements (Chen et al., 2014). Here, the atomic ratios of H/C, O/C, and (O+N)/C were, respectively, decreased from

0.52, 0.39 and 0.35(C-BC300) to 0.15, 0.21 and 0.19 (C-BC600).

The ratios of H/C and O/C can be used as carbonization indicators (Wang et al., 2015b). Thus, the low H/C and O/C for C-BC600 indicated that highly carbonized biochar was formed at this pyrolysis temperature. The atomic ratios of H/ C and (O+N)/C can serve as indices for aromaticity and polarity, respectively (Chen et al., 2005; Pujol et al., 2013). The decreasing trends for the atomic ratios H/C and (O+N)/C demonstrated that high-temperature pyrolysis is beneficial to produce C-BC that has enhanced aromaticity and decreased polarity, respectively. In previous studies, Spokas (2010) found that biochar having an O/C ratio between 0.2 and 0.6 was stable and had a 100–1,000 year half-life. Schimmelpfennig and Glaser (2012) showed that an O/C < 0.4 and an H/ C ratio <0.6 as well as C content >15% are associated with stable biochar. For all C-BCs in this study, the ratios of O/C, H/C, and C content fell within these intervals, suggesting that they have good stability and thus would be suitable for use as materials or additives for soil remediation. Content of Ca, Mg, K, and P ranged from 16,540.54–25,362.16, 4,752.36–5,897.53, 33,864.94–79.879.24, and 4,963.08–5,483.75mg/kg, respectively. Overall, the content of Ca, Mg, K, and P had an increasing trend from C-BC300 to C-BC600 ($p < 0.05$), which was consistent with previous studies (Cao and Harris, 2010; Hossain et al., 2011; Yuan et al., 2011; Cantrell et al., 2012).

Table-2 Elemental composition of C-BCs and Atomic Ratios

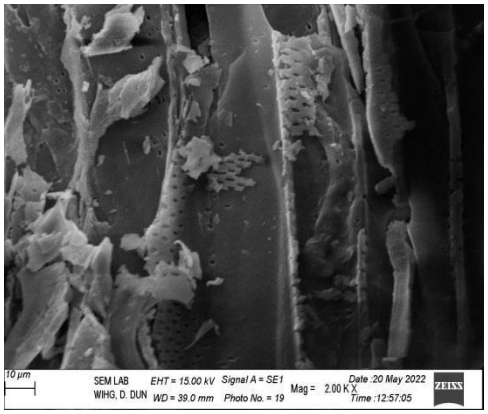
Biochar	C%	H%	N%	O%	H/C	O/C	(O+N)/C
C-BC300	53.07	4.321	1.75	25.07	0.52	0.39	0.35
C-BC400	63.25	3.16	1.32	20.60	0.29	0.27	0.25
C-BC500	75.28	1.01	1.39	17.97	0.17	0.24	0.20
C-BC600	79.17	0.85	1.25	16.43	0.15	0.21	0.19



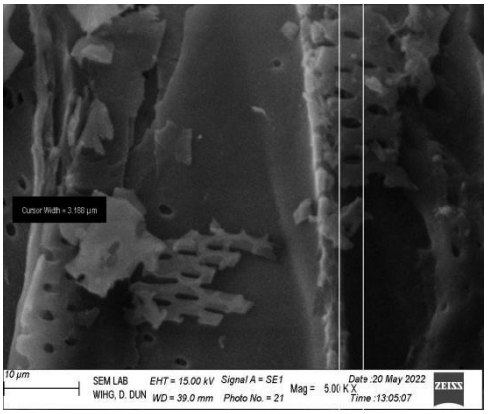
C, H, N, and O data of different C-BC respectively.

Surface morphology

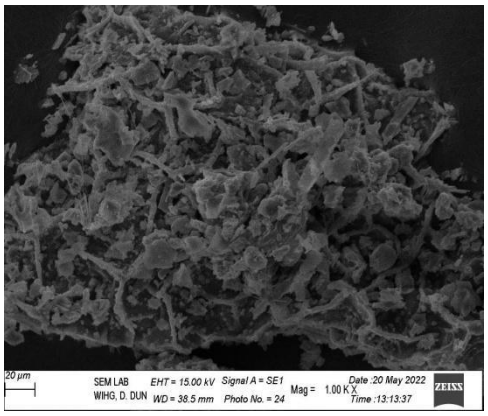
Morphologies of the four C-BCs are shown in Fig. 2. C-BC300 and C-BC400 retained the relatively complete tubular cell structure of Crofton weed, whereas this structure was gradually destroyed in C-BC500 and C-BC600 with the degree of destruction increasing with increasing temperatures as reported by Fan L et al. 2019. All C-BCs had a certain number of pore structures (Fig. (A), (B), (C), and (D)) formed from the large amounts of pyrolysis gas released from the C-BC surface during pyrolysis. However, excessive pyrolysis gas released through the surface of C-BC could lead to the expansion and coalescence of pores (Angın, 2013), which might result in the collapse of pore structures. Although the expansion and coalescence of pore structures are not visible in Fig. (C) and in Fig. (D), the decreased ISVs for C-BC500 and C-BC600 (Table 1) support this possibility to some extent.



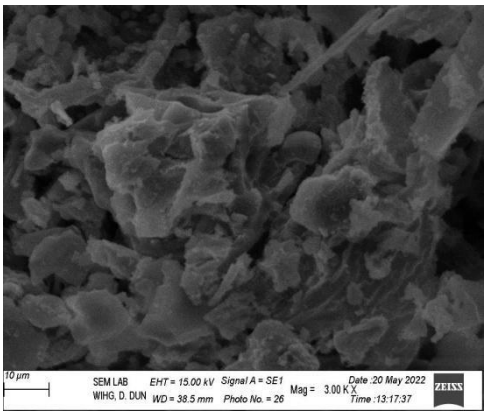
(A)



(B)



(B)



(D)

Figure - (A), (B), (C), and (D) represent the surface morphology of the C-BC400 and C-BC600 respectively.

The application of C-BC with suitable pore structures to soils can improve the physical properties of the soil by augmenting porosity, reducing bulk density, and increasing water-holding capacity. Moreover, the pore structure in C-BC can provide a good habitat for microbes, and in turn, increase the number and activity of beneficial microbes to improve the soil microbial environment. Based on our findings, C-BC prepared at low pyrolysis temperature (<400°C) would be suitable to produce biochar that has these properties.

Removal of Pb^{2+} and Cd^{2+} with C-BC

Percentage of Pb^{2+} removed by C-BC treatment of a 50 mg/L aqueous solution increased from 97.79% (C-BC300) to 99.52% (C-BC600) ($p< 0.05$), whereas the percentage of Cd^{2+} removed increased from 85.78% (C-BC300) to 97.56% (C-BC600) ($p< 0.05$) (Table 3). These results were consistent with those reported by Chi et al. (2017), and indicated that C-BC prepared using a high pyrolysis temperature had a better ability to remove $Pb^{2+}$and Cd^{2+}contaminants. However, the increasing trends for the percentage of $Pb^{2+}$and Cd^{2+} removed were not consistent with the trends for ISV (Table 1), implying that the $Pb^{2+}$and Cd^{2+} removal activity was not closely related to the surface area of C-BC. Cao and Harris (2010) reported that the main mechanism for the removal of $Pb^{2+}$with biochar involved an interaction between the phosphate in biochar with $Pb^{2+}$to form stable Pb phosphate minerals. Cui et al. (2016) showed that the formation of Cd-phosphate precipitate, cation exchange, and relevant functional groups together promoted the biochar-mediated removal of Cd^{2+}. Therefore, the increasing $Pb^{2+}$and Cd^{2+} removal activity by biochar could be related to the amounts of P, Ca, Mg, and K, and a certain amount of surface functional groups that are present on biochars produced using different pyrolysis temperatures.

Table - 3 Adsorption capacity of C-BC Biochar from Pb^{2+} and Cd^{2+} Solutions and Final pH of the solution.

Biochar	Pb^{2+} (%)	Final pH	Cd^{2+} (%)	Final pH
C-BC300	97.79± 0.18	7.45± 0.07	85.78±0.30	7.39± 0.23
C-BC400	98.36± 0.06	8.92± 0.02	95.31±0.28	7.64±0.18
C-BC500	99.05 ± 0.10	9.56 ±0.47	96.79±0.11	8.61±0.15
C-BC600	99.52± 0.20	9.71±0.06	97.56±0.18	8.95±0.45

Moreover, from C-BC300 to C-BC600, regardless of whether Pb or Cd removal was considered, the final pH tended to increase (Table 3). All of the C-BCs in this study had a final pH >7.0 for Pb^{2+}removal, whereas for Cd^{2+} removal all except C-BC300 raised the final pH >7.0. These results indicated that the pH in the solution can be increased by the presence of C-BC, particularly those C-BCs that were prepared at high pyrolysis temperatures. This significant pH elevation caused by C-BC is, on the one hand, conducive to deprotonation of the biochar surface, which helps capture $Pb^{2+}$and Cd^{2+} by C-BC (Yap et al., 2016), yet on the other hand, the elevated pH creates conditions for $Pb^{2+}$and Cd^{2+} precipitation (or surface precipitation) (Wang et al., 2015a). Therefore, the percentage of Pb^{2+} and Cd^{2+} removed by C-BC300 to C-BC600 was increased. In general, C-BC prepared using high pyrolysis temperatures may be more suitable for the removal of Pb^{2+}, ≥and Cd^{2+}.

Conclusions

As mentioned above, The C-BCs prepared at different temperatures exhibited the ability to remove $Pb^{2+}$and Cd^{2+} from aqueous solution. Increasing pyrolysis temperature was associated with enhanced ability to remove these heavy metals. Thus, C-BC, particularly C-BC prepared at high temperature, may be

used as a remediation material to reduce heavy metal pollution in future. Moreover, converting crofton weed to biochar would be a desirable approach to control the spread of this weed. However, to achieve practical application of crofton weed biochar, several issues must be addressed, including (1) efficient methods to collect crofton weed, (2) safe transport of croton weed that avoids spreading the weed to areas that are not yet affected, and (3) determination of ideal amounts of C-BC to different soils to achieve effective remediation.

Conflict of Interest

Authors have no conflict of interest of any kind

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Preliminary phytochemical screening of *Berberis asiatica* roots

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Abstract- Medicinal plants have bioactive compounds which are used to curing of various diseases. In this present investigation *Berberis asiatica* roots extract was studied. For phytoconstituents five separate solvents (100 ml of each) namely methanol, ethanol, chloroform, ethyl acetate and deionized waterwere used to obtain extracts from plant material. The extracts were subjected to qualitative phytochemical screening using standard procedure. Phytochemical screening reveals the presences of tannins, alkaloids, steroids Saponins, glycosides, flavonoids, anthraquinones, polyphenols and terpenoids.

Introduction

Almost all the prevailing traditional medicine systems rely upon the indigenous plants or plants derived products. With proper processing and prescription medicinal plants are being considered as safe to treat different ailments[1].From the past few decades year by year the world population is turning their attention towards the traditional medicine systems[2]. Because of their lesser side effects and cost effectiveness. Whenever a person suffering from any ailment that does not find an exact treatment within the modern medicine system such as Rabies, Alzheimer's, Diabetes, Hypertension and several type of Cancer[3-7]. In such situation he or she tries to seek the cure by taking prescription of the traditional medicine system[8].

During past few years a research towards traditional medicine system has been observed among the Diabetes suffering population of India. Numerous plants originated in India from Himalayan region, Western ghat and Gangetic planes have been reported that find their use in management of diabetes[9]. However, the current research and knowledge has established that the direct use of crude medicinal plants or plants derived products can confer the actual health benefits due to presence of other compounds also[10]. Therefore it is important to screen and identify the compounds present in those plants. This knowledge may further be utilized to developdifferent types of drugs, herbal medicines, health products, cosmetic products and supplementary foods. The

present work has been focused on the plant *berberis asiatica* which is not being much explored by the researchers. But, it is well known to the local tribal's of Garhwal Himalaya. They use its root and stem to treat different types of wounds including mouth ulcer and diabetes.

Berberis asiatica belongs to the family *Berberidaceae*is a short lived enduring shrub lesser then 10 meter height. It is native to the Western and Central Himalaya in India and Myanmar.Its vernacular names are daruharidra, kilmora, daruhaldi and kingod[11-12]. Its bright yellowroots and tomentose stem are used as a Ayurvedic preparation by the local population of Garhwal Himalaya and the fruits of the species are eaten as a dessert[13].

Materials and methods

Collection of plant material

The plant material was collectedfrom theforest of village Khola, block Khirsu (Longitude: 78.8679886 Latitude: 30.172 2334 and Elevation: 1766 m), Pauri Garhwal Uttrakhand, India during August 2021 and identified with the help of expert Taxonomist.[14]The collected plant roots were washed out in running tap water to remove the mud and microorganisms, shade dried for 15 days and chopped in to small pieces and further dried for another 15 days. Then these small pieces turned into coarse powder of roots using the household grinder and stored in air tight container till further use.

Chemicals and reagents

Iodine crystals (99%), Chloroform (99%), Sulphuric acid (98%), Hydrochloric acid (35%) (Himedia), Methanol (99%), Ethlye acetate (99%), Ethanol (99%) (MERK), Potassium iodide (99%), Ammonia solution (25%), Ferric chloride (98%), Lead acetate (99%) (Fisher scientific), Deionized distilled water(DI water) (milli Q), Benedict's quantitative reagent (Himedia), freshly prepared Wagner's reagent obtained by dissolving 6 gm of potassium iodide and 2 gmof iodine crystals. Similarly the Ferric chloride solution was prepared by dissolving 2 gm of Ferric chloride in 50 ml DI water.

Preparation of root extracts

Previously prepared coarse powder of roots turned into fine powder and5 gm of prepared powderwas extracted viafive separate solvents (100 ml of each) namelyMethanol, Ethanol,Chloroform, Ethyle acetate and DI water. Each of the extract was accomplished through magnetic stirring at the temperature 75° C for 1 hour. Further theextracts were filteredwith the help of Whatman filter paper no. 42 (Whatman International). All extracts were preserved in refrigerator at till further use.

Phytochemical screening

Each of the prepared extracts was treated as per the standard procedures to identify phytochemical components.

1) **Screeningof the tannins (Ferric chloride test):** In a test tube holding 2 ml of the prepared extract was treated with 4-5 drops of freshly prepared ferric chloride solution.Brownish green layer confirmed the presence of tannins[15].
2) **Screening of the alkaloids (Wagner's test):** A test tube filled with 2 ml of extract , 5-6 drops ofWagner's reagent were added slowly.The reddish-brown accelerate confirmed the acquaint ofalkaloids[16].
3) **Screening of the steroids (Salkowski's Test):** 2 ml of chloroform was mixed in 2 ml of extract; further similar volume of

concentrated $H_2SO_4$was added. The top layer revolve red and bottom layer revolve yellow with green glare, confirmed the presence of steroids[17].

4) **Screening of the saponins:** To identify the existence of saponins, 2ml of extract liquefyin 2 ml of Benedict's reagent. Blue –black acquaint indicates the presence of saponins[18].
5) **Screening of the cardiac glycosides:** To identify the presence of cardiac glycosides, 2 ml of extract liquefy with 2ml of chloroform in a test tube, after that add 1 ml of sulphuric acid added in it. Deep reddish brown color confirmed the presence of cardiac glycosides[19].
6) **Screening of the flavionds (Lead acetate solution Test):** Recognized the existence of flavonoids,2 ml of extract liquefy with 2 ml of 10 percent lead acetate. Yellowish green color confirmed the presence of flavonoids[20].
7) **Screening of the anthraquinones:** Firstly, 1 ml of extract simmers with10 percent of HCL for few moments by water bath process. Upon cooling to the room temperature the same amount of choloroform and few drops of Ammonia solution (10%) were added in it. A rose pinkcolor confirmed the present of anthraquinones[21].
8) **Phenol screening: (ferric chloride test)** To identify the existence of phenols, 1 ml of extract and 2 ml milli Q water combined in a test tube than few drops of 10 percent of ferric chloride in it. Appearance of blue or green color indicates presence of phenols [22].
9) **Examinationfor Terpenoids: (Salkowski's Test):** 2ml of extract is liquefy with 2ml of chloroform after that few drops of sulphuricacid consciously added in it.Presence of reddish brown color specify the terponoids[23].

Table-1 phytochemical examination of *Berberis asiatica* Roots

Phytochemicals	Methanol extract	Ethanol extract	Chloroform extract	Ethyl acetate extract	Aqueous extract
Tannins	_	+	+	+	+
Alkaloids	+	_	+	_	+
Steroids	_	_	_	_	_
Saponins	+	+	+	_	_
Cardiac glycosides	_	_	_	_	_
Flavonoids	+	+	+	_	+
Anthraquinones	_	_	_	_	_
Phenols	_	_	_	_	_
Terpenoids	_	_	_	_	_
+ component is present ; - component is absent					

Results and discussion

The screening accomplished with the five types of extraction solventson*Berberis asiatica* root powder. The presence of vital components having significant therapeutic values has been confirmed. The outcome is

summarized in Table-1.The screening process shows dissimilar results in different type of extraction solvents. The tannins are absent only in methanol extracted. Alkaloids present in methanol, water and chloroform extracts. Saponins presence found in methanol, ethanol and chloroform extract. Flavonoids absent only in ethyl acetate extract.Steroids, cardiac glycosides, anthraquinones, phenols, and terpenoids were absent in all extracts.

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Disclaimer Statment

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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Microbial contamination of eye make up product: Herbal Mascara a concern

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Abstract- Eyes are one of the most important features when it comes to beauty. Various cosmetics are used to enhance their level of attractiveness like mascaras, eyeliners, eye shadow among many more. Out of these above products mascara is the one which amplifies the eyelashes to look thick and long thus providing people with glamorous look to the eyes. But these cosmetic products must be safe to use by the consumers. In our study different brands of 15 mascara samples were randomly purchased from various cosmetic shops from suburbs of Delhi and examined microbiologically as per Indian Standard on cosmetics. Quantitativeresults of total microbial count ranged from 10^2 to 10^8cfu g^{-1}, whereas, in case of Yeast and Mould it was from 60 to 10^7cfu g^{-1}. Presence of microbial contamination in all examined mascara samples was found to be very high as compared to fungal count. Total microbial count, yeast & mould count exceeded the expected standard by considerable margin. Out of the 15 samples *Pseudomona-saeruginosa* was found in 6 samples whereas *Staphylococcus aureus* was found in 4 samples. However, none of the mascara samples were contaminated with *Escherichia coli*. The presence of *Pseudomonas aeruginosa*, *Candida albicans and Staphylococcus aureus* was found to be 40%, 47% and 27% respectively. *Staphylococcus aureus* is an organism associated with common infections caused due to any damage to the eye balls. Whereas, *Pseudomonas aeruginosa* is one of the most common agent in eye infections like conjunctivitis, keratitis and opthalmitis. Contamination in mascara may be a result of poor hygiene, contaminated raw materials or the susceptibility of the ingredients present in the cosmetic eye preparations.

Key Words: Mascaras, Microbial Contamination, Pathogens.

Introduction

According to The Federal Food and Drug Cosmetic Act criteria (2015), cosmetic means the articles intended to be rubbed, poured, sprinkled, or sprayed on, introduced into or otherwise applied to the human body or any part thereof for cleansing, beautifying, promoting attractiveness or altering the appearance and articles intended for use as a component of any such articles;except that such term shall not include soap(U.S. Food and Drug Administration (FDA), "The Federal Food and Drug Cosmetic Act Criteria" 2015).Beauty products should be easy to use, effective and safe. Make-up

products, especially those used in the eye area maytrigger a number of allergic and infectious reactions. The mainadverse effects include contact dermatitis by irritation, allergiccontact dermatitis and contact dermatitis by photosensitivity (photo toxicity), which may start an inflammatory reaction (Draelos*et al*, Saxena*et al*, Guin*et al*, Loden, *et al*, Biebl et al,Castanedo-Tardan*et al*). The cosmetics used on the ocular region arethe main cause of eyelid dermatitis, due to their pigments, resins, preservatives and vehicles (Draelos*et al*).

Mascara is one of the most popularcosmetic products, used to lengthen eyelashes and make themthicker, highlighting the feminine face (Rieger*et al*). However, this productis at a greater risk of contamination, being an aqueous-based formulation (Draelos*et al*). Also, the way it is handled may play a role in contamination, because of the greater chance of bacterial depositsoriginating from the environment and from the surface of the eyelashes making the product more susceptible to infections.

Many people use cosmetics unaware of the dangers that can threaten their health from their usage. From previous studies, researchers concluded that cosmetics such as eye makeup have the ability to induce microbial growth and possibly cause infections. Microorganisms can grow on almost every substances existing in nature and often able to attack or even decompose them, cosmetic ingredients are rich in nutrients that provide organic substrates in the form of sugar, starch, protein, amino acids, organic acids, alcohols, lipids etc. for microbial growth (Franca *et al*), addition to that ,water is a fundamental requirements for any microorganisms likely to contaminate the cosmetics products , thus untreated or non sterile water can support microbial growth leading to contamination of cosmetics products (Luis *et al*), generally microorganisms of interest in raw materials or cosmetic products grow best around neutral pH 7.0 and many yeast and molds are able to tolerate acid pH conditions(Razooki*et al*). To avoid microbial contamination of cosmetics during use and storage, the manufacturers add preservatives to their products. Laboratory evaluation of the effectiveness of the preservatives in mascaras by the usual microbiological procedures is difficult as many formulations are not readily solubilized by water (Ahearn*et al*).Antimicrobial preservatives are substances added to dosage forms to protect them from microbial contamination. However, inmany cosmetics no expiry date has been reported and may lose the preservativeactivity and became a potential risk for microbial contamination. But two different problems arise when preservatives are used in cosmetics, first are that micro-organisms can easily contaminate the cosmetics when the amounts of antimicrobial agents are kept low for safety and economy, and second are that serious problems of skin reactions produced by antimicrobial agents are caused when their amounts are increased for preventing microbial contamination. Some of the microbes like *Staphylococcus sp., Corynebacterium sp., Pneumococcus sp.,* are commonly found on or near the eye. *Staphylococcus epidermidis* and *Staphylococcus aureus* proliferate in contaminated mascaras.

The most common infections caused bythese microorganisms occur especially when the surface of the eyeballis damaged, in other words, traumatized (Draelos*et* al). *Pseudomonas aeruginosa*is the main agent of eye infections like conjunctivitis, keratitis and ophthalmitis, which may threaten the integrity of theeye, destroying tissues and

damaging visual acuity (Estevaet *al*). Infections by *P. aeruginosa* have been reported to occur due to contaminated mascara, trauma to the eye or bad hygiene (O'Donoghue*et al)*. Fungi also befound in contaminated mascaras, although less frequently thanbacteria, being related to immune-compromised people or thosewho wear contact lenses (Draelos*et al*, Estevaet *al*). Similar study also reported more of bacterial than fungal contamination (Nasser *et al*).

The quality and performance requirements for mascara are asfollows: They should (i) be non-irritating as they are applied soclose to the eyes, (ii) go on evenly and not harden the eyelashes orform blobs, (iii) make the eyelashes look thick and long, (iv) makethe eyelashes curl effectively, (v) have an appropriate lustre, (vi)have an appropriate drying time, (vii) not go on to the lower eyelidswhen dry and their appearance must not be spoiled by sweat,tears or rain, (viii) be easy to remove, (ix) be easy to use throughout their period of use, (x) not be contaminated by microorganisms(Mitsui *et al*).

The study involves: a) Collection of mascara samples from different location b) Enumeration of total aerobic microbial count as well as total yeast & mould count c) Isolation and identification of *Escherichia Coli*, *Pseudomonas aeruginosa, Staphylo coccus aureus* and *Candida albicans* from mascara samples d) Interpretation of the results for the benefit of human welfare by increasing general awerness among the people.

Material and Methods

Collection of mascara samples

Fifteen samples of Mascara (Fig. 1) were collected from different markets in Delhi region (Table-1). The samples were analyzed for microbiological parameters including total aerobic microbial Count, total yeast and mould Count,isolation and identification of pathogenic microorganisms like *Pseudomonas aeruginosa*, *Escherichia Coli, Staphylococcus aureus* and *Candida albicans* following respective "Indian Standard for Microbiological Examination of Cosmetics and Cosmetic Raw Materials".The materials and methods required for this study are listed here in accordance to their source of availability and grades:

Figure-1 Mascara samples taken from the Delhi market

Table-1 Sample collection from different location

S.No	Sample code	Name of location
1.	MK-1	Malkaganj, Delhi
2.	MK-2	Faridabad, Delhi
3.	MK-3	Tilak Nagar, Delhi
4.	MK-4	Paharganj, Delhi
5.	MK-5	Paharganj, Delhi
6.	MK-6	Paharganj, Delhi
7.	MK-7	Paharganj, Delhi
8.	MK-8	Paharganj, Delhi
9.	MK-9	Paharganj, Delhi
10.	MK-10	Mayapuri, Delhi
11.	MK-11	Paharganj, Delhi
12.	MK-12	Mayapuri, Delhi
13.	MK-13	Mayapuri, Delhi
14.	MK-14	Mayapuri, Delhi
15.	MK-15	Mayapuri, Delhi

Enumeration of Total Aerobic Microbial Count (TAMC)

1:10 dilution was prepared after homogenization of the sample. Then this was serially diluted up to 10^{-6} dilutions. 1ml of each dilution was transferred into sterile petri dishes. Molten media of Soyabean Casein Digest Agar was poured onto the respective plates as per Indian Standard and then incubated at 32^0C for 5days.After incubation the plates were observed for the bacterial colonies with the help of colony counter and then calculated as colony forming unit (cfu)/gram.

Enumeration of Total Yeast & Mould Count (TYMC)

1:10 dilution was prepared after homogenization of the sample. Then this wsa serially diluted up to 10^{-6} dilutions. 1ml of each dilution was transferred into sterile petri dishes. Molten media of Sabourauds Dextrose Agar was poured onto the respective plates as per Indian Standard and then incubated at 22^0C for 7days.After incubation the plates were observed for the bacterial colonies with the help of colony counter and then calculated as colony forming unit (cfu)/gram.

Isolation and identification of Pathogens

Detection of *Escherichia coli*

For the detection of *E.coli* approximately one gram was added in 9ml of Soyabean Casein Digest Broth and incubated at 32^0C for 48 hours. Further subcultured on Mac Conkey Agar plates and incubated at 32^0C for 48 hours. Plates was observed for appearance of pink colonies.Further confirmation done by gram staining and biochemical tests as per Indian Standard.

Detection *Staphylococcus aureus*

For the detection of *S. aureus* approximately one gram was added in 9ml of Soyabean Casein Digest Broth and incubated at 32^0C for 24 hours. Further subcultured on Baird Parker Agar plates and incubated at 32^0C for 72 hours. Plates was observed for appearance of black colonies with grey margin and clear halos. Further confirmation done by gram

staining and biochemical tests as per Indian Standard.

Detection of *Pseudomonas aeruginosa*

For the detection of *P. aeruginosa* approximately one gram was added in 9ml of Soyabean Casein Digest Broth and incubated at 32^0C for 24 hours. Further subcultured on Cetrimide Agar plates and incubated at 32^0C for 72 hours. Plates was observed for appearance of fluorescent colonies.Further confirmation done by gram staining and biochemical tests as per Indian Standard.

Detection of *Candida albicans*

For the detection of *C. albicans* approximately one gram was added in 9ml of Sabouraud Dextrose Broth and incubated at 72^0C for 24 hours. Further subcultured on Sabouraud Dextrose Agar plates and incubated at 32^0C for 72 hours. Plates were observed for appearance of white creamy colonies.Further confirmation done by gram staining and biochemical tests as per Indian Standard.

Results and Discussion

Highlighting and emphasizing the eye has been possible with a wide variety of eye cosmetics available. They include eye shadow, under eye concealers, eyeliners, mascaras, artificial eyelashes, eyebrow pencils (Draelos*et al*). Mascara has been the oldest and most commonly used option. This usually contains a mixture of waxes and pigments in addition to resins or petroleum distillates (Fagien*et al*). Effects of mascara are temporary. Risk of microbial contamination is always a problem. Contamination of microorganisms in cosmetics may cause spoilage of the product and when pathogenic they represent a serious health risk for consumers (Campana*et al*).Microbial contamination of cosmetic products is a matter of a great importance to the industry and it can become a major cause of both product and economic losses. The need of the microbial quality of cosmetics is well-clarified and well recognized.

Therefore, this study is aimed to evaluate the cosmetic products according to their microbial contents. Results of this study reflect the urgent need to reassess our methods to control the microbial contamination of cosmetics eye preparations. The results showed that Mascara an eye cosmetic preparation, when testedfound contaminated in varying degrees including bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* whereas all tested preparations were free from *Escherichia coli.* The total microbial count (Fig 3A &3B) of all detectedbacteria are ranging from 10^2- 10^8 cfu $g^{-1}$and yeast and mould count (Fig 3C &3D) in the range of 60- 10^7cfu g^{-1} (Table 2). It was observed in the present study that both total microbial count and yeast & mould count of Mascara were not in compliance with the specified requirements of the standard. However, presence of microbial contamination in all examined mascara samples was found to be very high as compared to fungal count.Fig 2 represents the range of TAMC & TYMC. Fungi can also be found in contaminated mascaras, although less frequently than bacteria being related to immune-compromised people or those who wear contact lenses [Esteva*et al*].

The study results revealed the drastic contaminating level of yeast and mould in six Mascara samples MK-1, MK-3, MK-4, MK-5, MK-6 and MK-11 which were in the range of 10^4-10^7cfu. g^{-1} whereas no yeast & mould was found in samples MK-2, MK-10, MK-12, MK-14 and MK-15.

Table-2 Microbiological profiling of Mascara Samples

Sample code	Total Aerobic Microbial Count cfu g^{-1}	Total Yeast & Mould Count cfu g^{-1}	*E.coli*	*P. aeruginosa*	*S.aureus*	*C.albicans*
MK-1	$1.0x10^7$	$9.0 x10^5$	Absent	Present	Absent	Absent
MK-2	$2.0x10^7$	Less than 10	Absent	Absent	Absent	Absent
MK-3	$1.0x10^7$	$8.0x10^4$	Absent	Absent	Absent	Present
MK-4	$3.0x10^6$	$1.3x10^5$	Absent	Present	Absent	Present
MK-5	$2.2x10^5$	$3.0x10^5$	Absent	Absent	Present	Present
MK-6	$3.2x10^7$	$1.5x10^6$	Absent	Present	Present	Present
MK-7	$5.6x10^4$	65	Absent	Absent	Present	Absent
MK-8	$2.1x10^4$	60	Absent	Present	Present	Present
MK-9	$1.2x10^7$	$1.6x10^3$	Absent	Present	Absent	Present
MK-10	$6.8 x 10^2$	Less than 10	Absent	Absent	Absent	Absent
MK-11	$1.8x10^8$	$3.7x10^7$	Absent	Present	Absent	Present
MK-12	$2.9x10^2$	Less than 10	Absent	Absent	Absent	Absent
MK-13	$8.6x10^2$	$9.2x10^2$	Absent	Absent	Absent	Absent
MK-14	Less than 10	Less than 10	Absent	Absent	Absent	Absent
MK-15	$1.2x10^2$	Less than 10	Absent	Absent	Absent	Absent

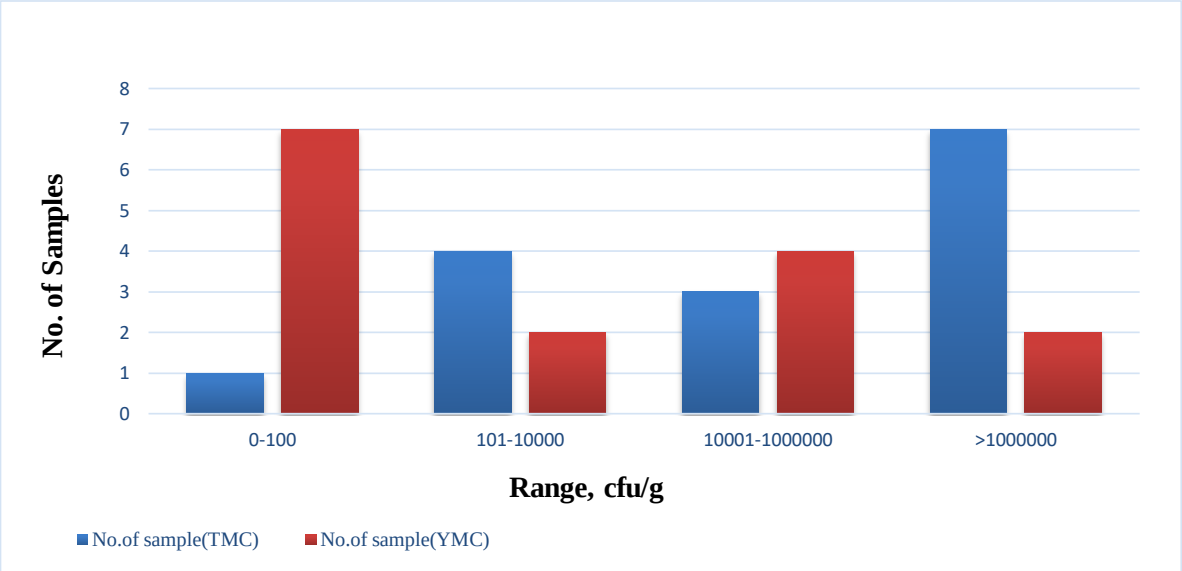

Figure- 2 Graphical representation of TAMC &TYMC in Mascara samples

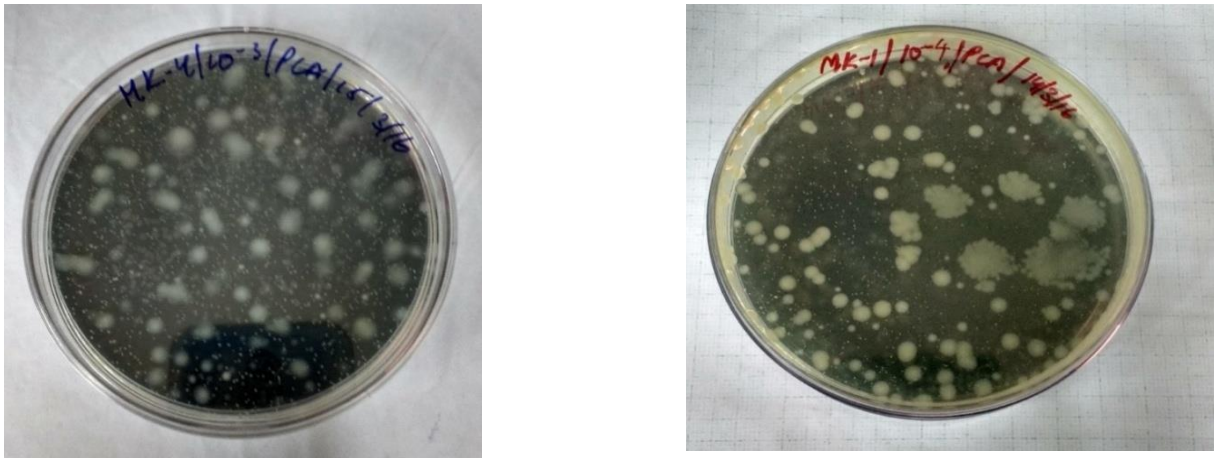

Figure- 3A and 3B Bacterial Colonies observed on Plate Count Agar

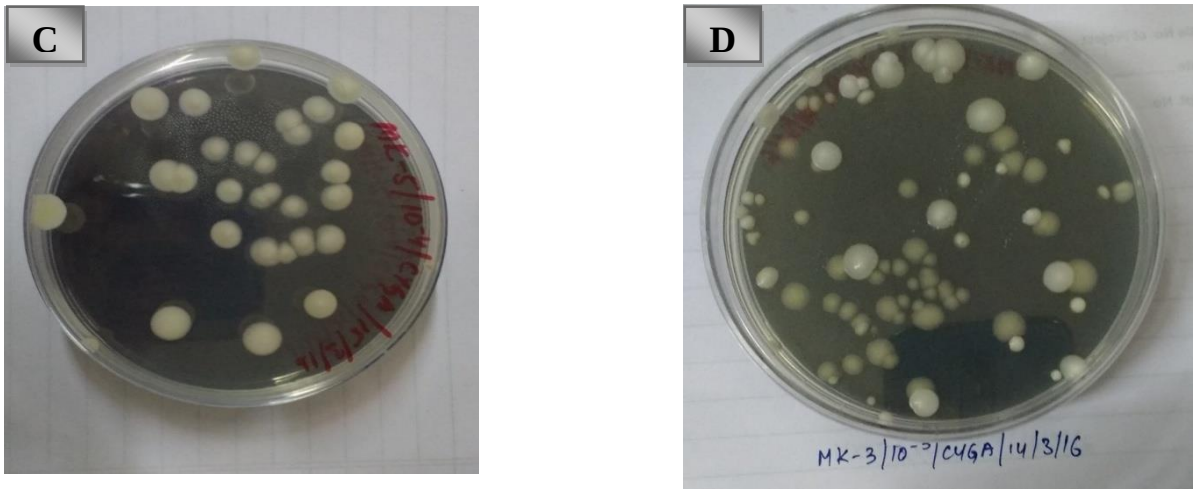

Figure- 3C and 3D Fungal Colonies observed on Chloramphenicol Yeast Glucose Agar

Table-3 Microbiological Limits of Eye products as per IS 14648-2011

Products	**Parameters**	**Limits**
Eye products (Products to be used in and around the eyes)	Total Microbial Count, cfu g^{-1}	100 (Max.)
	Yeast & Mould count, cfu g^{-1}	100 (Max.)
	Specified pathogens: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*and *Candida albicans*	Absent

Total aerobic microbial count, yeast & mould count exceeded the expected standard by considerable margin reflected in Table 2. The recommended microbiological standard for eye products (Mascara) is shown in Table-3 Biochemical characterization for identification of *Staphylococcus aureus* and *Pseudomonas aeruginosa*were shownin Table-4 and Table-5 respectively.

Table -4 Biochemical characteriztion of *Staphylococcus aureus*.

Sample Code	Gram Staining	Catalase Test	Coagulase Test
MK-5	Gram positive cocci in clusters	+	+
MK-6	Gram positive cocci in clusters	+	+
MK-7	Gram positive cocci in clusters	+	+
MK-8	Gram positive cocci in clusters	+	+
Positive Control *S. aureus* ATCC 6538	Gram positive cocci in clusters	+	+

Table-5 Biochemical characterization of *Pseudomonas aeruginosa*.

Sample Code	Gram Staining	Oxidase Test	Catalase Test	Growth On SMA	Hugh Leifson Test	Gelatin Liquefaction Test	Nitrate Test	Starch Hydrolysis
MK-1	Gram negative rods	+	+	+	+	+	+	-
MK-4	Gram negative rods	+	+	+	+	+	+	-
MK-6	Gram negative rods	+	+	+	+	+	+	-
MK-8	Gram negative rods	+	+	+	+	+	+	-
MK-9	Gram negative rods	+	+	+	+	+	+	-
MK-11	Gram negative rods	+	+	+	+	+	+	-
Positive Control *P. aeruginosa* ATCC 9021	Gram negative rods	+	+	+	+	+	+	-

(+) : Positive (-) : Negative

The microbial contamination in mascara sample indicates higher percentage of *Pseudomonas aeruginosa*, *Staphylococcus aureus*and *Candida albicans* showing an alarming situation for the cosmetic users. Our study revealed that out of 15 Mascara samples 9 samples were found to be contaminated with pathogenic micro-organisms. Out of all the samples studied it was found that 40% were contaminated with *Pseudomonas aeruginosa*, 27% with *Staphylococcus aureus*and 47% with *Candida albicans* whereas *Escherichia coli* were not detected (Fig 4). Although in six Mascara samples i.e. MK-2, MK-10, MK-12, MK-13, MK-14 and MK-15 no specified pathogen was detected (Table 2). According to this study, the results obtained showed that the presence of such higher counts and pathogen could lead to serious eye infections, damage of eye ball (Donoghue *et al*).

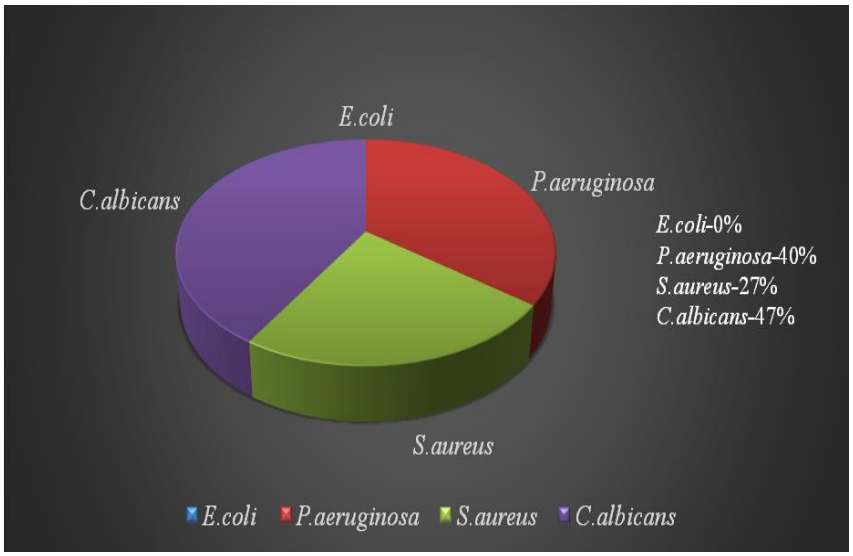

Figure-4 Percentage of pathogens in Mascara samples

The results of this study highlighted that microbiological contamination is a serious problem. Considering the risks generated by both the in appropriate usage and the possibility of in advertently using these products. There should be a public health campaigns warning of the need for the proper usage of these eye products. The contamination arises mainly because the consumers donot take proper care of the product or there is the lack of adherence to the requirements on the part of the industries that manufacturethe packaging.This may be a result of poor manufacturing practices, poor hygiene, contaminated raw materials or the succeptibility of the ingredients contained in the cosmetic eye preparation. Therefore, good manufacturing practices and hygiene must be carried out by the manufactures and personnel. Water must be tested continuously for microbial growth and raw materials should be tested before use specially those of natural origin and cosmetic eye preparation should be stored in an aseptic environment to avoid contamination before vending in the mark.

Conclusion and Recommendations

Microbiological safety is one of the most dynamic and critical parameter regarding quality of cosmetics. From the study conducted on Mascara samples it was found Total Microbial Count and Yeast and Mould count, exceeded beyond prescribed limits by Indian Standard on cosmetics. Moreover, presence of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and*Candida albicans* showed non-compliance of this product.In order to achieve of having safe cosmetics, there should be cooperative efforts from manufacturers, health authorities and consumers.

Channels between the quality controllers and the manufacturers should always be open to improve and check the conditions for the production of cosmetics. The consumer's role can be summarized in the following steps: the instructions recommended by the manufacturer should be followed carefully, good storage conditions should be established for the products during use and skin surfaces should be cleaned before and after using eye cosmetics. Therefore, good manufacturing practices and hygiene would be effective for the control of microbiological risks in Mascara. The nodal authorities should strictly follow the rules recommended by Drug and Cosmetic Act and should be restricted to the import and export of these cosmetic products causing health related problems.

Disclaimer Statment

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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Bioactive compound analysis in *Piper betle leaf*

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Abstract- Phytochemicals are bioactive compounds obtained from the plants and are widely applied in the traditional herbal medicine. These herbal medicines are used by the local people to cure the various diseases.*Piper betle* commonly known as paan is used as a herbal medicine from ancient time to till present days.The objective of the present study was to test phytochemicals as well as biochemical compound in leaves of *Piper betle*. *The results* showed that all the bioactive compound abundantly present i.e. tannin, saponins, flavonoids, terpenoids. Glycosides, proteins, carbohydrates and phenol in aqueous and other (methanol, acetone, diethyl ether, acetone) extracts. Whereas steroids are absent in all the extracts.

Key words: *Piper Betle*, Bioactive Compounds, Herbal Medicine.

Introduction

Paan, the treat – a betel leaf stuffed with a variety of ingredients – can be found in everywhere like people's homes, at restaurants, shopping centres and markets.*Tambul, tamalapaku, nagavalli, nagarbel, vettile* known by different names in different Indian languages. *Piper betle* commonly known as betel vine belongs to the family Piperaceae. It is a popular medicinal plant in Asia. India grows nearly 40 varieties out of the nearly 100 cultivated worldwide.

From the ancient time, analysis of biologically active natural yields from plants has attracted several natural product researchers. In the ayurvedic and Unani system of medicine, the betel plant is used as an anthelmintic, appetite stimulant, vermifuge, astringent, diarrhoea, aphrodisiac, breath freshener, carminative, cardiac tonic, dentifrice, in the prevention of diuretic emmenagogues, induction and increase of menstrual flow, laxative, strengthen gums, nerve tonic and also in the treatment of urinary disorders (Satyavati GV). Betel leaves may help in relieving headaches, fighting against cancer, healing wounds, may reduce gastric ulcers, diabetes, and allergies.(Karandeep Kaur et al, 2018)Betel leaves contain several phytochemicals and nutritional components like proteins, fats, minerals, fibre, carbohydrates, potassium, calcium, vitamin C and other nutrients.(Proshanta Guha,2022), which is recognized as a biologically active compound. These compounds have several significant pharmacological properties, in which antimicrobial, antidiabetic, antiulcer, anti-inflammatory, antimutagenic, and antioxidant properties are crucial once. Research on these compounds of betel leaves

have established their application as future active and additive ingredients in the pharmaceutical.

Material and method

Collection of plant material

The leaves of *Piper betle* were collected from the local market near at D.A.V(PG) College, Muzaffarnagar, UP, India and identified by the HOD of the Botany department, Dr. Sanjeev Kumar. The plant leaves were washed with running tap water.

Preparation of plant leaves extracts

The fresh leaves of the plant*Piper betle*were homogenised, and about 20 g of the sample from each variety was extracted separately with 200mL(10%) of aqueous (sterilized distilled water) and four different solvents: methanol, acetone, di ethyl ether and ethanol at room temperature. The extracts were filtered and filtrates were concentrated in a rotary evaporator at 45 °C.

Phytochemical and Biochemical screening of leaves of *Piper betle*

Preliminary qualitative screening of bioactive compounds present in the plant *Piper betle* was carried out with the following Chemical tests in the aqueous and four other (alcoholic and ether) extract using standards to identify the constituents as described by Sofowara (1993), Trease and Evans (1989), (Omoya and Akharaiyi, 2012),(Jyothiprabha and Venkatachalam, 2016). (Harborne and Williams, 2000).

Test of alkaloids (Dragendorff's Test)

5 ml each of the extracts, 5 ml of aqueous hydrochloric acid was added on a steam bath at 60°C for 5 min. The extract was filtered. Take one ml of the filtrate, few drops of Dragendorff's reagent were added. Appearance of Blue-black turbidity was positive for alkaloids.

Test of steroids (Libermann- Burchard Test)

1 ml of extract was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by the sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicates the presence of steroids.

Test of tannins (Braymer's Test)

5 ml each of the extracts were stirred separately with 100 ml distilled water and filtered. One millilitre ferric chloride reagent was added to the filtrate. A blue-black or blue green precipitate was an indication of the presence of tannins.

Test of terpenoids (Salkowski Test)

5 ml of extract was taken in a test tube and 2 ml of chloroform was added to it followed by the addition of 3 ml of concentrated sulphuric acid. Formation of reddish -brown layer at the junction of two solutions confirms the presence of terpenoids.

Test of flavonoids (Alkaline reagent Test)

2 ml of extract was treated with few drops of 1N sodium hydroxide solution and observed the formation of intense yellow colour. This yellow colour becomes colourless on addition ofdilute hydrochloric acid, indicating the presence of flavonoids.

Test of saponins (Foam Test)

5 ml each of the extracts were mixed with distilled water and shaken separately in a test tube. Frothing, which persists on warm heating was taken as preliminary evidence of the presence of the saponins.

Test of Phenols (Ferric chloride Test)

2ml of plant extract, 2ml of distilled water followed by 10 % $FeCl_3$ solution was added. Bluish black colour indicates the presence of phenol.

Test of Carbohydrate

Molisch's test- 5 mg extract was taken in test tube than the 1 ml of Molisch's reagent was added into it. Mixture was shaken properly. After that, 2ml of concentrated Sulfuric acid was poured carefully along the side of the test tube. Appearance of a violet ring at the interface indicated the presence of carbohydrate.

Benedict's test-Benedict's reagent was taken for the analysis of carbohydrate. the 5 mg extract was mixed with few drops of benedict's reagent, then allowed to boiled, the reddish-brown precipitate is found with the presence of the (reducing sugar)carbohydrates.

Test for proteins

Ninhydrin test- aqueous extract of *Piper betle* was mixed with 2 ml of 0.2% solution of Ninhydrin and boiled for 2 min on water bath, if violet colour appeared with the presence of amino acids and proteins in the aqueous extract.

Biuret's test- 5 mg extract was added with the few drops of biuret's reagent. The obtained mixture was shaken well and allowed to warm for 1-5 min. Appearance of red or violet colour indicated presence of proteins.

Test of Glycosides (Keller-kilani test)- Crude extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 2ml of concentrated H_2SO_4. A brown ring at the interphase indicated the presence of cardiac glycosides.

Results and Discussion

Results of the phytochemical screening of the plant are indicated in table. Presence of the phytochemicals is indicated by a + sign while a – sign indicate absence of the tested phytochemical.This work principally focuses on phytochemical screening in the leaves of *Piper betle* . The results are summarized and mentioned below. The current study was carried out on the *Piper betle* unconcealed the presence of medicinally active constituents. The phytochemical screening in the leaves of *Piper betle* showed that the abundant presence of all bioactive compoundi.e.tannin, saponins, alkaloids, flavonoids, terpenoids,phenol,glycosides, protein and carbohydrates in aqueous and other extracts. While steroids are absent in aqueous and other leaf extract.The latest analysis investigation has ascertained that the bioactive and inhibitorpotentials of those plants are attributed to the presence of phenols, flavonoids, alkaloids, terpenoids, saponin, and tannins (Agbor *et al*., 2011). Terpenoids are concerned with medication and antineoplastic functions thus employment of the plant to treat burns, skin diseases, and bug stings (BownDeni*et al*., 1995), Flavonoids are also present in all the extracts as a potent water-soluble antioxidant and free radical scavenger, which prevent oxidative cell damage and also have strong anticancer activity (Salah N,1995; Rio DA,1997]. Tannin rich medicinal plants are used as healing agents in a number of diseases Doughari JH (2012). Alkaloids comprising a large group of nitrogenous compounds are widely used as cancer chemotherapeutic agents, anaesthetics and Central Nervous Stimulants (Noble RL 1990; Madziga HA, 2010).

Table- Screening of bioactive compoundspresent in different extracts of leaves of*Piper betle*.

S.No.	Bioactive compound	Test name	Extracts				
			Aquoeus	Methanol	Acetone	Diethyl ether	Ethanol
1	Proteins	Ninhydrin test	+	+	+	+	+
		Biuret test	+	+	+	+	+
2	Carbohydrates	Molisch's Test	+	+	+	+	+
		Benedict Test	+	+	+	+	+
3	Alkaloids	Dragendorff test	+	+	+	+	+
4	Flavonoids	Alkaline reagent test	-	+	+	+	+
5	Steroids	Libermann Burchard Test	-	-	-	-	-
6	Tannin	Braymer's Test	-	+	+	+	+
7	Saponin	Foam Test	+	+	+	+	+
8	Phenols	Ferric Chloride Test	+	+	+	+	+
9	Terpenoids	Salkowski Test	+	+	+	+	+
10	Glycosides	Keller-kilani test	+	+	+	+	+

Conclusion

Natural products play a very important role within the field of recent medication analysis and development due to its low toxicity, simple handiness, and low price.Our results suggest that *Piper betle* can serve as potential source of bioactive healthy compounds and their consumption could be useful in the prevention of diseases. Determination of the natural phytochemicals and antimicrobial compounds can facilitate to develop of new drug candidates for antimicrobial medical aid.From the above studies, It is concluded that as the plants*Piper betle* studied, found to rich in phytochemicals, are full of pharmacological and medicinal significance and is used for herbal drugs.

Further study is required to find their potentials in the mentioned biological properties such as antidiabetic, anti-tumor, etc. It is concluded that as the plants studied, found to rich in phyto-chemicals, are full of pharmacological and medicinal significance. It is concluded that as the plants studied, found to rich in phytochemicals, are full of pharmacological and medicinal significance

Disclaimer Statment

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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Inhibitory activity of organic solvent extracts of *Murraya koenigii* on *Staphylococcus aureus*

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Abstract-*Staphylococcus aureus* is one of the most in famous and widespread bacterial pathogen. This pathogen can cause a wide variety of diseases, ranging from moderately severe skin infections to fatal pneumonia and sepsis. Treatment of *Staph. aureus* infections is complicated due to antibiotic resistance. There has been ongoing and increasing interest in the extraordinarily high number of toxins and other virulence determinants that *Staph. aureus* produces and how they impact disease. In the past few decades, a more dangerous form of *Staph.aureus* has emerged. This form is known as Methicillin-Resistant *Staph.aureus* and usually referred to by the acronym MRSA. Keeping in view of the problem of Antimicrobial Resistance(AMR).This study was designed to explore antibacterial activity of a common kitchen herb *Murraya koengii* against pathogenic gram positive *Staphylococcus aureus*.Four different plant extracts were prepared using solvents- Methanol, Hexane, Aqueous and Ethanol+Aqueous (1:1) and tested against *Staphylococcus aureus* by using Kirby bauer Disc diffusion method. Methicillin antibiotic disc was used as the positive control.

Keywords: *Murraya koenigii*, Antibiotic resistance, MRSA, Antimicrobial activity, Methicillin

Introduction

Unnecessary use of antibiotics in today's world is one of the major causes for the rising emergence of multi drug resistant pathogenic strains that do not respond to the usual line of treatment. Therefore, the need to search for new antimicrobials remains unchallenged. Currently, in addition to antibiotics and chemically synthesized drugs, the trend to look out for alternative medicines such as natural or herbal medicines is increasing because they may have fewer side effects or toxicity owing to their natural sources (Anita Joshi et al, 2009).

Curry leaves (*Murraya koenigii*) belongs to the Rutaceae family, and is native to India and the Southeast Asian Region.It is a popular leaf-spice used in very small quantities for their distinct aroma due to the presence of volatile oil and their ability to improve digestion. Curry leaves are natural flavoring agents with a number of important health benefits. They contain several medicinal properties such as anti-diabetic, antioxidant, antimicrobial, anti-fungal, anti-inflammatory, anti- carcinogenic and hepato-protective properties. The various notable pharmacological activities of the plant include activity on heart, anti-diabetic and cholesterol reducing property, antimicrobial activity, antiulcer activity, antioxidative

property, cytotoxic activity, antidiarrheal activity, phagocytic activity(Bhandari PR, 2012 and Disegha GC., et al 2014). These leaves are widely used in Asian cuisines for flavouring foods. The leaves have a slightly pungent, bitter and feebly acidic taste, and they retain their flavour and other qualities even after drying. Curry leaf is also used in many traditional cultures namely Indian. Ayurvedic and Unani prescriptions (Suman Singh et al, 2014).

Staph. aureus bacteremia has been noted to account for a greater number of deaths than that caused by acquired immune deficiency syndrome (AIDS), tuberculosis, and viral hepatitis combined (Klevens RM, Morrison MA, Nadle J, et al.) Other *Staph. aureus* infections, such as moderately severe skin infections, including furuncles, abscesses, and wound infections, are usually not life-threatening but may be accompanied by significant morbidity and pain. Due to their frequency they represent a considerable public health burden (McCaig LF, McDonald LC, Mandal S, et al.)

Staph. aureus infections are particularly problematic due to frequently occurring antibiotic resistance in *Staph. aureus* isolates, among which methicillin-resistant *S. aureus* (MRSA) are the most important clinically (Turner NA, Sharma-Kuinkel BK, Maskarinec SA, et al.). Infections by MRSA are accompanied by increased mortality, morbidity as compared to those caused by methicillin-sensitive *S. aureus* (MSSA) (Ippolito G, Leone S, LauriaFN, et al.)The rates of methicillin resistance among clinical isolates varies greatly by country, ranging from single-digit rates in Scandinavian countries to over 50% for example in the U.S. and China (Stefani S, Chung DR, Lindsay JA, et al).While hospital-associated MRSA infections are on the decline in the U.S., Europe, China, and many other countries, likely due to increased hygiene and surveillance measures, they are still on the rise in poorly developed countries, for example in Africa (Falagas ME, Karageorgopoulos DE, Leptidis J, et al).

Material and Methods

The plants used in this study were obtained from Himalaya Wellness Company, Dehradun. The curry leaves were shade dried for 3 days and powdered using an electric blender.

Methanol extraction: The methanol extract was made by, adding 50 gms of curry leaves powder in 250 ml of methanol (w/v). Flask containing samples were kept on rotary shaker for 24 hours.

Ethanol extraction: The ethanol extract was made by, adding 50 gms of curry leaves powder in250 ml of ethanol (w/v). Flask containing samples were kept on rotary shaker for 24 hours.

Hexane extraction: The extract was made by, adding 50 gms of curry leaves powder in250 ml of hexane (w/v). Flask containing samples were kept on rotary shaker for 24 hours.

Hydro-alcoholic extraction: 50gms of curry leaves powder in 250 ml of solvent containing ethanol and aqueous in the ratio 1:1(w/v). Flask containing samples were kept on rotary shaker for 24 hours.

After completion ofshaking the extracts were filtered using whattmanfilterpaper. The filtered extracts were collected and concentrated on waterbath at 60°-80°c to make the final volume of the curry leaf extracts for the experiment.

Determination of antimicrobial activity: Antibacterial activity of extracts of the

Murrayakoenigii was evaluated by the Kirby bauer Disc diffusion method. Nutrient agar media was prepared and inoculated with *Staph.aureus.* Inoculated media was poured in sterile petridish and kept until plates get solidified. The sterile disc wereplaced in extracts separately (40-50 µl microliter in each disc).After that Sample impregnated discswere placed on nutrient agar plates, using a sterile forcepand the plates were incubated at 35-37°c for 24 hours. The inhibition zone around each disc was measured in millimeters (mm). The standardMethicillin antibiotics disc used as positive control to test the curry leaves microbial inhibition activity.

Results and Discussions

The antibacterial effects of curry leaves (*Murraya koenigii*) extracts as tested on test strain samples and compared with known antibiotics are described with inhibition zones measured in millimeters.

Table -1

Extracts	**Zone of Inhibition** *Staphylococcus Aureus* **(Standard Atcc) Culture**	**Zone of Inhibition** *Staphylococcus Aureus* **(Clinical Isolates)**
Methanol	15 mm	11 mm
Hexane	10 mm	NA
Aqueous	10 mm	NA
Ethanol + Aqueous	16 mm	18 mm
Methicillin	25 mm	15 mm

Results of this study is as shown in table-1, in case of standard culture of *Staph.aureus* the highest zone of inhibition of 16 mm was found in hydro-alcoholic extract, followed by other extracts methanol (15 mm), hexane (10 mm), aqueous (10 mm). 25 mm zone inhibition towards Methicillin(Plate-1and 5). While Clinical isolate of *Staph aureus* showed highest zone of inhibition(18mm) towards Ethanol+Aqueous extract of *Murraya koengii* Plate-1 and 2.While no or very small zone of inhibition against Methicillin antibiotic.

Zones of Inhibition

Staphylococcus aureus **(Standard ATCC culture)**

Plate-1

Plate-2

Plate-3

Plate-4

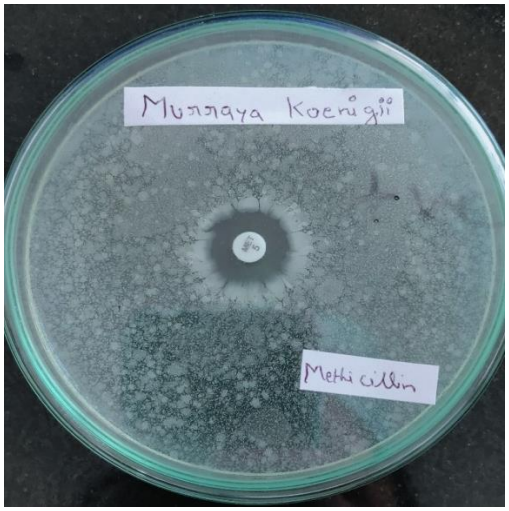

Plate-5

Zones of Inhibition

Staphylococcus aureus **(Clinical isolates)**

Plate-1

Plate-2

Indiscriminate use of antibiotics has led to the rising emergence of multi drug resistant pathogenic strains of bacteria causing diseases that challenge regular treatment protocols. Currently, in addition to antibiotics and chemically synthesized drugs, the trend to look out for alternative medicine in nature is increasing as the natural resources are less toxic and less deleterious to the overall health of human beings (Anita Joshi et al, 2009) In this context curry leaves have displayed immense potential as that natural alternative, especially with its antimicrobial property.Curry leaf extracts have demonstrated inhibition zone against *Staphylococcus aureus*.Subsequently, fully susceptible bacteria exhibited a large zone of growth inhibition around the disc; less sensitive (intermediate sensitive) isolates exhibited a smaller inhibition zone and resistant bacteria indicated no clear zones or grew up to the disc edge (Mohar Singh A et al, 2011&(Rajendran MP et al, 2014)).

Conclusion

Murrayakoenigii (Curry leaves) extracts have demonstrated antibacterial effects particularly on *Staphylococcus aureus*, as compared to antibiotics such as methicillin in our study. The hydro-alcoholic extract (ethanol + aqueous) of curry leaves were found to be most effective than other extracts against clinical isolate which is highly resistant towards antibiotic methicillin. *M.koenigii* has the potential to develop plant based antimicrobial drug it may be the best natural alternative to antibiotic therapy for *Staphylococcus aureus*. Therefore, curry leaves could be used as a natural remedy for the prevention of bacterial infections. Indeed this phenomenal plant may serve as a useful resource in the food industry and clinical medicine. Detailed research study is required on *Murrya koengii* to further explore its antibacterial activity and its future role as an alternative plant based drug against pathogenic Staphylococci.

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Effect of *Pueraria tuberosa* on female reproductive organs of albino rats

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Abstract- *Pueraria tuberosa* (Roxb ex Willd), Family – Fabaceae is popularly known as *Vidarikand*, English - *Kudzu*, a perennial herb found mostly in high Himalayas. It has many therapeutic properties including anti-fertility (Gupta *et al.*, 2005) in male albino rats, but, there is no mention about effect on female animals. Effect of crude root powder (*kand*) of this plant is reported in this paper by authors. Histological studies of the ovary and uterus were undertaken after oral administration of aqueous solution of the *kand* (25, 50 and 75 mg/kg/day/rat) for 30 days. The naked eye observation revealed no change and when examined histologically, there was an evidence of follicular atresia with absence of primary and secondary follicles at 50 and 75 mg kg dose for 30 days. There was no severe regressive change observed in the histoarchitecture of uterus. It appears that the plant may be used in female fertility regulation.

Keywords: Herbal drugs, Phytomedicine, Female reproduction, Contraception, *Pueraria tuberosa* D.C.

Introduction

Pueraria tuberosa D.C., family – Fabaceae (Hindi - Vidarikand; English - Kudzu) is a perennial herbaceous twinner plant found throughout India, Nepal and Pakistan and also in other Asian countries including China. At Himalayan mountain ranges. It is found upto the height of 4000 feet. It has big sized tuberous roots often rounded in shape and slightly sweet in taste. It is highly nutritious. According to Ayurveda, the tubers are aphrodisiac, diuretic and galactogogue (Kirikar and Basu, 1935). Modern researchers have reported its effects on many health problems namely anticonvulsant (Basavaraj *et al.*, 2011), antidiabetic, (Oza and Kulkarni, 2018a), anti-inflammatory (Tripathi *et al.*, 2013), antistress (Verma *et al.*, 2012), cardioprotective (Patel *et al.*, 2018), hypolipidemic (Tanwar *et al.*, 2008), immunomodulatory (Patel *et al.*,

2016), neuroprotective (Xing *et al.*, 2011), wound healing (Kambhoja and Murthy, 2007). Concerning with reproduction, antifertility effect of this plant was reported by Gupta *et al.*, (2005) in male albino rats. Considering the above point, the effect of tubers of *Pueraria tuberosa* was carried out on female reproductive organs of albino rats (*Rattus rattus norvegicus*). The results are reported in this paper.

Phytochemistry

In the crude tuber extracts of *P. tuberosa* the following chemicals-alkaloids, anthracene, anthracyanine, anthraquinone, glycosides, carbohydrates, catecholic compounds, coumarines, flavonoides, sugars and volatile oils have been reported. Viji and Paulsamy (2015, 2018) reported above mentioned compounds including phenols and tannins using HPTLC. Pandey and Tripathi (2010) reported tuberocin, puerarostan, β-sitosterol from ethanolic tuber extract. Liquid chromatography – mass spectrometry (LC-MS) analysis of ethanolic extract was found to contain puerarin, diadzein and formononetin (Chauhan *et al.*, 2013). Two most active (pharmacologically as well as therapu-etically) chemical compounds were reported, first, Puerarone (Fig. 1a) by Maji *et al.* (2014) and second, tuberostan (Fig. 1b). These compounds are flavonoids and extracted from aqueous tuber decoction of *P. tuberose (Fig.-2)*.

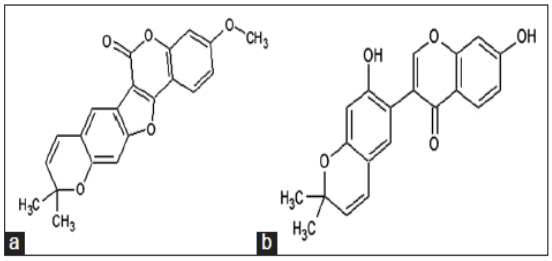

Figure- 1 Chemical structures of potential phytochemicals from *Pueraria tuberosa*: (a) Puerarone; (b) Tuberostan

Figure- 2 Tuberous root of *Pueraria tuberosa* (Roxb ex Willd) D.C.

Material and Methods

The tuber roots of *Pueraria tuberosa* D.C. were collected from Chakrata tehsil of Dehradun district of Uttarakhand in and around forested area of Tiuni. The tubers (roots) were identified by Botanists of Botanical Survey of India (B.S.I.), Northern circle, Kalagarh Road, Dehradun, Uttarakhand (India). Freshly collected air dried and mechanically powdered tuberous (tuber powder) was used as a drug. The doses 25, 50 and 75 mg/kg work prepared by adding gum acacia powder (05 mg/dose) as vehicle. Each dose was dissolved in distilled water in such a way that the dose was equivalent to 2 ml of drug solution and administered orally daily with the help of catheter tube fitted into a specially designed syringe-needle for 30 days. Control female rats (05-rats)

received vehicle only for experimental period.

Regularly cyclic adult female rats (100-120 gms body weight) were selected for the experiment. They were maintained under uniform husbandry conditions with free access of food (Hindustan Lever Limited) and tap water ad libitum. The three doses 25, 50 and 75 mg/kg were given (as described above) to group II, III and IV of rats respectively for 30 days. 05 rats were included in each group. Female rats of control group (group I) received vehicle (gum acacia powder) in the similar manner.

Albino rats were maintained as per the protocol outlined in publications of the committee for the purpose of control and supervision of experiments on animals. Standard guidelines and approval was obtained from Institutional Animal Ethical Committee appointed by the Principal for laboratory animals.

All the female rats in each group, 24 hours after the last day's dose, were sacrificed by the decapitation. The ovaries and uterii were dissected out, freed from surrounding tissues, bloated on filter paper and weighed quickly on semi-micro balance. For histological studies, ovaries and uterii were fixed in Bouin's fluid, dehydrated, paraffin embedded tissue sections were cut at 6 micron and stained with Ehrlich's Haemotoxylene and Eosine. Prepared slides of ovary and uterus were photographed and histopathological changes were described.

The body weight and organ weight prior to start of experiment and last day of experiment (30th day) was taken and recorded. The significance of difference of weight between treated and control rats was assessed by the student 't' test taking $p < 0.05$ as significant.

Results

Body and Organ Weight Changes

The results are presented in table-1. The female rats of control group did not show any change i.e. reduction in the body weight. It was maintain throughout the experimental period. Similarly, no significant reduction in body weight was noted at any dose level of *Pueraria* tuberosa, tubers powder dissolved in distilled water as solution administered for 30 days. However, the ovarian weight was reduced significantly ($p < 0.05$) after the treatment with a doses 50 and 75 mg/kg/day for 30 days. No significant reduction of weight in uterus was found at the given dose level.

Histopathological Changes In Reproductive Organs

Control: The histoarchitecture of ovaries of control rats revealed the normal cellular structures with organised germinal epithelium, all types of follicles, developing, maturing and fully mature or gravid follicles with antrum and ovum, a few atretic follicles, interstitial cells, normal vascularity and loose stroma (Figure-1). The histoarchitecture of uterii of control group of rats presented the normal features. The endometrium which surround the lumen made up of columnar epithelial cells. Lumen was wide, uterine glands were normal, tortuous and distributed in the stroma of myometrium. The musculature (myometrium) was well developed and vascularity appeared normal (Figure- 4).

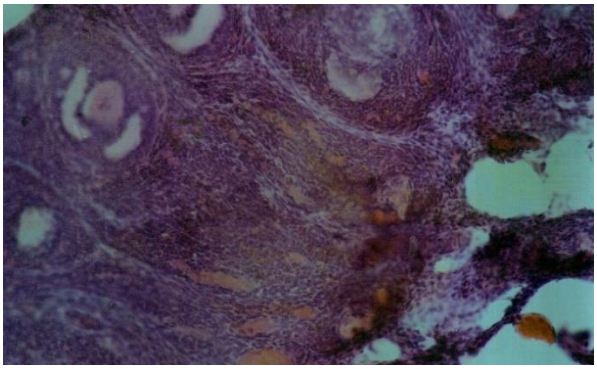

Figure-1 T.S. of ovary of control albino rats showing normal histoarchitecture with Primary (Primordial), secondary (developing) and mature (Graffian) follicles. A few atretic (degenerated) follicles, stroma and vascularity appears normal. Corpora lutea are also seen. X

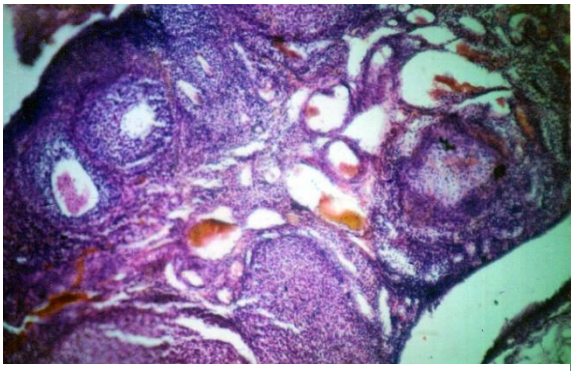

Figure- 2 T.S. of ovary of treated albino rats with *Pueraria tuberosa*, tuber aqueous solution at dose of 50 mg/kg for 30 days showing follicular atresia of primary and secondary follicles, absence of corpora lutea in loose stroma and normal vascularity. X 150

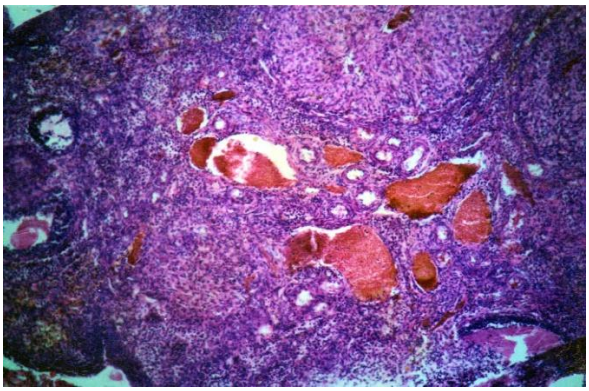

Figure-3 T.S. of ovary of treated albino rats with *Pueraria tuberosa*, tuber aqueous solution at dose of 75 mg/kg for 30 days showing mass atrophy of follicles, absence of corpora lutea compact stroma and less vascularity. X 150

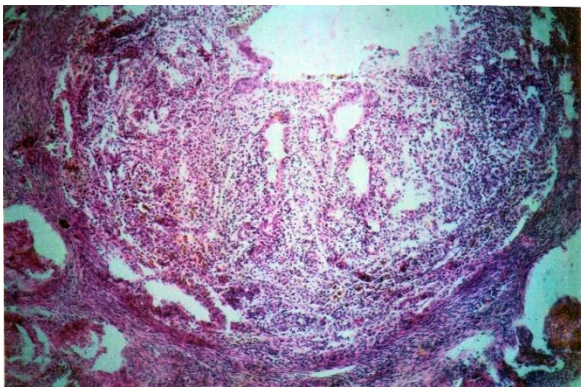

Fig.4. T.S. of uterus of control albino rats showing normal structural features. The endometrium showing columnar epithelial cells, tortuous uterine glands, normal musculature, loose stroma, wide uterine lumen and normal vascularity.. X

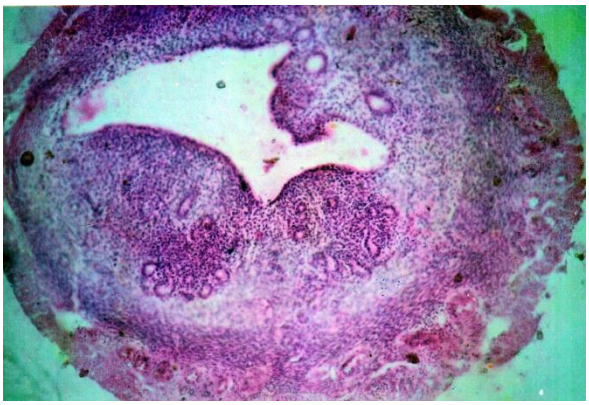

Figure-5 T.S. of uterus of treated albino rats with *Pueraria tuberosa*, tuber aqueous solution for 30 days at dose of 50 mg/kg showing less histopathological changes in the uterine elements like endometrium, uterine glands and uterine lumen. X 150

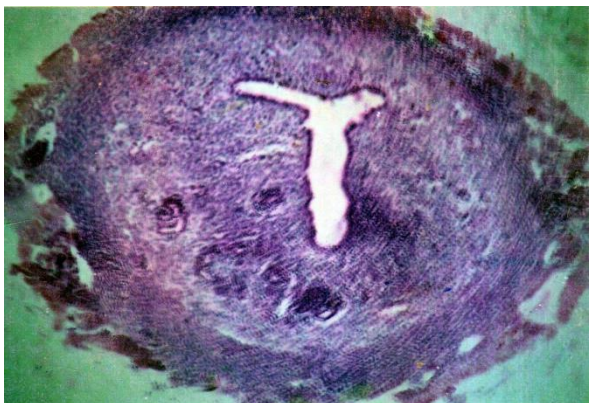

Figure-6 T.S. of uterus of treated albino rats with *Pueraria tuberosa*, tuber aqueous solution for 30 days showing mild histopathological changes in the uterine elements like distorted endometrium, shrunken uterine glands, reduced uterine lumen, stroma and vascularity. X 150

Treated (by *Pueraria tuberosa*)

The administration of 25 mg/kg/day dose of *P. tuberosa* aqueous solution for 30 days caused no histopathological changes in the ovaries. Many developing follicles can be seen with a few atretic follicles. The ovaries of rats treated with higher doses (50 and 75 mg/kg/day) for 30 days showed marked follicular atresia (degeneration of follicles) of primary and secondary follicles. Graffian (Gravid) follicles were few and appeared not much affected (Fig.2) but highly affected in the ovaries (Figure-3). There were no corpora lutea in both the cases. Primordial oocyte population was significantly reduced and degenerated. The vascularity, stromas were not much affected and appeared normal.

The administration of 25 mg/kg/day dose of *P. tuberosa* aqueous solution for 30 days did not cause histopathological changes in the uterii of treated rats. It did not differ much from the picture of uterus of control group of rats. Similarly, the doses 50 mg/kg and 75 mg/kg/day for 30 days caused mild regressive changes in the uterine elements. The endometrium, the lumen which is fully distorted appeared normal. The uterine glands were slightly reduced and not much regressed. The musculature (myometrium) and vascularity were also not much affected (Figure- 5,6).

Table-1 Effect of *Pueraria tuberosa* tuber powder as aqueous solution on body weight (gms) and genital organ weight (milli gms) of female rats treated with various doses for 30 days. 05 rats were used in each group. Values are mean ± S.E.

Doses (mg/kg)	Body weight (gms)		Genital organ weight (milli gms)	
	Initial	Final	Ovaries	Uterii
Control	110.30 ± 06.40	130.15 ± 11.20	84.27 ± 17.92	110.25 ± 10.35
25	105.20 07.50	102.10 ± 07.15	80.25 ± 13.98	108.17 ± 25.90
50	112.52 ± 17.20	110.13 ± 09.12	53.15 ± 17.92*	95.25 ± 15.13
75	115.10 ± 10.23	112.17 ± 10.15	50.20 ± 25.65*	90.14 ± 37.52

* $p < 0.05$

Discussion

In the present study which deals with effect of *Pueraria tuberosa* D.C. tuber root crude powder as aqueous solution/ suspension on reproductive organs i.e. ovaries and uteri of female albino rats. According to studies, the ovarian and uterine weight was reduced by administration of the above plant with the increasing dose level 25 mg, 50 mg and 75 mg/kg/day ($p < 0.05$). The doses 50 and 75 mg/kg caused deleterious/regressive effect on ovarian elements such as primordial, developing, mature and Graffian follicles. The doses increased formation of atretic follicles. i.e. follicular atresia.

The formation of follicles with normal ova, ovulation. fertilization, implantation and change in the estrous cycle and genital organ weight (ovary and uterus) are controlled by hormones, oestrogen and progesterone (Lerner, 1969). Pincus *et al.* (1956) reported that the reduction of ovarian weight was due to suppression of endogenous oestrogen or progesterogenic action. Pandey (1990) reported that the

administration of *Adhatoda vasica* leaves as aqueous extract caused widespread damage to all ovarian elements including reduction in weight. Singh (1017) observed atrophic changes in ovary and uterus caused due to administration of *Stevia rebaudiana* leaves. Present study of histopathological changes in female reproductive organs are comparable to the studies made by Chakraborti *et al.* (1968) when female albino rats fed with green leaves of *Artobotrys odoratissimus* Linn. The follicular atresia and other degenerative changes in ovary which have similarity with present observations. The results reported in the present study on *Pueraria tuberosa* are similar with results reported by Dixit (1977) by administration of *Malva viscus conzattii* flower extract in female genital organs of Indian gerbil (*Meriones hurrianae* Jerdon). Singh and Singh (1992) also reported similar histopathological in the ovary and uterus after administration of *Cassia fistula* flower extract in female albino rats within 30 days at different doses. The dose 25 mg/kg dose for 30 days did not cause histopathological and other changes in reproductive organs of female albino rats but 50 and 75 mg/kg doses caused deleterous/ regressive changes in the present study.

Conclusion

As stated above *Pueraria tuberosa* D.C. is very popular for traditional remedy among people of India and China including other Asian countries. Its tubers and leaves are used as folk medicine. It has shown medicinal value for anticancer, anticonvulsant, antidiabetic, anti-inflammatory, antioxidant, antistress, cardioprotective, hepatoprotective, immu-nemodulatory, neuroprotective and wound healing. Antifertility activity in male albino rats was already reported and effect of tubers on female reproductive organs i.e. ovaries and uterii are reported in this paper. It has two very important and active phytochemical, besides many others, known as flavonoides – Puerarone and Tuberostan. According to the present study, the tubers of *P. tuberosa* have shown regressive deleterious effect on ovary and uterus of female albino rats. It is concluded that the plant material – tubers of *Pueraria tuberosa* may be useful to control female reproductive function for family planning.

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Disclaimer Statment

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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Cover page plants details

Rheum emodi (Revand chini)

India is one of the twelve mega-biodiversity countries of the world having rich vegetation with a wide variety of medicinal plants and a tradition of plant-based knowledge distributed amongst a vast number of ethnic groups. *Rheum emodi* or Himalayan rhubarb is a perennial herb belongs to family Polygonaceae. It has been used in various traditional systems as laxative, tonic, diuretic and to treat fever, cough, indigestion, menstrual disorder since antiquity. This paper consists of literature of Rheum emodi regarding its ethno botany, folkloric uses, chemical properties and pharmacological studies. The most common constituents of *Rheum emodi* are anthraquinone (rhein, chrysophanol, aloe-emodin, emodin, physcion, and their glycosides) and stilbene (picetannol, resveratrol and their glycosides). Studies have shown that Rheum emodi possess anticancer, antioxidant, anti-inflammatory, antimicrobial, antifungal, antidyslipidemic, ant platelet, ant diabetic, antiulcer, hepatoprotective, immune enhancing and nephroprotective activities. These studies raised the therapeutic efficacy of rhubarb in diverse ailments. It is essential that this medicinal plant should study more extensively to confirm these therapeutic effects and to check the traditional claim that this plant have significant medicinal properties as is reported in the traditional knowledge of the indigenous people that the plant is used in the treatment of gastrointestinal infections, respiratory infections, liver and skin infections.*Rheum emodi* is a mild purgative, astringent, tonic, laxative, stomachic, and aperient. Powdered rhizomes are sprinkled over ulcers for quick healing.It is also reported to be a potent anti- inflammatory drug.

Curcuma longa Linn *(Haldi)*

Curcuma longa Linn. (*C. longa*), popularly known as turmeric, belongs to the Zingiberaceae family and has a long historical background of having healing properties against many diseases. In Unani and Ayurveda medicine, *C. longa* has been used for liver obstruction and jaundice, and has been applied externally for ulcers and inflammation. Additionally, it is employed in several other ailments such as cough, cold, dental issues, indigestion, skin infections, blood purification, asthma, piles, bronchitis, tumor, wounds, and hepatic disorders, and is used as an antiseptic. Curcumin, a major constituent of *C. longa*, is well known for its therapeutic potential in numerous disorders. However, there is a lack of literature on the therapeutic potential of *C. longa* in contrast to curcumin. Hence, the present review aimed to provide in-depth information by highlighting knowledge gaps in traditional and scientific evidence about *C. longa* in relation to curcumin. The relationship to one another in terms of biological action includes their antioxidant, anti-inflammatory, neuroprotective, anticancer, hepatoprotective, cardio-protective, immunomodulatory, antifertility, antimicrobial, antiallergic, antidermatophytic, and antidepressant properties. Furthermore, in-depth discussion of *C. longa* on its taxonomic categorization, traditional uses, botanical description, phytochemical ingredients, pharmacology, toxicity, and safety aspects in relation to its major compound curcumin is needed to explore the trends and perspectives for future research. Considering all of the promising evidence to date, there is still a lack of supportive evidence especially from clinical trials on the adjunct use of *C. longa* and curcumin. This prompts further preclinical and clinical investigations on curcumin.

Vinca rosea (Sadabahar)

Catharanthusroseus (synonymous with *Vincarosea*) is a perennial plant commonly seen in tropical countries. Seven species of this genus are native to Madagascar and one species is native to Southern Asia.[1] It is more commonly known as Madagascar periwinkle. The local name in Malaysia is *KemuntingCina*. The National Cancer Council of Malaysia (MajlisKanserNasional, MAKNA) uses the periwinkle logo as its symbol of hope for cancer patients.

This plant produces beautiful flowers with a variety of colours such as purple, pink and white and commonly planted for decorative purposes. Historically Madagascar periwinkle had been used for various treatments, e.g. diabetes mellitus, high blood pressure and infection.

The stem of Madagascar periwinkle produced a milky sap which is the source of over 70 different indole alkaloids. Two of the common anti-cancer drugs which are derived from this plant are vincristine and vinblastine (they are named after *Vinca*). Vincristine is used in the chemotherapeutic regime for Hodgkin's lymphoma while vinblastine is used for childhood leukemia. These vinca alkaloid bind to tubulin dimers and inhibiting microtubule structures of the cells, thus inhibiting the metaphase of cellular mitosis. Their Main side effects of these drugs are peripheral neuropathy, hair loss, hyponatremia and constipation.

Piper nigrum (Kali Mirch)

Black pepper (*Piper nigrum* L.) commonly known as the "King of Spices", belonging to the family Piperaceae, is one of the most popular spices used worldwide and is native to southern India. The name "pepper" originated from the Sanskrit word "*Pipali*", and other Indian vernacular names are Milagu (Tamil), Kari Menasu (Kannada), KuruMulagu (Malayalam), Miriyam (Telugu), and Kali Mirch (Hindi). Most of the production of black pepper occurs in India, Malaysia, Indonesia, China, Thailand, Sri Lanka, Vietnam, Brazil and Madagascar. Black pepper is one of the vital spicy ingredients in foods, especially in Asian countries, and it also possesses potential applications in traditional medicine, perfumery, preservatives and insecticides.

Plant-based food products are storehouses of several bioactive constituents such as phenolics, flavonoids, terpenes and sterols. These constituents have been evaluated for their biological and antibacterial effects. In traditional medicines, black pepper has been reported to have a gastrointestinal activity to increase appetite, to antidote cough, cold, dyspnea throat diseases, discontinuous fever, dysentery, stomachache, worms and piles and is used as anti-inflammatory, antipyretic, and to treat epilepsy and snakebite.

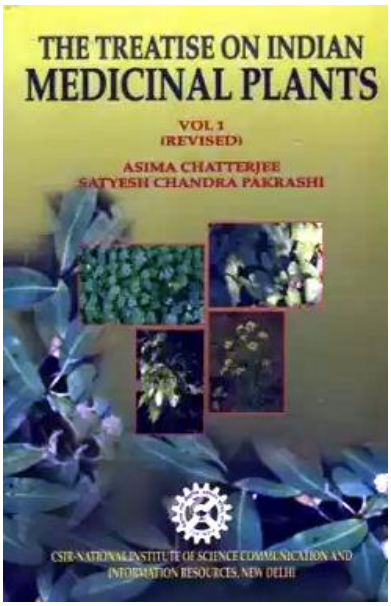
The aroma and pungency of black pepper are mainly attributed to piperine and volatile oils. Piperine is the major bioactive component in black pepper, and its displays various therapeutic benefits including antiplatelet, antihypertensive, anticancer, antioxidant, analgesic, antidepressants and anti-diarrheal. The alkaloid piperine improves the therapeutic value of several drugs, vaccines and nutrients by enhancing bioavailability through inhibiting numerous digestive enzymes. Likewise, piperine aids in digestion through stimulating pancreatic and intestinal enzymes, and enriches cognitive skills and fertility. Furthermore,

piperine is recognized as delivering several therapeutic activities distinct from other chemical components.

Black pepper essential oil constitutes approximately 0.4–7 % of the berry dry weight and is beneficial for the management of rheumatism, cold, tiredness, muscular pains and infection. It was also used as a nerve stimulant to enhance blood circulation. Both white pepper and black pepper contained 2–7 % piperine . The volatile oil constituent piperamides and nerolidol exhibited insecticidal activities. β-caryophyllene displayed anaesthetic effects, and piperine was used in perfumes.

BOOK REVEIW

THE TREATISE OF INDIAN MEDICINAL PLANTS



Asima Chaterjee

Satyesh Chandra Pakrashi

The publishing of this work was undertaken at the ivitiative of and encouragement from **Dr. A. P. Mitra, D.G., C.S.I.R.**

This is the first of the six volumes, **THE TREATISE OF INDIAN MEDICINAL PLANTS** published by **CSIR. Professor (Mrs.) Asima Chaterjee** is the Editor in Chief, **Dr. S.C. Pakrashi** as the Co- Editor. Each volume incorporates:

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ii. The authentic Sanskrit Slokas both in Devnagri and Roman Scripts with English translaton of the Slokas explaining the use of individual plants species in treatment of diseases. One hundred and eleven different herbs have been described.
iii. Plant species have been arranged according to botanical classification.

This book covers 200 pages. A good attempt has been made to give important Chemical Constituents reported till 1989. Although the basic concept of Ayurveda terms are not literally the same as those used in the modern medicine, viz; Vata,Pitta, Kapha etc, they have been used as such in the english translation of the slokas. The sequence of therapeutic uses of herbs (plants) as described in the slokas has been maintained in the translation.

Dr.I.P.Saxena
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