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Lansing, Michigan 48909  
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# *Nature and Science*

ISSN: 1545-0740

Volume 6 – Number 3 (Cumulated No. 20), July 20, 2008

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## **Impacts Of Industrial Effluent On Quality Of Well Water Within Asa Dam Industrial Estate, Ilorin Nigeria**

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### **ABSTRACT**

The impact of industrial effluent on the quality of ground water (well) within an Industrial Estate was studied. The quality was assessed in terms of physicochemical parameters and bacteriological parameter. Three wells within the industrial were examined in the course of the study. Results obtained showed that the turbidity varied between 1.5 to 250 NTU and colour ranged from 211 to 2519 Pt- Co. The total, suspended and dissolved solids content were high. The conductivity ranged from 161 to 731  $\mu\text{s}$ , while pH ranged from 6.9 to 7.3. Calcium and Magnesium ions as well as chloride ion content of the water were high. The dissolved oxygen content ranged from 6 to 9mg/l. Bacteriological indices showed that the well water were highly contaminated having high total bacterial counts (1200- 1375 cfu/ml). The well water showed presence of faecal coliform (*E. coli*) and had high coliform counts (1600 - >1800 MPN/100ml). It was observed that the wells were negatively affected by the effluent discharged within the industrial plant. [Nature and Science. 2008;6(3):1-5]. ISSN: 1545-0740.

**Key words:** Industrial effluent, well, Ground water, Bacterial count.

### **INTRODUCTION**

The importance of water in the control of diseases had long been recognized (Hofkes, 1981; WHO, 1996). Water is a factor of production in virtually all enterprise, including agriculture, industry and the services sector (UNESCO, 2006). The importance of safe drinking water is underlined by the assertion that: "safe drinking water is the birthright of all humankind – as much a birthright as clean air" (TWAS, 2002). It also reported that the majority of the world's population, especially in most parts of Africa and Asia, does not have access to safe drinking water and that as much as 6 million children dies daily as result of waterborne diseases linked to scarcity of safe drinking water or sanitation (TWAS, 2002). WHO (2004) pointed out that diseases related to contamination of drinking-water constitute a major burden on human health: and that interventions to improve the quality of drinking-water provide significant benefits to health.

For most communities the most secure source of safe drinking water is pipe-borne water from municipal water treatment plants. Often, most of water treatment facilities do not deliver or fail to meet the water requirements of the served community; due to corruption, lack of maintenance or increased population. The scarcity of piped water has made communities to find alternative sources of water: ground water sources being a ready source. Wells are a common ground water source readily explored to meet community water requirement or make up the short fall.

Wells are categorized based on the nature of construction: open dug wells are generally considered the worst type of groundwater sources in terms of faecal contamination and bacteriological analysis. Dug wells with windlass or hand pumped or mechanically pumped well are generally regarded to be less prone to contamination (WHO, 2004). WHO (1997) assert that open or poorly covered well heads pose the commonest risk to well-water quality; the possibility of the water being contaminated is further increased by the use of inappropriate water-lifting devices by consumers. The commonest physical defects leading to faecal contamination of dug wells are associated with damage to, or lack of, a concrete plinth, and with breaks in the parapet wall and in the drainage channel (WHO, 1997). The most serious source of pollution of well water is contamination by human waste from latrines and septic tanks resulting in increased levels of microorganisms, including pathogens. Other likely sources of contamination include runoffs, agrochemicals such as pesticides and nitrates used on farm lands and industrial effluents. Contamination of well water due to under seepage has reported in the Niger Delta area of Nigeria (Ibe and Agbamu, 1999). Seepage from effluent bearing surface water would readily contaminate wells located close to the surface water.

Arising from the drive for industrialization, parts of Ilorin town are designated industrial estate/ area to accommodate the industries. One of such industrial estate has the course of River Asa running

through its whole length. The river flows through Ilorin town almost dividing it into two halves (Olayemi, 1994). This makes it readily prone to abuse as effluent receptacle leading to contamination. Studies have shown that the River's water quality is affected by the discharge of the effluents (Eniola and Olayemi, 1999). This is consistent with the observation of Sangodoyin (1991) that effluents discharge alters the physical, chemical and biological nature of receiving water body. Wells are a vital and common source of water in Ilorin, some of these wells are located along the course of River Asa.

In this study, the effect of the discharge of effluent into river Asa on the quality of water of wells within the immediate catchment of the river was investigated. Water samples from wells within the industrial estate were subjected to physicochemical and bacteriological investigations to ascertain the effect of the effluent on the quality of the well water.

## MATERIALS AND METHODS

Open dug well with concrete apron (plinth) around the well head were involved in the study. Water samples from the wells were collected into clean sterile 250ml sampling bottles as described by WHO (1997). The pH, colours (Pt-Co), turbidity, temperature, total Hardness, calcium hardness, magnesium hardness, calcium ion magnesium ion, chloride and conductivity were determined. The suspended, dissolved and total solid contents of the water were determined as described by ASTM (1985). The total heterotrophic bacteria counts were determined using the pour plate method (APHA, 1992). The coliform counts were determined as Most Probable Number (MPN) using the multiple tube fermentation test (APHA, 1992).

## RESULTS

The physicochemical characteristics of the well water are shown on Table 1. The bacteriological characteristics are shown on Figure 1. Water from the wells were found to be close to neutral (pH 6.9 to 7.3) with high bacterial count (1200- 1375 cfu/ml). The coliform count was high (1600 - >1800 MPN/100ml) and faecal coliform (*E. coli*) was isolated. The variation in the total suspended and dissolved solids contents of the wells as well as the dissolved oxygen contents of the well water are shown on Figure 2.

**Table 1. Physicochemical Characteristics of the water from Wells within Asa Dam Industrial Estate, Ilorin.**

Parameters measured	W <sub>1</sub>	W <sub>2</sub>	W <sub>3</sub>
pH	6.9	7.3	6.9
Colour (Pt-Co)	211	2519	240
Turbidity (N.T.U)	1.5	250	4.6
Temperature (°C)	27	28	28
Total Hardness (mg/l)	149	153	37
Calcium Hardness (mg/l)	102	96	34
Magnesium Hardness (mg/l)	46	57	4
Calcium ion (mg/l)	410	383	135
Magnesium ion (mg/l)	37	46	3
Conductivity (us)	338	731	161
Chloride (mg/l)	155	12	2

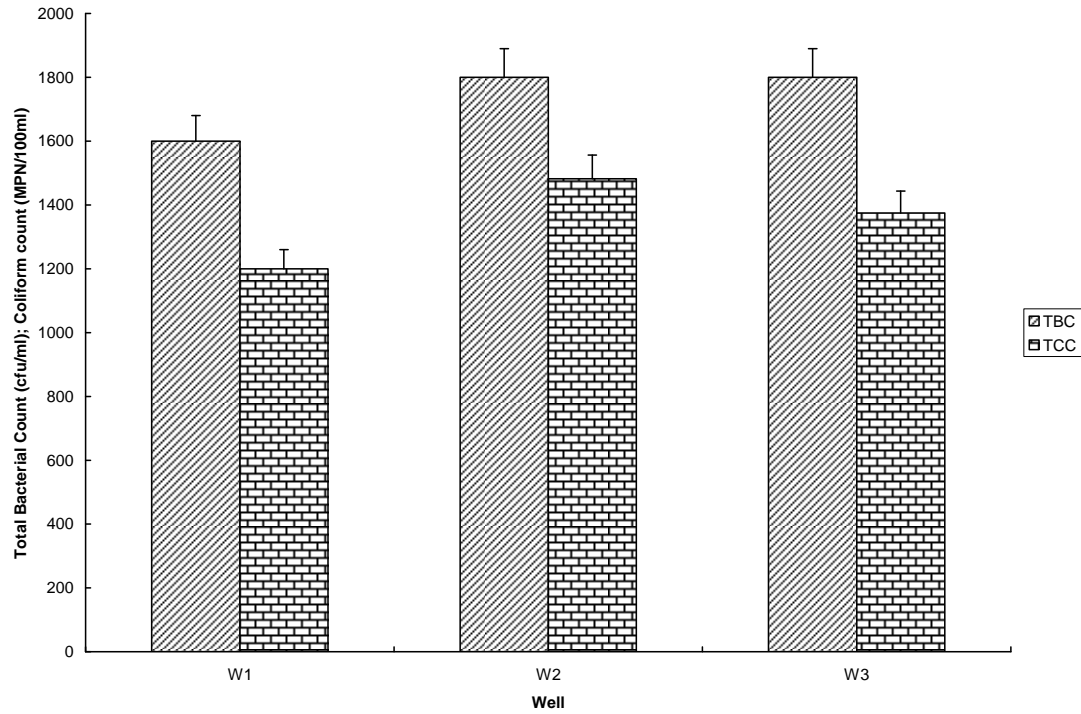


Figure 1: Bacteriological Characteristics of the Water from Wells within Asa Dam Industrial Estate, Ilorin.

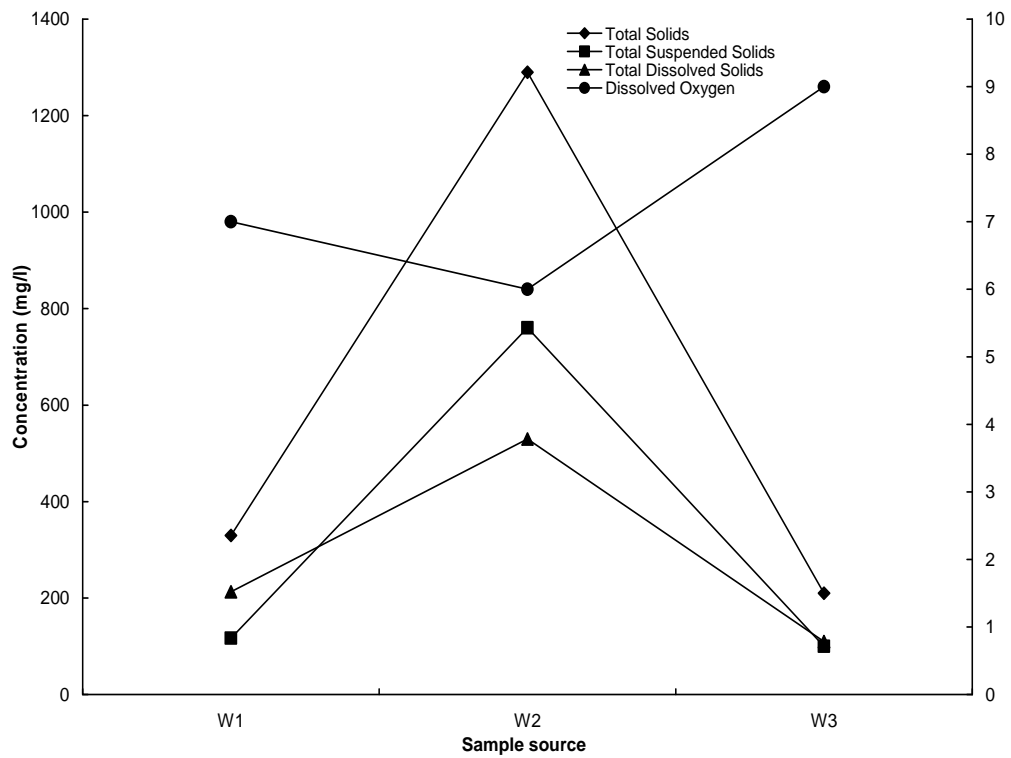


Figure 2. Variation in Total, Suspended and Dissolved Solids Contents of the Wells

## Discussion

Water from the wells was observed to be coloured and turbid with the value ranges of 211- 2519 Pt-Co and 1.5 – 250mg/l respectively. Thin films of oil present on the water surface appear to make the value of the colour to be very high. The high turbidity value is as a result of increase in the type and concentration of the suspended matter released by the industry. The content of total solids, suspended and dissolved solids were also high. This is attributable to the industrial waste discharged into the surface water and suggests some of the content of the effluent have found their way into the ground water. Well water containing high total solids, total suspended solids and total dissolved solids are not fit for drinking, laundry work and livestock purpose. The high conductivity values suggest that the dissolved solids are mostly mineral salts. The high chloride is also suggestive of the use of large quantity of Chlorine or its associated compounds in activities within the industrial estate. The high bacterial count is suggestive of presence of organic matter (Gray, 1989, Olayemi, 1994). The values of dissolved oxygen obtained suggest that the water was not overtaxed by the quantity of degradable material in it and also that it was being well re-oxygenated.

Bacteriological speaking water from the wells fall short of the WHO (1997) recommended guideline standard for drinking water. It requires that water intended for drinking should not contain any pathogen or microorganisms indicative of faecal contamination. All the water samples examined contained faecal coliform (*E. coli*) and high population of heterotrophic bacteria, which is consisted with WHO (2004) report that open dug wells are contaminated, with levels of at least 100 faecal coliforms per 100 ml. This is not necessarily a result of the citing of the well along the river course but a reflection of the human activities taking place around the catchment of the wells. The unringed nature of the wells makes contamination by seepage from the soil more likely. The WHO (2004) recommends that wells are ringed and provided with an apron around the head to minimize contamination. The bacteriological quality of the wells requires that they be subjected to treatment if they are to be used for drinking and domestic purpose.

## Conclusion

The results obtained showed that the water from the well were not fit for human consumption and their qualities were affected by the presence of the wells within the industrial estate and proximity to river that serves for disposal of industrial effluent.

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June 13, 2008

**Dwinding of an endangered orchid *Dactylorhiza hatagirea* (D.Don) Soo: A case study from Tungnath Alpine meadows of Garhwal Himalaya, India**

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**ABSTRACT:** The Central Himalayan region has been rich in biological wealth and would become an uplift resource of socio-economic status of the Himalayan people. Presence of a varied number of medicinal plants indicates its significance. Due to various levels of disturbances, destruction of number of economically important plants in these alpine meadows is continued like declining of *Dactylorhiza hatagirea* (D.Don) Soo, in its natural population. Out of six study sites, only two sites showed its presence, which indicates its declining health from natural population. [Nature and Science. 2008;6(3):6-9]. ISSN: 1545-0740.

**Key words:** Alpine meadows, study sites, natural population, density, orchid, grazing pressure

## INTRODUCTION

The alpine region forms the uppermost catchments of the Himalayan Rivers which supports million of people in the lower hills as well as in plain of north India. Therefore health of the alpine ecosystem has direct bearing on the life-support system, environmental stability, biodiversity and human welfare in the region (Rawat, 2005). The various changes in the Himalayan forests are appearing in their structure, density and composition due to global warming, uncontrolled lopping and felling of trees for fuel wood, fodder and grazing (Gaur 1982, Bargali et al. 1998; Kumar et al. 2004). Changes in climate, exploitation of several plants for medicine and grazing pressure in alpine region of Garhwal Himalaya have led to drastic changes in vegetation composition and population of species during last few decades (Nautiyal et al. 2004). *Dactylorhiza hatagirea* (D. Don) Soo (Family Orchidaceae), a high value medicinal orchid, is reported to occur in temperate to alpine regions (2500-5000 m) in India, Pakistan and Nepal (Bhatt et al. 2005). *Dactylorhiza hatagirea* (D. Don) Soo, earlier known *Orchis latifolia* Hook (Vij et al. 1992). It is a terrestrial orchid and commonly it is known as Salampanja and Hatajari in Garhwal Himalaya. The tubers of this species, commonly sold as 'Salampanja' are known to yield a high quality 'Salep' which is extensively used in local medicine as nervine tonic for its astringent and aphrodisiac properties (Vij et al 1992). It has been categorized as critically endangered (Kala, 2000), rare (Samant et al 2001) and listed under appendix II of CITES (Uniyal et al 2002). This study aims to assess the quantum of availability of a therapeutically important orchid *D. hatagirea* (D.Don) Soo, in its natural habitats.

## MATERIAL AND METHODS

Tungnath (30° 30' N - 79° 15' E and elevation 3300- 4200 m ) represent an alpine zone of the Garhwal Himalaya (Sundriyal, 1994). In this region our study area covers an elevation range of 3500 to 4000 m. The rocks around Tungnath alpine meadows are mainly mylonitized gneisses, augengneisses, schist, granite and highly folded having a north west – south west trend (Valdia 1980). The heavy snowfall, frost, drought, low

oxygen and carbon dioxide are the common features of an alpine environment (Billings 1973). The present study deals with a quantitative analysis of herb species in different sites of Tungnath Alpine meadows to assess the quantum of availability of *D. hatagirea* in its natural habitats (Table: 1). Phytosociological data for herbs were quantitatively analyzed in six study sites on northern-west aspect by placing random sampling 40, 1x1 m quadrat. Quadrats data were analyzed for density, frequency and abundance (Muller-Dombois and Ellenberg, 1974).

## RESULTS

A total of 24 herbs species were encountered across the study sites. Out of six study sites, only two sites showed presence of *D. hatagirea*. Observable grazing pressure was recorded at all study sites. The maximum density was of *Phleum alpinum* L. (141.52-201.28 ind/m<sup>2</sup>) followed by *Gaultheria trichophylla* Royle, (14.2-75.0 ind/m<sup>2</sup>), *Danthonia cachaemyriana* Jaub. and Spach, (8.32-40.32 ind/m<sup>2</sup>), *Plantago depressa* Willd. (15.0-58.4 ind/m<sup>2</sup>) and *Ainsliea aptera* DC (2.80-32.2 ind/m<sup>2</sup>) and dominant herbs in all study sites. The target species i.e. *D. hatagirea* showed minimum density (0.70-1.8 ind/m<sup>2</sup>) in all study sites (Table: 2).

**Table: 1 Site description indicating altitudinal range, aspect and dominant herb species**

Study sites	Altitudinal range (m)*	Aspect	Dominant herb species
1	3500-3600	NW*	<i>Phleum alpinum</i> , <i>Gaultheria trichophylla</i> , <i>Plantago depressa</i>
2	3600-3690	NW	<i>Phleum alpinum</i> , <i>Ainsliea aptera</i> , <i>Gaultheria trichophylla</i>
3	3940-4000	NW	<i>Phleum alpinum</i> , <i>Potentilla peduncularis</i> , <i>Danthonia cachaemyriana</i>
4	3600-3650	NW	<i>Phleum alpinum</i> , <i>Plantago depressa</i> <i>Geum elatum</i>
5	3700-3800	NW	<i>Phleum alpinum</i> , <i>Plantago depressa</i> , <i>Geum elatum</i>
6	3550-3600	NW	<i>Phleum alpinum</i> , <i>Gaultheria trichophylla</i> , <i>Danthonia cachaemyriana</i>

\* m = meter, NW\* = north-west

**Table: 2 Density of *D. hatagirea* and dominant herbs in different study sites**

Sites	Density of <i>D. hatagirea</i> ( ind/ m2)*	Density of Dominant herb's (ind/m2)*
1	-	<i>Phleum alpinum</i> (160.12), <i>Gaultheria trichophylla</i> (75.00), <i>Plantago depressa</i> ( 30.32), <i>Danthonia cachaemyriana</i> ( 26.72)
2	-	<i>Phleum alpinum</i> ( 201.28), <i>Ainsliea aptera</i> ( 32.20), <i>Gaultheria trichophylla</i> ( 23.60), <i>Danthonia cachaemyriana</i> ( 10.60)
3	1.8	<i>Phleum alpinum</i> (141.52), <i>Potentilla peduncularis</i> (41.92), <i>Tanacetum longifolium</i> ( 32.72), <i>Danthonia cachaemyriana</i> ( 34.32)
4	-	<i>Phleum alpinum</i> (190.52), <i>Plantago depressa</i> (36.12), <i>Tanacetum longifolium</i> ( 16.80), <i>Geum elatum</i> (17.60)
5	0.7	<i>Phleum alpinum</i> (174.32), <i>Plantago depressa</i> (58.40), <i>Geum elatum</i> (17.72)
6	-	<i>Phleum alpinum</i> (196.72), <i>Plantago depressa</i> (15.0), <i>Gaultheria trichophylla</i> (20.40), <i>Danthonia cachaemyriana</i> ( 40.32)

\* (ind/m2) = individual per meter square

## DISCUSSION

On the basis of field visit, past records and observable grazing pressure, our study sites are fallen within the category of unprotected area. The density of *D. hatagirea* ranged from 0.70- 1.8 ind/m2 in these sites which was comparatively less from the reported density of *D. hatagirea* (*Orchis latifolia*) i.e. 2.66 ind/m2 in grazed sites and 3.2 ind/m2 in ungrazed sites at Tungnath (Nautiyal et al. 2004). Bhatt et al (2005) also reported 2.02-2.19 ind/m2 density in protected area and 1.13-1.64 ind/m2 in unprotected area in west Himalaya for *D. hatagirea*. These data shows that there is decrease in number of plants of this species with time. Tungnath is one of the famous religious shrines of Hindus where large herds of sheep, goat and buffalo reach every year during May-October for summer grazing (Nautiyal et al. 2004). Therefore the low density in unprotected areas may be due to heavy grazing pressure.

According to local people the Himalayan Monal, *Lophophorus impejanus* also known as the Impeyan Monal or Impeyan Pheasant, destroy its underground part i.e. tubers for food. It was also observed by the first two authors during their field visit. This and other levels of disturbances like grazing pressure because of its palatable nature, over exploitation due to its high medicinal value, and unawareness of the proper procedure of collection and propagation etc. are the major factors for declining of this species from its natural habitats. Chhetri et al. (2005), also reported that, the Sandakphu area in the Singalila range is a natural habitat of precious medicinal plants like *Aconitum*, *Picrorhiza*, *Nardostachys*, *Dactylorhiza*, etc., which are being destroyed by grazing.

Therefore it is a need to promote cultivation, propagation and conservation of this species. Using *in-situ* as well as *ex-situ* conservation efforts we can propagate and conserve this species and would become an ecologically as well as economically important plants of High Altitudes.

## ACKNOWLEDGEMENT

The financial support received from UCOST Dehradun is gratefully acknowledged.

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6/21/2008

## Optimization of flavonoids extraction from the leaves of *Tabernaemontana heyneana* Wall. using L<sub>16</sub>

### Orthogonal design

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**Abstract:** The study has been carried out to investigate the effects of single factors such as temperature, extraction time, concentration of ethanol, material ratio and no. of extractions on the contents of flavonoids present in the leaves of *Tabernaemontana heyneana* Wall. were investigated in this study. On this basis, an L<sub>16</sub> orthogonal design of experiment was adopted to determine the optimal conditions for the extraction of flavonoids. The amount of flavonoids extracted reached its maxima when extracted at 85°C for 2hrs by using 75%ethanol with a material ratio of 1:05 and 4 times of extraction. The TLC performed for the optimized extracts showed the presence of rutin, quercetin related compounds. The PTLC of the optimized extracts also proved the presence of flavonoids, especially high levels of rutin related compounds. [Nature and Science. 2008;6(3):10-21]. ISSN: 1545-0740.

**Keywords:** Flavonoids, orthogonal experiments, Single-factor experiments, *Tabernaemontana heyneana* Wall.

## 1. INTRODUCTION

Humans have gathered food and medicinal herbs ever since their arrival on earth and were guided then by instinct, followed by experience, and also by rational thought (Havsteen, 2002). For millions of years, mankind has fared quite well using this approach, but after the development of science and technology, people felt that the current state of affairs was quite satisfactory and hence, they failed to support research and education adequately (Harborne, 1988). Therefore, it is time to examine more closely what we are eating, how diseases can be treated more efficiently, and how we can effectively conserve our natural resources. One of our natural resources is the plants in remote forests, some of which may contain compounds of potential medical use. One such compound is flavonoids which appear to play a major role in the successful medical treatments of ancient times and their use has persevered till date (Dixon *et al.*, 1998). Flavonoids are a group of polyphenolic compounds possessing low molecular weight that exhibit a common benzo- $\gamma$ -pyrone structure. They are categorized into various subclasses including flavones, flavonols, flavanones, isoflavanones, isoflavanoids, anthocyanidins, and catechins (Hodnick *et al.*, 1988; Cook and Samman, 1996). The average human diet contains a considerable amount of flavonoids and the major dietary sources are fruits (i.e., orange, grapefruit, apple, and strawberry), vegetables (i.e. onion, broccoli, green pepper and tomato), soybeans and different herbs.

One of the prominent and medically most useful properties of many flavonoids is their ability to scavenge free radicals (Van Acker *et al.*, 1996). A free radical is molecule containing one or more unpaired electrons in atomic or molecular orbitals that includes super oxide (O<sub>2</sub><sup>-</sup>), hydroxyl radicals (OH<sup>•</sup>) and H<sub>2</sub>O<sub>2</sub>, collectively known as reactive oxygen species (ROS) (Sathishkumar *et al.*, 2008). These ROS may induce oxidative damage to various macromolecules like polyunsaturated fatty acids in cell membranes, carbohydrates, proteins and DNA which results in homeostatic imbalance. The flavonoids are essential constituents of the cells of all higher plants (Brouillard and Cheminat, 1988). They resemble in their regulatory properties most of the lipid-soluble vitamins, but serve in addition, due to their color and odor, as communicators with the environment (Middleton and Teramura, 1993). The effect of flavonoids on plant growth, which is known, is atleast partly indirect and associated with the action of the auxins. It was reported that flavonoids can improve the blood circulation and lower the blood pressure (Yaqin Xu *et al.*, 2005).

*Tabernaemontana heyneana wall.* (Apocynaceae) known as kundalam paalai in Tamil, is known to possess antimicrobial activity against skin diseases, venereal diseases, respiratory problems, nervous disorders and various other diseases (Ignacimuthu and Ayyanar, 2005; Ignacimuthu *et al.*, 2006). The stem bark decoction is used for cleaning cuts and wounds before dressing them (Chandrashekar *et al.*, 1995). The mixture of leaf and stem powder of this plant along with the stem bark of *Ficus racemosa*, *Ficus benghalensis*, *Madhuca longifolia*, is heated with coconut oil and applied externally to cure skin diseases (Ignacimuthu and Ayyanar, 2005; Ignacimuthu *et al.*, 2006). Similarly the same mixture along with the stem bark of *Strychnos nux-vomica* and fruits of *Carica papaya* were taken internally to induce abortion (Ignacimuthu and Ayyanar, 2005; Ignacimuthu *et al.*, 2006).

In many cases, it is difficult to find quickly suitable experimental conditions for a given separation task. Prediction of separation conditions is not yet straightforward. Therefore, good experimental design becomes increasingly important. Orthogonal design which only focuses on the main effects of the factors, allows the number of experiments to be drastically reduced. In separation science, this kind of experimental design has already shown its usefulness in liquid chromatography and capillary electrophoresis (Hu Zhide *et al.*, 2002).

At present, there are no scientific reports on the extraction of flavonoids from the leaves of *Tabernaemontana heyneana Wall.* In this study, the optimal conditions to extract flavonoids from the leaves of *Tabernaemontana heyneana Wall.* were investigated systematically in order to explore a proper process to utilize the *Tabernaemontana heyneana Wall.* leaves in the area of healthcare.

## **2. MATERIALS AND METHODS**

### **2.1 Plant material**

The plant was collected from the medicinal garden of Kumaraguru College of Technology, Coimbatore, India and the species was identified, confirmed by Botanical Survey of India (BSI), Southern Circle, Coimbatore, India and a voucher specimen (No. DBT 001) was deposited at Department of Biotechnology, Kumaraguru College of Technology, Coimbatore, India.

### **2.2 Extraction process**

The main factors that affect the extraction of flavonoids like temperature, extraction time, materials ratio (weight of the leaves: volume of the extracting agent), extracting agent (%) and the no. of extraction were studied individually. The optimum extraction conditions were then determined by  $L_{16} (4^5)$  orthogonal design of experiments i.e., four levels and five different parameters. A single factor analysis of variance (One way ANOVA) was adopted to investigate the effect of each factor in the extraction of flavonoids.

### **2.3 Estimation of total flavonoids (TFC)**

TFC was estimated spectrophotometrically (Zhishen *et al.*, 1999) with slight modifications (Beckman DU 530 UV/ Vis spectrophotometer, USA). About 0.1ml of the diluted sample added distilled water to make the volume to 5ml and 0.3 ml 5%  $\text{NaNO}_2$  was added to this. 3ml of 10%  $\text{AlCl}_3$  was added 5 minutes later. After 6 minutes, 2 ml of 1 M NaOH was added and the absorbance was measured at 510 nm. Rutin was used as a standard for constructing a calibration curve. Data were reported as mean  $\pm$  SD for three replicate measurements.

### **2.4 Identification of flavonoids by thin layer chromatography (TLC)**

Chromatographies of the optimized extracts were run one dimensionally in the mobile phase solvent (ethyl acetate - ethanol - water, 5:1:5, v/ v/ v) at room temperature of 20-25°C. The concentrated extracts were spotted on the lower left of the TLC plate and the diameter of the spot in each chromatogram was

normally about 5mm. Authentic markers of flavonol (quercetin) and flavonoid glycoside (rutin) obtained commercially were co-chromatographed. Identification of the flavonoids in the extracts was identified under UV light after the application of ammonia (Adam *et al.*, 2002; Guorong Fan *et al.*, 2006). A similar preparative thin layer chromatography (PTLC) was also performed to confirm the results of TLC.

### 3. RESULTS AND DISCUSSIONS

Flavonoids are a broad group of secondary metabolites with varied and important roles in plant physiology as well as they have gained recent interest because of their broad pharmacological activity. Putative therapeutic effects of many traditional medicines may be ascribed to the presence of flavonoids (Saskia Van Acker *et al.*, 1996; Schultz *et al.*, 2008). Flavonoids and other plant phenolics are reported, in addition to their free radical scavenging activity, to have multiple biological activities including vasodilatory, anticarcinogenic, antiinflammatory, antibacterial, immune-stimulating, antiallergic, antiviral, and estrogenic effects, as well as being inhibitors of phospholipase A2, cyclooxygenase, and lipoxygenase (Catherine Rice-Evans *et al.*, 1996). Plant flavonoids usually occur in plants as glycosides, although in some circumstances they may occur as free aglycones. Most glycosides are O-glycosides, with the most common monoglycoside being at the 7-position.

Previous reports for the extraction optimization of rutin, a flavonoid glycoside from the leaves of buck wheat revealed that a Solid:liquid ratio of 1:20 for 4 hours at 60°C was required for higher rutin yield. In this methanol is used as a solvent for extracting the rutin. Huo (Chinese Patent 1217329, 1999) described an extraction of rutin from tartary buckwheat seeds by washing with water, coarse grinding, coarse screening, soaking in water, drying in the air, fine grinding, soaking in edible alcohol, extracting below 60°C. Balandina *et al.*, (1982) extracted rutin from buckwheat seeds with hot water to remove the desired product and crystallized it. In general, a full evaluation of the effect of five different parameters at four levels on the yield would require 1024 (4<sup>5</sup>) experiments. In order to reduce the number of experiments, an L<sub>16</sub> (4<sup>5</sup>) orthogonal design graph was used. In this way, only 16 experiments were necessary to run (Chen *et al.*, 2007).

#### 3.1 Effect of temperature on the extraction of flavonoids

Fig.1 showed the contents of raw flavonoids tended to increase gradually with a rise in the temperature range from 55°C to 85°C. The contents of flavonoids gradually increased with a rise in the temperature in a range of 55°C to 85°C with a 10°C temperature interval. It may be probable that the greater speed of the molecule movements in higher temperature so that flavonoids diffused more quickly from cell to extracting agent. But the flavonoids could be oxidized at temperature of surpassing 80° so that the contents of flavonoids extracted started to decrease gradually (Yaqin Xu *et al.*, 2005). Temperature's effect on extraction is dual. On one hand, higher temperature can accelerate the solvent flow and thus increase the flavonoids content and on the other hand, higher temperature can decrease the fluid density that may reduce the extraction efficiency (He Guo-qing *et al.*, 2005). Hence, it was found that 85°C was the optimum temperature for extracting the raw flavonoids.

#### 3.2 Effect of flavonoids extraction time

The result of Fig.2 showed that the contents of flavonoids extracted for 2h reached maxima and prolonged extraction may not yield an increased content. The contents of flavonoids extracted for 2hrs reached its maxima. Furthermore a decrease in the flavonoids content was noticed for 3hrs extraction and a sudden increase in their content was observed for 4hrs extraction time. This increase in the flavonoids content may be due to the synergistic effect of other parameters involved. A similar report by Chen *et al.*, (2007) revealed that 2hrs was the optimal extraction time for the extraction of a hypotensive drug geneposide from the bark of *Eucommia ulmoides* tree.

#### 3.3 Effect of material ratio on the extraction of flavonoids

Fig.3 showed the contents of raw flavonoids extracted were maxima at 1:05 materials ratio. Further increase in the material ratio leads to a gradual decrease in the flavonoids content revealing a saturated condition. A significant rise in the flavonoids content was observed with the material ratio of 1:05. However, a gradual decrease in the flavonoids content was noticed when there is an increase in the material ratio. This decrease might be due to the fact that when the material ratio reached a certain level, the extract has well dissolved in the solution that may lead the contents of the extract become saturated and prevent further increase (Yaqin Xu *et al.*, 2005).

### **3.4 Effect of extracting agent (ethanol) on the extraction of flavonoids**

The result of Fig.4 revealed that the contents of raw flavonoids extract increases with the concentration of ethanol i.e., 55% to 75%. A decrease in the flavonoids content was noticed further more, i.e., beyond 75%. Considering one of the aims of this work is to propose a suitable solvent for extracting the raw flavonoids. Among various solvents, ethanol was selected as a right choice because it is environmentally benign and relatively safe to human health (He Guo-qing *et al.*, 2005). Ethanol interacts with the flavonoids probably through non-covalent interactions and promotes a rapid diffusion into the solution (Luque de Castro and Tena, 1996). Various concentration of ethanol used exhibited different effect in changing the fluid polarity and thus had diverse effect on the solubility enhancement of the flavonoids (He Guo-qing *et al.*, 2005). The optimal extraction yield may be fulfilled when the polarity of the fluid and its flavonoids are coincident. In this study, the results indicated that the optimal ethanol concentration for extraction flavonoids was found to be 75%.

### **3.5 Effect of no. of extractions on flavonoids**

The contents of raw flavonoids extract increases with the no. of extractions i.e., a gradual rise is noticed from 1 time to 4 times. Obviously, when the no. of extraction times increased the yield of the respective bioactive principle may be increased (Chen *et al.*, 2007). In this investigation, the raw flavonoids content was increased by 4 times of the extraction.

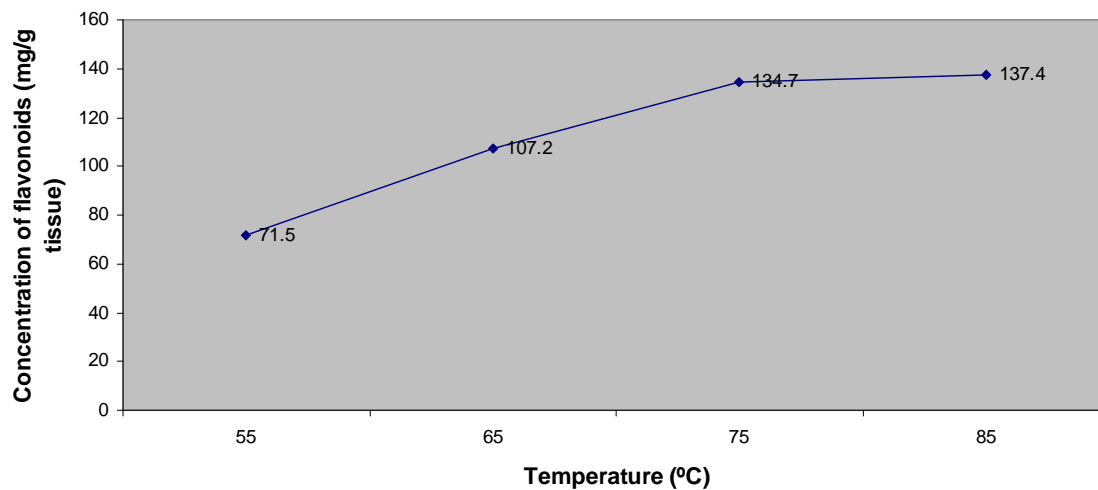
### **3.6 Optimization of flavonoids extraction using L<sub>16</sub> orthogonal design**

The parameters and the orthogonal design of experiment for the extraction of flavonoids were given in the Table 1 and Table 2. The results were made in the form of range analysis and one way ANOVA by SPSS software. The results were depicted in Table 3 and Table 4. The order of the effect of factors on flavonoids extraction was A>D>E>B>C. The temperature had the greatest effect on the extraction procedure and it was found to be significantly different at 5% level. An equivalent effect was observed in the material ratio change, even though it was not proved to be significant difference at 5% level. The other factors such as solvent (%), extraction duration and no. of extractions did not play a vital role in extracting the flavonoids to a higher yield. The optimum extraction conditions obtained from the statistical analysis were A<sub>4</sub>B<sub>2</sub>C<sub>3</sub>D<sub>1</sub>E<sub>4</sub>. It means that 85°C, 2hrs extraction duration, a material ratio 1:05, 75% ethanol concentration and 4 times of extraction were the optimum conditions for flavonoids recovery.

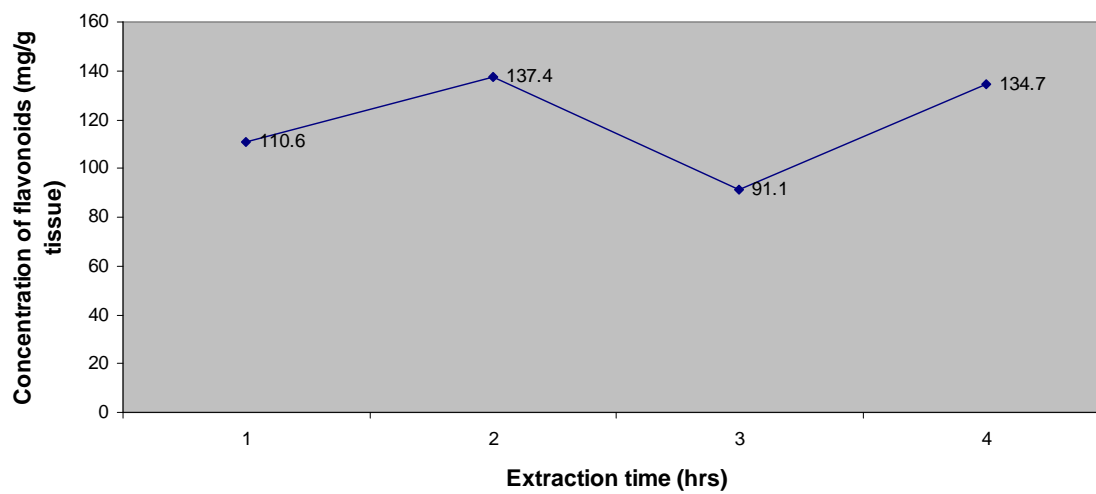
### **3.7 TLC and PTLC results**

The results of TLC and PTLC revealed the presence of flavonoid glycosides, flavonols and phenolic acids in the optimized extracts (Fig.5 and Fig.6).

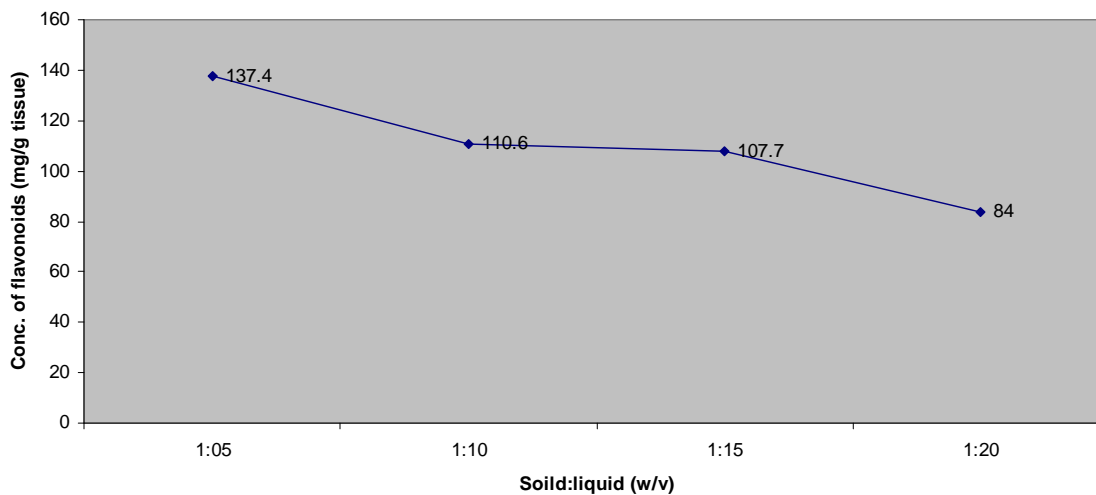
**Fig.1 Effect of temperature on flavonoids extraction**



**Fig.2 Effect of different extraction time**



**Fig.3 Effect of soild:liquid (w/v) in the extraction of flavonoids**



**Fig.4 Effect of ethanol on the extraction of flavonoids**

