International Journal of Pharmacological Research
ISSN: 2277-3312
www.ssjournals.com
Journal DOI:10.7439/ijpr

Phytochemical, trace metals assessment and antimicrobial efficacy of *Barleria cristata*

S. Baskar*, G. Selvan, R. Anbarasu, V. Raja

P.G and Research Department of Physics, Thanthai Hans Roever College, Perambalur – 621 212, Tamil Nadu, India

Corresponding author*

S. Baskar
P.G and Research Department of Physics,
Thanthai Hans Roever College,
Perambalur – 621 212, Tamil Nadu, India
E-mail: sivabaskar85@gmail.com

Abstract

Traditional plants contain various secondary metabolites which have shown significant pharmacological activity. In the present paper, we analysed the phytochemical, trace metal and antimicrobial activity of ethanolic leaf extracts of *Barleria cristata*. The significance of high antimicrobial activity is due to their presence of secondary metabolites in this herb. The phytochemical screening revealed the presence of steroids, triterpenes, alkaloids, phenols, flavonoids, saponin, tannins and amino acid. The antimicrobial activity of *B. cristata* ethanol extract was examined with six various pathogenic microorganisms such as gram positive, gram negative and fungal strains using the disk diffusion test. The two tested concentrations such as 0.60 and 1.20 mg/disc produce zone of inhibition on Muller Hinton Agar (MHA) and Potato Dextrose Agar (PDA) plates for bacteria and fungi, respectively. In this study, higher (1.20 mg) concentration got greater sensitivity than lower (0.60 mg) concentration against all strains. The trace metal analyses of the plants were also carried out. The mean concentration of trace metals such as cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), nickel (Ni), lead (Pb) and zinc (Zn) were 0.05, BDL, 0.07, 0.88, BDL, 0.23 and 0.24 mg kg^{-1}, respectively. Therefore, it is signified that *B. cristata* plant extract is safe to be used as an antimicrobial agent.
Keywords: *Barleria cristata*, Antimicrobial activity, Phytochemistry, Trace metals, Methanol extract.

1. Introduction

India is endowed with a wealth of medicinal plants, which have been a valuable source of natural products for maintaining human health. Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [1]. Medicinal plants are an important source of therapeutic remedies for various ailments. Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th Century [2]. The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization.

The earliest mention of medicinal use of plants in Hindu culture is founds in “Rigveda”, which is said to have been written between 4500 - 1600 B.C. and is supposed to be the oldest repository of human knowledge. A special feature of higher plants is their ability to produce a large number of organic chemicals of high structural diversity of the so-called secondary metabolites [3]. Such metabolites are divided into three different categories based on their mechanism of function viz., bacteriostatic, antimicrobial and chemotherapeutic [4]. Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. This Herbal and natural products have been used in folk medicine for centuries throughout the world, but there are relatively lower incidences of adverse reactions to plant preparations compared to modern conventional pharmaceuticals, this coupled with their reduced cost, is encouraging for both the consuming public and national health care institutions to consider plant medicines as alternatives to synthetic drugs [5].

Natural antimicrobials have been often derived from plants, microorganisms or animal tissues [6][7]. Microbes are closely associated with the health and welfare of people. Some are beneficial, while others are detrimental. Due to the increasing therapeutic problem in the treatment of infectious diseases, the search for new drugs from natural sources becomes imperative, because most rampant killer diseases in developing countries are of microbiological origin [8]. Natural products from plants may offer new agents for antibiotic use. The antibacterial and antifungal studies of the plant extracts and pure compounds have been carried out by the agar well diffusion method [9]. In this way, several studies on antibacterial and antifungal substances from plants have been conducted by a number of researchers [10]. Medicinal plants occupy a distinct role in the life of people since ancient times [11].

Air pollution has been described as an additional stress on plants since they often respond to atmospheric

contamination in the same way as they respond to drought and other environment stress. Especially, the role of cement pollutants causing injury to plants either by direct toxic effect or modifying the host physiology rendering it more susceptible to infection [12]. In severe case of pollution, the injury symptoms were expressed as foliar necrosis or completely disappearance of the plant. Samal and Santra[13] in 2002, have also previously studied the impact of air pollution on plants with reference to foliar anatomical and biochemical changes by experimenting on various sensitive plants.

On the other hand, certain metals such as Cu, Zn, Mn and Fe are essential for plant growth and some are not play an important role in the plant physiology [14][15]. But, these metals were easily deposited/ accumulated in the soil, water and plants due to the pollutions. These metals were easily transferred from plants to human food chain and were caused to harmful effects on humans [16]. Even though, the efficacy of the plant depends on its habitat, soil type, environmental effect and etc. Therefore, it should be needed to screen the activity of medicinal plants before they are used for therapeutic purposes.

B.cristata grows as a shrub 60 –100 cm tall. The leaves are dark green on the upper surface and pale green on the lower surface. They are elliptic to narrowly ovate. The flowers are about 5 cm long, funnel-shaped in violet, pink, or white color. The fruits are about 1.5 cm long ellipsoid capsules. They become glabrous and glossy at maturity. It is native to a wide area ranging from Southern China to India and Myanmar. Cultivated as an ornamental plant in villages and gardens, it has become naturalized in Hawaii, where it grows in dry habitats. In Fiji, where it is known as "tombithi" and in Christmas Island (Indian Ocean), the shrub grows also as a ruderal species along roadsides and disturbed areas from near sea level to about 100 m. The present study was emphasized the phytochemical constituent, trace metal concentration and antimicrobial activity of ethanolic extracts from the aerial parts of *B. cristata*.

2. Materials and Methods

2.1 Plant Material and extraction

B. cristata leaves were collected in October 2015 from Tiruchirappalli district of Tamilnadu and dried at 31°C for 10 days. The shade dried *B. cristata* powder (100 g) was successively extracted with ethanol by soxhlet apparatus. The extract was filtered through membrane filter (0.45 m size) with the aid of a suction pump. The obtained filtrate was evaporated to dryness at 38°C. The extract was then weighed, dissolved in the minimal volume of dimethyl sulphoxide and used for screening of phytochemical, trace metal and antimicrobial activity.

2.2 Phytochemical screening

2.2.1 Qualitative analysis

The preliminary phytochemical investigation was carried out by the methods described by Koperuncholan and Ahmed John 2011[17]. The solvent extracts were subjected to routine qualitative chemical analysis to identify the nature of phytochemical constituents present in sample.

Steroids: A 3 ml of test solution and minimum quantity of chloroform was added with 3-4 drops of acetic anhydride and one drop of concentrated H_2SO_4. Purple color thus formed changes into blue or green color indicating the presence of steroids.

Triterpenoids: A 3 ml of test solution was added with a piece of tin and 2 drops of thionyl chloride. Formation of violet or purple colour indicates the presence of triterpenoids.

Reducing Sugars: A 3 ml of test solution was added with a 2 ml of Fehling's reagent and 2 ml of water. Formation of reddish orange color indicates the presence of reducing sugar.

Sugars: A 3 ml of the test solution was added with very small quantity of anthrone reagent and a few drops of concentrated H_2SO_4 and heated. Formation of green or purple color indicates the presence of sugars.

Alkaloids: A 3 ml of test solution was taken with 2N HCl. Aqueous layer formed was decanted and then added with one or a few drops of Mayer's reagent. Formation of white precipitate or turbidity indicates the presence of alkaloids.

Phenols: A 3 ml of test solution in alcohol was added with one drop of neutral ferric chloride (5%) solution. Formation of intense blue color indicates the presence of phenols.

Flavonoids: A 3 ml of test solution in alcohol was added with a bit of magnesium and one (or) two drops of concentrated HCl and heated. Formation of red or orange color indicates the presence of flavonoids.

Saponins: A 3 ml of test solution was added with water and shacked. Formation of foamy lather indicates the presence of Saponins.

Tannins: A 3 ml of test solution was added with water and lead acetate. Formation of white precipitate indicates the presence of tannins.

Anthroquinones: A 3 ml of test solution was added with magnesium acetate. Formation of pink color indicates the presence of anthroquinones.

Amino Acids: A 3 ml of test solution was added with 1% ninhydrin in alcohol. Formation of blue or violet color indicates the presence of amino acids.

Catechins: A 3 ml of test solution in alcohol was added with Ehrlich reagent and a few drops of concentrated HCl. Formation of pink color indicate the presence of catechins.

2.2.2 Quantitative analysis of phytoconstituents

The chlorophyll pigments in the leaves were estimated following the method of Arnon. The optical density was read at 645 nm and 663 nm wavelengths on a spectrophotometer. The Amino acids were estimated by ninhydrin method which is calorimetrically measured at 570nm. Proteins were estimated by Bradford method and the absorbance was measured at 595nm against blank/ sample. Carbohydrates were estimated by anthrone method which can be measured by using colorimetrically at 620 nm (or) by using a red filter. All the trials were performed thrice and the mean values were presented.

2.3 Testing of trace metals concentration in plants

The *B. cristata* plant sample was collected from the Tiruchirappalli district, Tamil Nadu. The plant leaves were carefully removed and washed with sterile distilled water, separately. The cleaned leaves were dried in shadow area and were grinned with agate mortar and pestle. The powered plant samples were stored in sterile plastic container. The 1 g of powdered plant samples was treated with aqua-regia mixture (hydrochloric acid + nitric acid) in Teflon bomb and was incubated at 140 °C for 2-3 days. After incubation, the reaction mixture was filtered with nitrocellulose (0.45 µm) filter paper by Millipore vacuum filtration unit. Then the extraction was test for trace metals (Fe, Cu, Zn, Pd, Cd, Cr and Ni) analysis. The extraction of the studied metals in the solutions was determined by the 797 VA Computrace voltametry, Metrohm.

2.4 Testing of antimicrobial activity

The test strains were: *Aeromonas liquefaciens* MTCC 2645 (B1), *Enterococcus faecalis* MTCC 439 (B2), *Klebsiella pneumonia* NCIM 2883 (B3), *Micrococcus luteus* NCIM 2871 (B4), *Salmonella typhimurium* NCIM 2501 (B5), *Vibrio cholerae* MTCC 3906 (B6), *Candida albicans* MTCC 1637 (F1), *Cryptococcus* sp. MTCC 7076 (F2), *Microsporum canis* MTCC 3270 (F3), *Trichophyton rubrum* MTCC 3272 (F4). The cultures were obtained from MTCC (Microbial Type Culture Collection), Chandigarh and NCIM (National Collection of Industrial Microorganisms), Pune, India. Microbial strains were tested for antimicrobial sensitivity using the disc diffusion method [18]-[21]. The antibacterial and antifungal activity of test samples was analyzed against certain microorganisms on muller hinton agar (MHA) and potato dextrose agar (PDA), respectively [22][23]. A sterile cotton swab was used to inoculate the standardized microbial suspension on surface of agar plate. The 0.60 and 1.20 mg/disk of sample coated disks were placed in agar plates, separately. For negative control study, the methanol solvent was used. The plates were incubated at 37±1°C for 24–48 h (for bacteria) and 25 ±1°C for 48-72 h (for fungus)[24][25]. After incubation, the zone of inhibition was measured with ruler. The assays were performed in triplicate and the average values are presented. Methicillin – 10mcg (for bacteria) and Itraconazole – 10mcg (for fungus) was used as positive control [26]. All the media, standard discs and sterile disc were purchased from Hi-Media (Mumbai, India).

3. Results and discussion

3.1 Phytochemical constituents of Secondary metabolites

Traditional herbal medicines are remains one of the most ancient living practiced widely in India. Medicinal herbs consist of wide variety of chemical compounds. Hence this present study was conducted to study the in vitro phytochemical, trace metal and antimicrobial activity of medicinal plant used by Indian peoples to show that the therapeutic properties of *B. cristata* used in traditional medicine coincide with laboratory findings. The ethanolic extract of *B. cristata* was subjected for phytochemical analysis, the results shows that positive for tests of steroids, triterpenes, alkaloids, phenols, flavonoids, saponins, tannins and amino acid but shows negative results for reducing sugars, sugars, catechins, anthroquinones were discussed in the table -1 the quantitative reports were discussed in the table -2.

Hundreds of distinct steroids are found in plants, animals and fungi. All steroids are made in cells either from the sterolslanosterol (animals and fungi, see below right) or from cycloartenol (plants). Both lanosterol and cycloartenol are derived from the cyclization of thetriterpene squalene. Steroids along with phospholipids function as components of cell membranes. Steroids such as cholesterol decrease membrane fluidity [27]. Terpenes are released by trees more actively in warmer weather, acting as a natural form of cloud seeding. The clouds reflect sunlight, allowing the forest to regulate its temperature [28]. Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants, and animals, and are part of the group of natural products (also called secondary metabolites). Many alkaloids can be purified from crude extracts by acid-base extraction. Many alkaloids are toxic to other organisms. They often have pharmacological effects and are used as medications, as recreational drugs, or in entheogenic rituals [17].

Flavonoids are one class of secondary plant metabolites that are also known as Vitamin P or citrin. These metabolites are mostly used in plants to produce yellow and other pigments which play a big role in colouring the plants. In addition, Flavonoids are readily ingested by humans and they seem to display important anti-inflammatory, anti-allergic and anti-cancer activities [29]. Tannase is an enzyme that Nierenstein used to produce m-digallic acid from gallotannins [17][37].

3.2 Trace metal analysis

Soil pH, anions and cations is very important because it influences the availability and plant uptake of micronutrients including heavy metals [30]. Toxic chemicals and trace metals are very important pollutants which affects all ecosystem at large extent [31]. The accumulation of trace metals in large quantities to the green plants causes physiological, biochemical, growth and functional changes to the large extent [32]. The results of the trace metal concentrations are summarized in table 3. The mean concentration of trace metals such as cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), nickel (Ni), lead (Pb) and zinc (Zn) were 0.05, BDL, 0.07, 0.88, BDL, 0.23 and 0.24 mg kg^{-1}, respectively. Through the natural process of bio-magnifications, minute quantities of metals become part of the various food chains and concentrations become elevated to levels which can prove to be toxic to human, animal, plant and other living organisms [33]. In particularly, higher trace metal in the plants caused progressive reduction in the photosynthetic ability of leaves, closure of leaf stomata, and productivity of plants, ascorbic acid content and chlorophyll content [34][35].

3.3 Antimicrobial activity

The antimicrobial activity of *B. cristata* ethanol solvent sample was examined with various pathogenic microorganisms using the disk diffusion test. The results of the antimicrobial activities are summarized in table 4. The two tested concentrations such as 0.60 and 1.20 mg/disc produce zone of inhibition on MHA and PDA plates for bacteria and fungi, respectively. In the present study, higher (1.20 mg) concentration of sample got greater sensitivity than (0.60 mg) lower concentration in all the tested microorganisms. Koperuncholan co-workers (2010) stated that the solvent extraction of plant were affected the bacterial strains in the higher concentration such as 2.5 and 5.0 mg/well. But in this study, we conformed that the low concentrations (0.60 and 1.20 mg/disk) of the *B. cristata* ethanol extract were highly affect the microbial growth. In this study, all the pathogens were fairly affected and nil effect was not observed in the test samples. In bacteria, the test sample was most effective against *Salmonella typhimurium* NCIM 2501 (B5) while smaller effect was noticed from *Micrococcus luteus* NCIM 2871 (B4). In fungi, this was effective against *Trichophyton rubrum* MTCC 3272 (F4) whereas smaller effect was observed in *Cryptococcus* sp. MTCC 7076 (F2). There is no antimicrobial activity in negative control such as ethanol.

Table 1: Qualitative phytochemical constituent of *B. cristata*

Phytochemical Constituents	ethanol
Steroids	+
Triterpenes	+
Reducing sugars	-
Sugars	-
Alkaloids	+
Phenolics	+
Catechins	-
Flavonoids	+
Saponins	+
Tannins	+
Anthraquinones	-
Amino acids	+

+ = Present; - =Absent

Table 2: Quantitative phytochemical constituent of *B. cristata*

Biochemical constituents	***B. cristata* (mg/g)**
Chlorophyll A	0.229
Chlorophyll B	0.872
Total Chlorophyll	1.101
Amino acid	165.0
Protein	2.670
Carbohydrate	1.905
Phenol	0.026

Table 3: Concentration of trace metals in *B. cristata*

Sampling Site	Sample Name	Cd	Cr	Cu	Fe	Ni	Pb	Zn
Tiruchirappalli Tamil Nadu	*Barleria cristata*	0.05	BDL	0.07	0.88	BDL	0.23	0.24

BDL – Below detectable limit

Table 4: Antimicrobial activity of the ethanolic solvent extract of *B. cristata* leaves

S. No	Test Microorganisms	Ethanol extract mg/disc		PC	Diseases	Route of Transmission
	Bacteria	0.60	1.20	10 mcg		
1.	*Aeromonas liquefaciens* B1	11	12	14	Wound Infections / Gastroenteritis	Water / Food
2.	*Enterococcus fecalis* B2	12	13	8	Endocarditis / Epididymal Infections	Water / Food
3.	*Klebsiella pneumoniae* B3	12	14	28	Acute diarrhoea / Dysentery	Water / Food
4.	*Micrococcus luteus* B4	10	11	38	Skin & Pulmonary infections	Soil / Water / Air / Food
5.	*Salmonella typhimurium* B5	14	16	0	Typhoid	Water / Food
6.	*Vibrio cholarae* B6	11	14	16	Cholera	Water / Food
	Fungi					
7.	*Candida albicans* F1	11	14	10	Skin infection / Gastrointestinal tract Infection	Air / Wound / Soil / Water
8.	*Cryptococcus* sp. F2	9	10	9	Bronchiectasis / Endophthalmitis.	Air / Wound / Soil / Water
9.	*Microsporum canis* F3	12	14	9	Tinea capitis /Ringworm	Air / Wound / Soil / Water
10.	*Trichophyton rubrum* F4	13	15	7	Tinea corporis / Tinea pedis	Air / Wound / Soil / Water

PC - Positive Control [Using antibiotic disc; Bacteria – Methicillin (10mcg/disc); Fungi – Itraconazole (10mcg/disc)]

4. Conclusion

This study has revealed that *B. cristata* presence of secondary metabolites such as steroids, triterpenes, alkaloids, phenols, flavonoids, and tannins are helps to the excellent antimicrobial properties which can be used as antimicrobial agents in new drugs for therapy of infectious diseases in humans. The result of the antimicrobial report explains the use of this plant in folk medicine for the treatment of different diseases and it could be a source of new antibiotic compounds being nontoxic and less expensive than the modern drugs. The trace metal concentrations were not crossing the standard levels and it gives the alarming for bioaccumulation/biomagnifications studies.

Acknowledgement

The authors thank the Biospark Biotechnological Research Center (BBRC), Tiruchirapalli, Tamil Nadu, India for antimicrobial studies.

Reference

[1] Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. *J. Appl. Microbiol*., 1999; 86(6): 985.

[2] Zaika LL. Species and herbs, their antimicrobial activity and its determination. *J. Food Safety*, 1975; 9: 97-118.

[3] Evans JS, Pattison E, Morris P (1986). Antimicrobial agents from plant cell culture. In: Stafford PMA, Fowler M (eds) Secondary metabolites in plant cell culture, Cambridge University, London.

[4] Castello MC, Phatak A, Chandra N, Sharon M. Antimicrobial activity of crude extracts from plant parts and corresponding calli of Bixa orellana L. *Indian J. Exp. Biol.* 2002; 40: 1378-1381.

[5] Nair R, Kalariya T, Chanda S. Antibacterial activity of some selected Indian medicinal flora. *Turk. J. Biol.* 2005; 29: 41-47.

[6] Gordon MC, David JN. Natural product drug discovery in the next millennium. Pharma Pharm. Biol. 2001; 139: 8-17.

[7] Koperuncholan M and Manogaran M. Edible plant mediated biosynthesis of silver and gold nanoflakes against human pathogens, *World Journal of Pharmaceutical Research* 2015, 4(1):1757-1775.

[8] Gundidza M, Gaza N. Antimicrobial activity of *Dalbergia melanoxylon* extracts. *J. Ethnopharmacol* 1993; 40: 127-130.
[9] Bauer AW, Kirby WMM. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.* 1966; 45: 493-496.
[10] Barnabas CG, Nagarajan S. Antimicrobial activity of flavonoids of some medicinal plants. *Fitoterapia* 1988; 3: 508-510.
[11] Latha M, Pari L. Preventive effect of Cassia auriculata L. flowers on brain lipid peroxidation in rats treated with sterptozotoc. *Mol. Cell. Biochem* 2003; 243: 23-28.
[12] Gupta AK and Mishra RM. Effect of lime kiln's air pollution on some plant species. *Pollution Research*. 1994; 13(1):1-9.
[13] Samal AC and SC Sautra. Air quality of Kalyani township and its impact on surrounding vegetation. *Indian J Environ Hlth*. 2002; 44:71-76.
[14] Shaw, A.J. Heavy Metal Tolerance in Plants: Evolutionary Aspects. CRC Press, Boca Raton, FL1990.
[15] Koperuncholan M and Ahmed John S. Biosynthesis of Silver and Gold Nanoparticles and Antimicrobial Studies of Some Ethno medicinal Plants in South-Eastern Slope of Western Ghats. IJPI'S Journal of Pharmacognosy and Herbal Formulations, 2011; 1(5):10-15.
[16] Ihekoronye, A.I., and Ngoddy, P.O. Integrated Food Science and Technology for the Tropics. Macmillan Publishers, 1985; 75–77.
[17] Koperuncholan M and Ahmed John S. Antimicrobial and Phytochemical Screening in *Myristica dactyloides* Gaertn. *Journal of Pharmacy Research* 2011; 4: 398-400.
[18] Vignesh, G, Arunachalam, S, Vignesh, S, Arthur James, R. BSA binding and antimicrobial studies of branched polyethyleneimine - copper (II) bipyridine / phenanthroline complexes. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 2012; 96: 108 - 116.
[19] Vignesh, G, Sugumar, K, Arunachalam, S, Vignesh, S, Arthur James, R. A Comparative Study on the Binding of Single and Double Chain Surfactant Cobalt (III) Complexes with Bovine Serum Albumin, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2013; 113: 415 – 422.
[20] Lakshmi praba, J, Arunachalam, S, Riyazuddin, R, Divya, R, Vignesh, S, Akbarsha, A, Arthur James, R. DNA/ RNA binding and anticancer/ antimicrobial activities of polymer-copper (II) complexes. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy.* 2013; 109: 23–31.
[21] Muthukumar K, Vignesh S, Dahms HU, Gokul MS, Palanichamy S, Subramanian G, Arthur James R. Antifouling assesments on biogenic nanoparticles: A filed study from polluted offshore platform. *Marine Pollution Bulletin* 2015 http://dx.doi.org/10.1016/j.mar.bul.2015.08.033.
[22] Anitha, R, Karthikeyan, B, Pandiyarajan, T, Vignesh, S, Arthur James, R, Vishwanathan, K, Murari, B.M. Antifungal studies on bio-compatible polymer encapsulated silver nanoparticles. International Journal of Nanoscience 2011; 10 (4): 1-5.
[23] Beevi, M.H., Vignesh, S, Pandiyarajan, T, Jegatheesan, P, Arthur James, R, Giridharan, N.V., Karthikeyan, B. Synthesis and antifungal studies on CuO nanostructures. *Advanced Materials Research*.2012; 488-489, 666 - 670.
[24] Vignesh G, Pradeep I, Arunachalam S, Vignesh S, Arthur James R, Arun R and Premkumar K. Biological and protein-binding studies of newly synthesized polymer–cobalt(III) complexes. *Luminescence* 2015. DOI 10.1002/bio.2992.
[25] Vignesh G, Sugumar K, Arunachalam S, Vignesh S, Arthur James R, Arun R and Premkumar K. Studies on the synthesis, characterization, human serum albumin binding and biological activity of single chain surfactant–cobalt(III) complexes. *Luminescence* 2015 DOI 10.1002/bio.2991.
[26] Pandiyarajan, T, Udaybhaskar, R, Vignesh, S, Arthur James, R, Karthikeyan, B. Concentration dependent antimicrobial activities of CuO nanoflakes. *Material science and engineering* C. 2013; 33(4): 2020–2024.
[27] Sadava D, Hillis DM, Heller HC, Berenbaum MR. Life: The Science of Biology 9th Edition. San Francisco: Freeman. pp. 2011; 105–114.
[28] Adam, David (October 31, 2008). "Scientists discover cloud-thickening chemicals in trees that could offer a new weapon in the fight against global warming". The Guardian.
[29] Koperuncholan M, Sathish Kumar P, Sathiyanarayanan G, Vivek G. Phytochemical Screening and Antimicrobial Studies of Some Ethno medicinal Plants in South-Eastern Slope of Western Ghats. *International journal of Medicobiologial* Research 2010; 1: 48-59.
[30] Kirkham, M.B., Cadmium in plants on polluted soils: Effects of soil factors, hyper accumulation and amendments. *Geoderma*, 2006; 137(1-2):19-32.

[31] White, P.A, and Claxton, L.D. Mutagens in contaminated soil: a review. Mutation Research- Reviews in Mutation Research. 2004; 567: 227-345.

[32] Prasad, M.S.V., Subramanian, R.B. and Inamdar, J.A. Effect of cement kiln dust on *Cajanus cajan* (L.) Mill. Species. *Indian Jr. Environ. Hlth*. 1991; 33: 11-21.

[33] Bryan, E.W. The effects of heavy metals (other than mercury) on marine and estuarine organisms. *Journal of London*. 1971; 177: 389–394.

[34] Gostin, I., Ivanescu, L. Structural and micromorphological Changes. *Int. J. Energy Environ* 2007; 4: 219–226.

[35] Ramesh V, Ahmed John S and Koperuncholan M. Impact of cement industries dust on selective green plants: A case study in Ariyalur industrial zone, *International Journal of Pharmaceutical, Chemical and Biological Sciences* 2014; 4:152-158.

[36] Ahmed John S and Koperuncholan M. Antibacterial Activities of various solvent extracts from *Impatiens balsamina*. *International Journal of Pharma and Bio Sciences*, 2012; 3:401-406.

[37] Nierenstein, M. "A biological synthesis of m-digallic acid". *The Biochemical Journal* 1932; 26 (4): 1093–1094. PMC 1261008. PMID 16744910.

[38] Ahmed John S and Koperuncholan M. Direct Root Regeneration and Indirect Organogenesis in *Silybum marianum* and Preliminary Phytochemical, Antibacterial Studies of Its Callus. *The International Journal of Pharmaceutics* 2012; 2: 52-57.