

Clinical Evaluation of the Antihyperlipidemic Effects of Ethanolic Extract from *Crossandra infundibuliformis* Leaves and Stems in a High Fat Diet-Induced Hyperlipidemia Model

Dr. R.Naga Kishore, M. Sree vidhya, K. Subhashini, N. Srividya

Department of pharmacy, Geethanjali college of pharmacy, Cheeryal, Keesara, Medchal, Hyderabad –501301

Abstract:

Hyperlipidemia is a disarray of lipid metabolism produced by elevation of plasma concentration of any or all lipid profile or lipoproteins rise in plasma treacher collins syndrome (TCs), and Triglycerides (TGs) levels; it is additionally called hyperlipoproteinemia. It is defined as increase serum TC, TG, VLDL, LDL and HDL which are responsible for different complications like: heart attack, coronary artery syndrome, stroke, atherosclerosis, myocardial infarction, and pancreatitis. Hyperlipidemia can be either primary or secondary type, the primary syndrome may be treated by hypolipidemic drugs, but secondary induced by diabetes, hypothyroidism or renal lipid nephrosis which treated by treating the original disease moderately than hyperlipidemia. Genetic disorders and lifestyle diet rich in calories, fat, and cholesterol play a vital role to cause dyslipidemia. Investigation of hypolipidemic effect of ethanolic extract of *Crossandra infundibuliformis* leaves and stems (ECILS) in the high-fat diet (cholesterol 2%, sodium cholate 1%, sucrose 48%, peanut oil, methionine 4%, and 47% normal laboratory feed). The mice feed with high-fat diet containing (cholesterol 2%, sodium cholate 1%, sucrose 48%, peanut oil, methionine 4%, and 47% normal standard laboratory feed) for 1-month. Then they are checked for the blood parameter levels like TC, TGs, low-density lipoproteins (LDL), very-low-density lipoprotein (VLDL), and low-density lipoproteins (HDL). Ethanolic extracts at a low dose (100 mg/kg), significantly reduced the levels of TC, TGL, LDL, VLDL, and increased the levels of HDL and reduced the body weights on 30th day, and at medium, high doses it reduced the levels of TC, TGL, LDL, VLDL and increased the levels of HDL and reduced the body weights.

Keywords: Atherosclerosis, Hyperlipidemia, nephrosis, extract of *Crossandra infundibuliformis* leaves and stems (ECILS), lowdensity lipoproteins (LDL), very-low-density lipoprotein (VLDL), and low-density lipoproteins (HDL).

Introduction:

Crossandra infundibuliformis is a well-known plant in India for its therapeutics Profits even in fields of medicine inclusive of Ayurveda and siddha yoga of traditional Medicine in the entire world for the treatment of several ailments. Every single part of this plant is used to remove the active phytochemistry. It revealed the existence of secondary metabolites as well as other compounds like tannins, saponins, alkaloids, flavonoids, steroids, glycosides, terpenoids, and carbohydrates. The Acanthaceae family includes *Crossandra infundibuliformis*. It has been demonstrated that this plant has several therapeutic qualities. The popular tropical flower *Crossandra infundibuliformis* is also called as "Firecracker" because, in high humidity, the seed

Pods that are typically developed after flowering tend to burst. It can resist extreme temperatures, grows to a height of 2 metres, and can withstand high humidity levels. Due to its perennial nature, continuous blossoming throughout the year, minimal fertiliser use, and higher profitability, it is of considerable importance when compared to other flower crops.

Hyperlipidemia is the state of increased levels of TC, TG, LDLs, and VLDL. Human lifestyle diseases include hyperlipidemia. Due to its numerous problems, including cardiovascular diseases including myocardial infarction (MI), congestive heart failure (CHF), hypertension, and atherosclerosis, it has a significant negative impact on human health. Diabetes mellitus (DM) and hyperlipidemia are also related.

Worldwide, atherosclerosis is the leading cause of death and disease. Heart failure can be brought on by a number of causes, including a diet heavy in saturated fats and cholesterol, age, family history, hypertension, and lifestyle choices. However, the majority of coronary heart diseases (CHDs) are caused by elevated levels of cholesterol, including TC, TG, and LDL cholesterol. About 31% less CHD incidence and 33% less CHD mortality can be caused by a 20% reduction in blood cholesterol levels. Lipids have important roles to perform in the body. Additionally, a side effect of diabetes is the induction of hyperlipidemia. The drugs that have some antioxidant and anti-diabetic action therefore also have a positive impact on hyperlipidemia. Inhibitors of hydroxymethylglutaryl-CoA (HMG CoA) reductase have been used to treat hyperlipidemia, and atorvastatin is one of the most widely used inhibitors.

Flavonoid-rich sources have the potential to be used in the treatment of hyperlipidemia, according to a literature review. According to a thorough review of the literature, *Crossandra infundibuliformis* is a reliable source of flavonoids. In order to test the plant's hypolipidemic potential against high-fat diet-induced hyperlipidemia in male rats, we used the leaves and stems of *Crossandra infundibuliformis*. A perennial herb native to India, Sri Lanka, and other Asian countries, *Crossandra infundibuliformis* (Acanthaceae) is typically grown as a flowering plant. It is an evergreen subshrub with polished and wavy-margined leaves and fan-shaped blossoms that may appear very frequently. This bloom's colour ranges from a standard orange shade to a coral salmon shade to a red shade and even emerald turquoise. Since ancient times, these plant components have been used to cure various forms of stress, such as irritability, and they are also renowned for their ability to heal wounds. When the dry case of the plant meets with water, it tears open like a sparkler, leading to the plants receiving their common name of sparkler. This plant is also notable for its sexual enhancer action and for its reducing movement. The goal of the current study is to examine the antihyperlipidemic properties of an ethanol extract of *Crossandra infundibuliformis*' leaves and stems.

Plant description:

Part I:

It is an upright, evergreen subshrub that can reach a height of 1 m. The fan-shaped blooms can bloom at any time during the plant's lifespan, and the leaves have glossy, wavy margins. Flowers have 3 to 5 asymmetrical petals and an erratic form. They develop from four-sided stalked spikes and have a 2 cm stalk that resembles a tube. The common colour orange is followed by salmon-orange, apricot, coral, red, yellow, and even turquoise flowers. This plant

is grown as a houseplant in temperate climates and needs a minimum temperature of 10°C. It is typically grown in pots, although growing it in beds might look nice.

Part II:

Crossandra is a flowering plant in family Acanthaceae, chromosome number of triploid ($2n = 30$) and tetraploid ($2n = 40$). It is a small, evergreen ornamental shrub growing 1 m (3 ft) producing beautiful flowers almost round the year. It has a remarkable range of colours varying from orange, pink, red, yellow and double-coloured blue types with a white throat. The leaves are glossy, oval in shape, upright and deep-green in colour. The flowers are unusually shaped with 3 to 5 asymmetrical petals. Inflorescence is hairy and the florets grow from four-sided stalked spikes with prominent bracts. The corolla is cylindrical, more-or-less curved; the stamens are fringed, four in pairs.

Plant profile:

Biological source: it is obtained by fresh and dried whole plants namely as *Crossandra infundibuliformis* belongs to family Acanthaceae

Species description *Crossandra infundibuliformis*:

The leaves are glossy and most striking, Flowers throughout the year. The deheaded and pinching back maintain a bushy shape and keep blooming with each new flush of growth.

1). *Crossandra greenstockii*:

Busheled crossandra is a miniature plant that is occasionally mistaken for a shrub. Around a golden centre, its orange petals open widely. From basal rosettes of broad green, irregularly lobed leaves, flower stalks rise tall.

2). *Crossandra pungens*:

It is a 60 cm tall thick subshrub. Oval, dull-green leaves with white veins are present. The spikes are crowded, the bracts' margins are roughly ovate and bristly to prickly, and the blooms are orange.

3). *Crossandra flava*:

It is a green, upright, unbranched shrub that is between 15 and 20 cm tall. The leaves are opposite, glabrous, oblong to lanceolate, and dark green in hue. Upper leaves are sessile, whereas below leaves are stalked. The 4-sided spike has yellow-green bracts that are spiky. Bright yellow flowers with a heavily constricted and jointed corolla tube are present.

4). *Crossandra guineensis*:

It grows freely and reaches a height of 30 to 60 cm. The bracts are violet in hue and have no spines. It may be expanded. both in a sunny environment and in some shade.

5). *Crossandra nilotica*:

The plants stand about 60 cm tall. Semi-woody stem. Ellipsoid, glossy, dark green leaves. Dense spikes of brick red blooms are produced. A lip is formed by the lower three petals.

6). *Crossandra undulaefolia*:

It is a little, 30-90 cm tall, perennial shrub with short branches. The leaves are pointy, 5–12 cm long, dark green, and glossy. Four-sided, 7.5 to 12.5 cm long spikes. Spiny bracts are pronounced on flowers. Because of the cracking sound made when the seed pod splits, it is also known as the "Fire cracker plant."

Origin and Distribution:

This plant is indigenous to Sri Lanka, Malaysia, and Southern India. Malenadu and Kerala in southern India are where it is most frequently found. In Tamil Nadu, crossandra is thought to be grown on an estimated 1317 hectares, producing 2634 tonnes annually at a productivity of 2 tonnes per hectare. In the districts of Coimbatore, Madurai, Villupuram, Cuddalore, Pondicherry, Trichy, and Thiruvannamalai in South India, the crop is mostly grown.

Table 1: The morphological Characteristics of *Crossandra infundibuliformis*.

Properties	
Common Name	Crossandra, firecracker flower
Plant Type	Evergreen perennial flower
Mature Size	1 to 3 feet tall and 1 to 2 feet tall
Soil Type	Rich, loamy, well-draining
Sun Expose	Part shade
Soil pH	5.8 to 6.8
Bloom Time	April to October
Flower Color	Orange, apricot, salmon pink, red
Hardiness Zones	10 to 11
Native	Southern
Area	India, Sri Lanka

Taxonomic Hierarchy.

Taxonomical Classification

Kingdom: plantae

Division: Tracheophyta Order: Lamiales

Family: Acanthaceae Genus: *Crossandra*

Species: *infundibuliformis*

Materials and Methods:

Procurement and Authentication of Plant The leaves and stems of *Crossandra infundibuliformis* were collected from Guntur, Andhra Pradesh. The plant material was identified and authenticated by Dr. P.V. Prasanna, Plant Taxologist, Scientist-F and HOD, Professor, Ministry of Environment, Botanical Survey of India, Hyderabad, Telangana.

Preparation of Plants Extract:

The leaves and stems of *Crossandra infundibuliformis* were collected and washed thoroughly with distilled water to make sure the leaves and stem are free of dust and are shade-dried. The dried leaves and stems are then powdered finely using a mechanical grinder. And then, the required quantity of the powder is subjected to solvent extraction with ethanol. The obtained extract is dried completely using desiccators.

Animals and Treatment:

Animals

Healthy male Sprague Dawley rats weighing between 250 and 300 g were procured and maintained in polypropylene cages at an ambient temperature of $22 \pm 1^\circ\text{C}$ and relative humidity of 50–60% with a 12 hours light/dark cycle in registered animal house. The animal experiments were carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals approved under the number (1048/a/07/CPCSEA), India and approved by the Institutional Animal Ethics Committee (IAEC). Throughout the experimental period, the animals were fed with standard pellet diet and water ad libitum.

Experimental Design:

Animals were randomly divided into six groups containing six ($n = 6$) animals each and treated as explained in Table 1. Body weights were recorded once a week throughout the experimental study. At the end of each week, blood samples were collected from the retro-orbital plexus. Blood samples were kept aside for approximately 1-hour at room temperature and centrifuged at 2500 rpm at 4°C for 15 minutes to separate the serum from blood. The serum samples were used for the estimation of biochemical parameters such as TC, HDL, LDL, VLDL, and TGs. At the end of the study, animals were sacrificed by cervical dislocation and cut open to isolate the liver and weighed immediately. Then the liver was fixed in 10% formalin and was used for histopathological study.

Table 1: Treatment Groups

S. No.	Name of the group	Treatment
1	Normal	Received standard pellet diet for 30 days.
2	HFD	Received high-fat diet for 30 days.
3	HFD + Standard atorvastatin	Received high fat diet + Standard Atorvastatin (20 mg/kg b.wt, p.o) for 30 days.
4	HFD + Test dose 100 mg/kg	Received high fat diet + 100 mg mg/kg b. wt , p.o of plant extract for 30 days.
5	HFD + Test dose 200 mg/kg	Received high fat diet + 200 mg mg/kg b. wt , p.o of plant extract for 30 days.
6	HFD + Test dose 400 mg/kg	Received high fat diet + 400 mg mg/kg b. wt , p.o of plant extract for 30 days.

Table 2: Blood parameters (TC, TGs, HDL, LDL, and VLDL), Bodyweight, before disease-induced hyperlipidemia in experimental rats

S. No.	Group	Body weight	TC	TG	HDL	LDL	VLDL
1	Normal	152 ± 0.12	61.12 ± 2.8	72.25 ± 1.9	29.65 ± 1.6	17.02 ± 2.4	16.08 ± 3.7
2	HFD	150 ± 1.12**	65.59 ± 1.5**	72.12 ± 1.0**	32.30 ± 1.3**	18.88 ± 3.2**	16.12 ± 1.2**
3	HFD ± Atorvastatin 20 mg/kg	156 ± 0.36*	62.32 ± 3.8*	72.12 ± 1.9*	45.25 ± 2.3*	30.36 ± 1.5*	24.23 ± 1.5*
4	HFD ± Test dose 100 mg/kg	160 ± 2.35*	62.32 ± 1.6*	84.32 ± 3.5*	29.68 ± 1.9*	15.77 ± 2.3	23.03 ± 2.6**
5	HFD ± Test dose 200 mg/kg	158 ± 1.22*	72.23 ± 1.5*	70.1 ± 2.3*	30.25 ± 1.5*	27.96 ± 2.9	22.6 ± 2.06*
6	HFD ± Test dose 400 mg/kg	162 ± 0.66*	65.75 ± 1.2*	72.8 ± 2.6*	36.2 ± 2.1*	15.31 ± 1.8*	27.1 ± 2.3*

Statistical Analysis:

The results were expressed as mean ± S.D. [Standard error of the mean (SEM)]. Statistical analysis was calculated using one-way ANOVA followed by post hoc Dunnett's test for multiple comparisons, and statistical significance was set at $p < 0.05$. Values are represented as mean ± SEM; a (***) - $p < 0.0001$, b (**) - $p < 0.01$, c (*) - $p < 0.05$.

Results:

Estimation of Serum Biochemical Parameters The estimated values of biochemical parameters in all the experimental rats before and after the treatment period are summarized in Tables 2 and 3, Figs. 1 and 2.

Histopathological Findings:

Histopathological changes in the liver of high-fat diet-induced hyperlipidemia after 30 days treatment is shown in Fig. 3.

Discussion:

It has been well established that nutrition plays an important role in the etiology of hyperlipidemia and atherosclerosis. In our study, we choose cholesterol diet, which contains the common ingredients in our daily food. Cholesterol feeding has been often used to elevate serum or tissue cholesterol levels to assess the hypercholesterolemia related metabolic disturbances in animals. Cholesterol feeding alone, however, does not affect the serum TG level. It is assumed that a high level of saturated fat, in addition to cholesterol, is required to elevate serum TG levels in a rat model significantly. The extract exhibited a significant control of serum lipid profiles in rats. From the obtained result, it was observed that keeping the animal on HFD significantly increased the TC, TG, LDL-C level in serum ($p < 0.05$) as compared to rats on normal diet. When high functioning depression HFD was co-administered with ECILS, the elevated levels of TC, TG, and LDL-C conditions have shown a considerable decline. It was noted that TC, TG, and LDL-C lowering activity of ECILS (400 mg/kg) was more

significant as compared to the other two lower doses. There was a significant elevation in plasma HDL-C in ECILS treated rats as compared to HFD rats, thus indicating the efficacy of ECILS in preventing the elevation seen in various components of lipid profile under experimentally induced hyperlipidemia. Ample of evidence exists with respect to the fact that HDL cholesterol is inversely related to the total body cholesterol and a reduction of plasma HDL cholesterol concentration may accelerate the development of atherosclerosis leading to ischaemic heart diseases, by impairing the clearing of cholesterol from the arterial wall. Flavonoids are reported to increase HDL-C concentration and decrease in LDL and VLDL levels in hypercholesterolaemic rats. Flavonoids and polyphenols found in our ECILS could, therefore, be considered favorable in increasing HDL and decreasing LDL and VLDL in ECILS treated rats. The findings in the study in this context with the previous studies are quite comparable.

Hypercholesterolaemia is the state been depicted by an expanded greasy substances called lipids, rise in plasma TCs and TGs levels it is additionally called hyper lipoproteinemia. The present study was designed to investigate the hypolipidemic effect of ethanolic extract of *Crossandra infundibuliformis* leaves and stem (ECILS) in high fat diet (cholesterol 2%, sodium cholate 1%, sucrose 48%, peanut oil, methionine 4%, and 47% normal laboratory feed) . The rats feed with High-fat diet containing (cholesterol 2%, sodium cholate 1%, sucrose 48%, peanut oil, methionine 4%, and 47% normal standard laboratory feed) for 1 month, and then they are checked for the blood parameter levels like TC, TGs, LDL, VLDL, and HDL. Ethanolic extracts at low dose (100 mg/kg), significantly reduced the levels of TC, TGL, LDL, VLDL & increased the levels of HDL and reduced the body weights on 30st day, and at medium, high doses it reduced the levels of TC, TGL, LDL, VLDL & increased the levels of HDL and reduced the body weights.

Evaluation of Antihyperlipidemic Activity of Ethanolic Extract of *Crossandra infundibuliformis* Leaves and Stems...

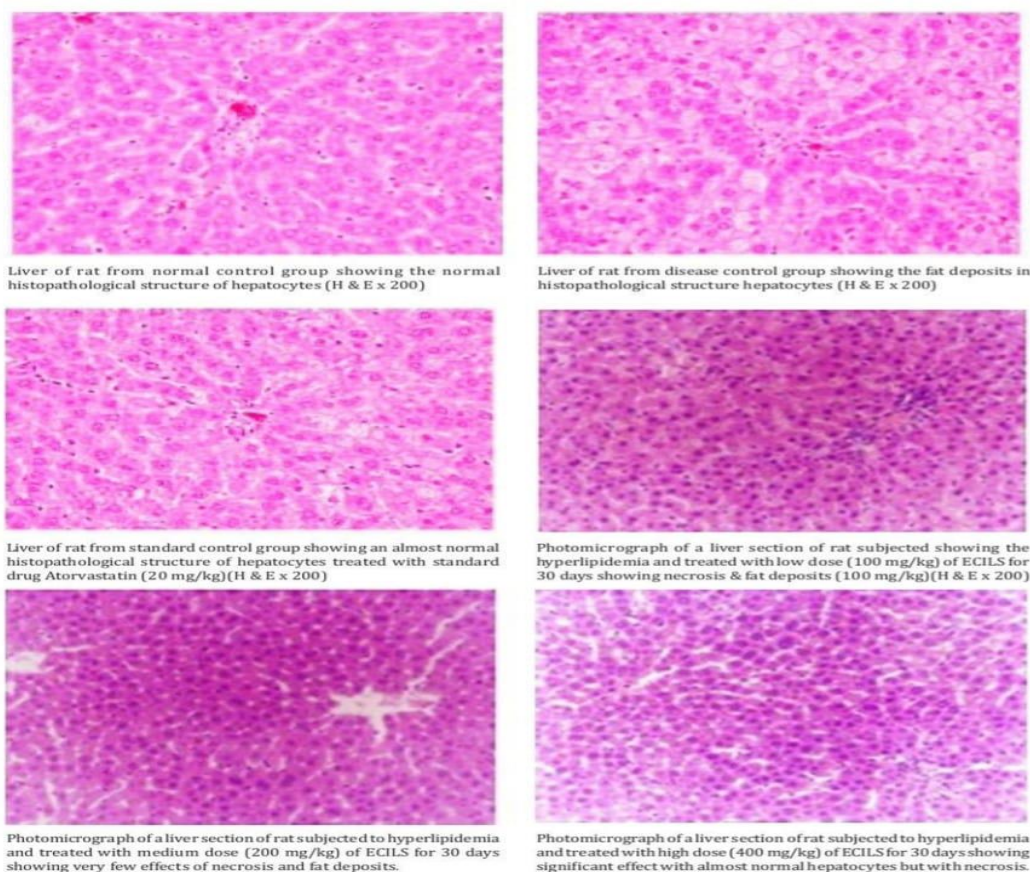


Fig. 3: Effect of ECILS and other treatment groups on histopathology of the liver after 30th day of treatment

Conclusion:

The result of this present study revealed that the ethanoic extract of *Crossandra infundibuliformis* leaves and stems improved the serum lipid profile in rats by decreasing serum TC, TG, LDL-C, and increasing serum HDL-C. This finding provides some biochemical basis for the use of leaves and stems extract of *Crossandra infundibuliformis* as an antihyperlipidemic agent having a preventive and curative effect against hyperlipidemia. Further, studies are required to gain more insight into the possible mechanism of action.

Reference:

1. Grundy SM, Vega GL. Hypertriglyceridemia: causes and relation to coronary heart disease. In Seminars in thrombosis and hemostasis 1988 Apr (Vol. 14, No. 02, pp. 149-164). Copyright© 1988 by Thieme Medical Publishers, Inc.
2. Robinson MT. Nutrition Evidence-Based Guidelines for Treating Hyperlipidemia in Children with Type 1 Diabetes. The Diabetes Educator. 2009 May;35(3):408-419

3. Zafar Alam Mahmood, Syed WA, Mohamaad S, Zafar M. Hyperlipidimia development and consequences, *Int J Mol Sci.* 2009;15(3).
4. Shamir R, Fisher EA. Dietary therapy for children with hypercholesterolemia. *American family physician.* 2000 Feb 1;61(3):675-682.
5. Brown MS, Goldstein JL. Lipoprotein receptors in the liver. Control signals for plasma cholesterol traffic. *The Journal of clinical investigation.* 1983 Sep 1;72(3):743-7.
6. Sivagamasundari R. Anti-Hyperlipidemic Activity of Aerial Parts of *Delonix elata* on High Cholesterol Diet Induced Hyperlipidemia in Rats (Doctoral dissertation, Mohamed Sathak AJ College of Pharmacy, Chennai).
7. Vinay Kumar, Abul K Abbas and Nelson F. *Patholog. basis of disease.* 7th Edition. New Delhi: Elsevier. 2002. 46-49.
8. Coughlan BJ, Sorrentino MJ. Does hypertriglyceridemia increase risk for CAD? Growing evidence suggests it plays a role. *Postgraduate medicine.* 2000 Dec 20;108(7):77.
9. Kinoshita B, Glick H, Garland G. Cholesterol and coronary heart disease: predicting risks by levels and ratios. *Annals of internal medicine.* 1994 Nov 1;121(9):641-647.
10. Fungwe TV, Cagen LM, Cook GA, Wilcox HG, Heimberg M. Dietary cholesterol stimulates hepatic biosynthesis of triglyceride and reduces oxidation of fatty acids in the rat. *Journal of lipid research.* 1993 Jun 1;34(6):933-941.
11. Aruna P, Rajangam J, Rani PG, Manivannan MI. Nutrient studies in *Crossandra* (*Crossandra infundibuliformis* L. Nees). *Asian Journal of Horticulture.* 2007;2(2):169-177.
12. Elamathi R, Deepa T, Kavitha R, Kamalakannan P, Sridhar S, Suresh Kumar J. Phytochemical screening and antimicrobial activity of leaf extracts of *Crossandra infundibuliformis* (L.) nees on common bacterial and fungal pathogens. *Int j curr sci.* 2011;1:72-77.
13. Gundamaraju R, Verma TM. Evaluation of wound healing activity of *Crossandra infundibuliformis* flower extract on albino rats. *International Journal of Pharmaceutical Sciences and Research.* 2012 Nov 1;3(11):4545.
14. Vadivel E, Sunita V. In vitro Anticancer and Insecticidal Activity of *Crossandra infundibuliformis*. *Journal of chemical and pharmaceutical research.* 2016;8:260-264.
15. Vasantha G. The antidiabetic effect of leaf and stem extract of *Crossandra infundibuliformis* in alloxan-induced diabetic rats and diabetic nephropathy in male rats. *International Journal of Green Pharmacy (IJGP).* 2020 Mar 16;14(1):79-86..
16. Huang X, Vaag A, Hansson M, Weng J, Laurila ES, Groop L. Impaired insulin-stimulated expression of the glycogen synthase gene in skeletal muscle of type 2 diabetic patients is acquired rather than inherited. *The Journal of Clinical Endocrinology & Metabolism.* 2000 Apr 1;85(4):1584-1590.
17. Priya SV, Saratha R. *Crossandra Infundibuliformis* Leaves as Corrosion Inhibitor for Mild Steel in 1 M HCl and 0.5 M H₂SO₄. *J. Environ. Nanotechnol.*;3(1):30-36.

18. Patil KG, Jaishree V, Tejaswi HP. Evaluation of Phenolic Content and Antioxidant Property of *Crossandra infundibuliformis* Leaves Extracts. American Journal of Plant Sciences. 2014 Apr 1;2014.
19. Prasad K. Hypocholesterolemic and antiatherosclerotic effect of flax lignan complex isolated from flaxseed. Atherosclerosis. 2005 Apr 1;179(2):269-275.
20. Grundy SM, Vega GL. Hypertriglyceridemia: causes and relation to coronary heart disease. In Seminars in thrombosis and hemostasis 1988 Apr (Vol. 14, No. 02, pp. 149-164). Copyright© 1988 by Thieme Medical Publishers, Inc.
21. Bacharach PS, Denke MA, Stein EA, Rifkin BM. Lipid and Dyslipoproteinemia in Clinical Diagnosis and Management by Laboratory Methods. Journal of Pharmacy research. 2001;29:224-248.
21. Karuppaiah, P., Effect of bioregulators and Feso₄ on growth, yield and quality of *Crossandra* (*Crossandra infundibuliformis* L.). Horticulture International Journal, 2019, 3(1),60–80.
22. Karuppaiah, P., Effect of bioregulators and Feso₄ on growth, yield and quality of *Crossandra* (*Crossandra infundibuliformis* L.). Horticulture International Journal, 2019b, 3(1),13–19.
23. Pharmacognosy, Phytochemicals and Traditional Use. Journal of Advances in Medical and Pharmaceutical Sciences, 2015, 4(4),1–13.