Kidney diseases: prevalence, incidence and aetiology

Chronic kidney diseases become apparent, as a global health problem, resource-constrained nations struggle to address this challenge due to the significant cost of life-sustaining treatments and long-term renal replacement therapies. Early identification of chronic kidney disease is needed to prevent disease progression and reduces secondary complications like the risk of the cardiovascular morbidity and mortality (Vivekanand *et al.*, 2013).

It is reported nearly 34,000 cases and 4,500 deaths have been reported in the last 10 years even as the state government and medical institutions which struggle to provide dialysis to the underprivileged afflicted population. Early detection of chronic kidney disease also requires the development of cost-effective approaches. Because of a shortage of trained nephrologists, general practitioners are involved in caring for patients with chronic kidney disease. In India for about 1.2 billion population, have only about 900 nephrologists. This means that general physician or other specialists will look after the vast majority of patients with kidney problems (Ganguli, 2016).

According to the Global Burden of Disease study chronic kidney disease in 2010 kidney disease which ranked 27th in the list of causes of total number of global deaths in 1990 (age-standardised annual death rate of 15·7 per 100 000), but rose to 18th in 2010 (annual death rate 16·3 per 100 000) (Lozano *et al.*, 2013). The kidney diseases can be classified mainly diabetic and non-diabetic kidney disease shown in figure 1.

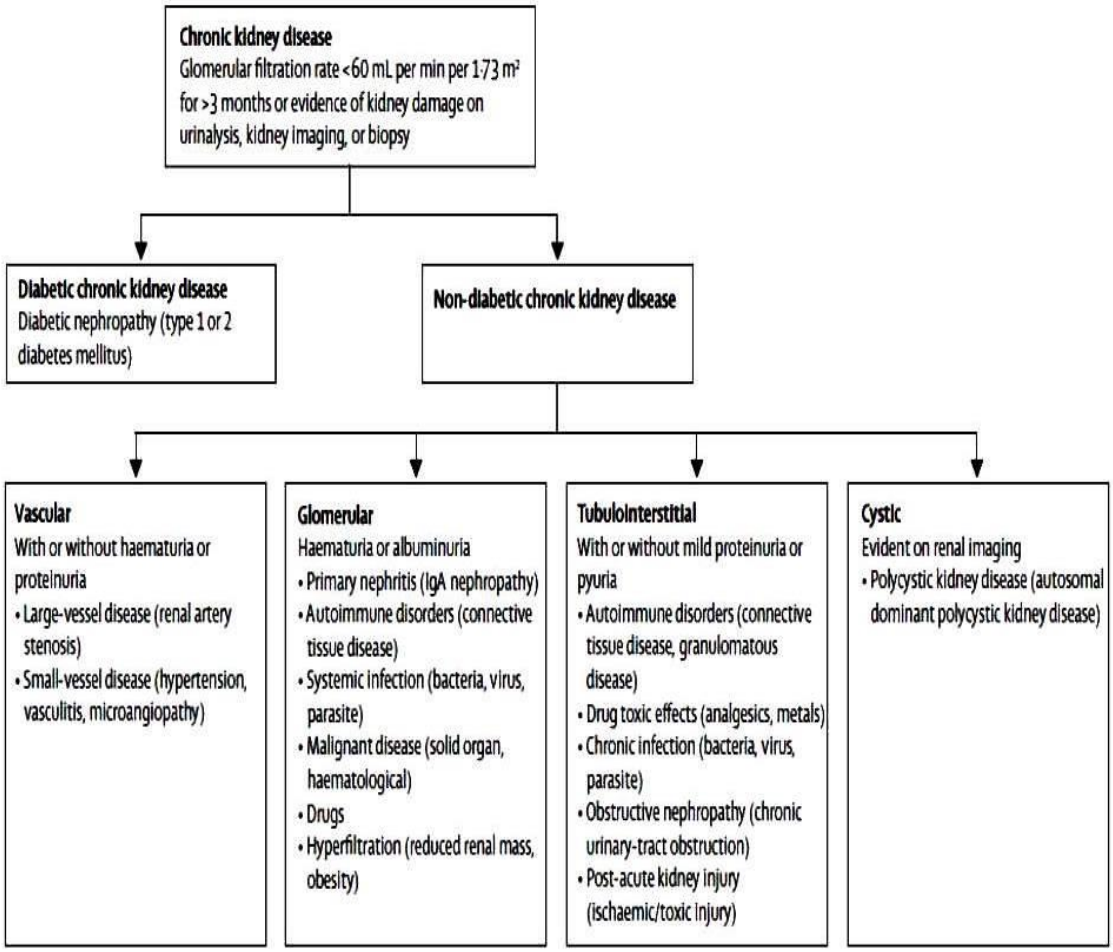

Figure 1: Classification of chronic kidney disease and symptoms.

Several systemic diseases such as hypertension, metabolic syndrome, diabetes mellitus and hypercholesterolemia may also lead to nephropathy. Chemotherapeutic agents, infection, antibiotics and radiocontrast agents mainly excreted from the kidney. Environmental toxins mostly heavy metals such as mercury and lead, occupational chemicals such as radiation, urban fine particles, smoking as well as alcohol consumption induce renal damage. In recent years, oxidative stress induced nephrotoxicity have become one of the most popular research topics in the molecular mechanism of kidney diseases (Ozbek, 2012).

Drug-Induced Nephrotoxicity

The kidney is an essential organ required by the body to control major systems including the regulation of the extracellular environment, maintenance of homeostasis, such as detoxification and excretion of toxic metabolites and drugs (Ferguson *et al*., 2008). Therefore, the kidneys are considered to be the major target organ for exogenous or endogenous toxicants (Finn and Porter, 2003; Galley, 2000). Kidney diseases are considered a global health issue worldwide. They contribute to approximately 850,000 deaths per year, making them the 12th leading cause of death (Nasri, 2014; Schieppati and Remuzzi, 2005). Drug-induced nephrotoxicity is now days increasingly recognized as a significant contributor to kidney diseases including acute kidney injury (AKI) and chronic kidney disease (CKD). Nephrotoxicity has a wide spectrum of toxic mechanism, reflecting damage to different nephron segments based upon individual drug mechanisms. Both tubular and glomerular injuries are recognized as targets for drug toxicity and may result in acute or chronic functional damage (Awdishu, 2017). Nephrotoxicity focuses primarily on drug-induced nephropathy. Prospective cohort studies of kidney disease have documented the frequency of drug-induced nephrotoxicity to be approximately 14-26% in adult populations worldwide (Hoste *et al.*, 2015; Uchino *et al.*, 2005; Mehta *et al.*, 2004).

Pathophysiology of Cisplatin induced nephrotoxicity

Cisplatin (*cis*-diamminedichloroplatinum (II)), is a metallic (platinum) coordination compound. It is a major antineoplastic drug used for the treatment of solid tumours. In the 1960s, it was discovered to have cytotoxic properties in bacteria. The first in-vivo tests performed at the Chester Beatty Institute in London, United Kingdom, Cisplatin was taken on by the US National Cancer Institute (NCI) for clinical testing. The first

patients were treated with cisplatin in 1971. Approval was granted in 1978 by the US Food and Drug Administration (FDA). Cisplatin has made a major impact in the chemotherapeutic treatment of testicular and ovarian cancers and is widely used today as the chemotherapeutic drug (Kelland, 2007).

The chief dose-limiting side effect of cisplatin is nephrotoxicity, 20% of patients receiving high-dose cisplatin suffer severely from renal dysfunction. The mechanism for this renal cell injury by platinum containing drugs has been the focus of intense investigation for many years. Understanding the mechanism for nephrotoxicity provides a model for investigating drug-induced nephrotoxicity for further studies and allows the clinicians to prevent or treat this problem better (Schrier *et al.*, 2004; Thadhani *et al.*, 1996; Merouani *et al.*, 1996).

Uptake of Cisplatin into Renal Cells

The kidney accumulates cisplatin to a larger extent than other organs of the body and is also the major route for its excretion. The cisplatin concentration in proximal tubular epithelial cells is about 5 times more than the serum concentration. The major site of renal toxicity is the S3 segment of the proximal tubule followed by the distal collecting tubule and then the S1 segment of the proximal tubule. In addition to a transporter-mediated transportation process, cisplatin also enters the cell through passive diffusion intracellularly, the highest concentrations of cisplatin are found in the mitochondria, cytosol, nuclei and microsomes (Kroning *et al*., 2000; Gately and Howell, 1993).

The critical transporter for cisplatin in kidney is the organic cation transporter (OCT 2), it carries cisplatin to proximal tubules in both animals and humans. Transportation

is mediated by these membrane proteins is polyspecific, electrogenic, voltage-dependent, bi-directional, pH-independent, Na-independent. Three isoforms of organic cation transporter have been identified in humans. OCT1 is the main isoform of the liver, OCT2 is the main OCT in the kidney and OCT3 is widely expressed in some organs, especially in the placenta (Xin *et al*., 2007).

Cisplatin Metabolism

Cisplatin is converted to a nephrotoxic molecule in proximal tubule cells of the kidney and leads to toxicity. Cisplatin is conjugated to glutathione and then metabolized to a reactive thiol through a γ-glutamyl transpeptidase located on the cell surface and cysteine *S*-conjugate β-lyase an intracellular enzyme and finally to a potent nephrotoxin. Inhibition of these 2 enzymes has no effect on the uptake of cisplatin into the renal cells but it reduces nephrotoxicity.

Subcellular mechanisms of cisplatin induced kidney toxicity

Inhibition of protein synthesis

Protein synthesis inhibition seems to be post transcriptional event in non- proliferating cells or normal cells of the kidney, like renal tubular epithelial cells, this is the predominantly biochemical manifestation for cisplatin toxicity in proximal tubular cells. The suppression of protein synthesis may involve the ability of cisplatin to disturb ribosome assembly in-vitro by interference with the joining of the 48S and 60S subunits. The mechanism of cisplatin cytotoxicity in tumour cells is different from normal cells which are mainly by inhibition of DNA and RNA synthesis and formation of adduct with DNA (Valderra *et al*., 1996).

Glutathione and protein-SH depletion

Intracellular glutathione pool consists of cytosolic (30%) and mitochondrial (70%). Cisplatin or other platinum containing chemotherapeutic agents are able to form conjugates with thiol group by this mechanism, the dissociation of one of the chlorine atom which results in a positive charge on the platinum that will attract the negatively charged Sulphur on the cysteine moiety of the GSH (Mistry *et al*., 1989). Binding of cisplatin by GSH and protein- sulfhydryl groups results in cellular damage. Reduced glutathione is present as the major cellular oxidant defense system and it is also a potent factor in the restricting of lipid peroxidation. Mitochondrial GSH becomes essential in the regulation of inner membrane permeability by keeping intra-mitochondrial SH groups in the reduced state. The interaction of the platinum compound with sulfhydryl groups is the second essential factor promoting cytotoxicity. Due to the molecule's high affinity to SH groups, its chloride moieties are replaced by sulfhydryl-groups. The formation of stable protein-S-drug adducts results in dysfunction of membrane-associated proteins and cytoplasmic proteins (e.g. Na+/phosphate and Na+/glucose co-transporters) and decreases the activity of essential enzyme systems like glutathione-S transferase reductase and peroxidase. In addition, stable glutathione-cisplatin adducts lead to a decline the amount of reduced glutathione available to scavenge free reactive oxygen metabolites. This may efficiently affect the cellular oxidant defense systems, eventually leading to lipid peroxidation. Thus, the extent of cellular damage seems to be mainly depending upon the amount of cellular reactive -SH groups (Zhang and Lindup, 1993; Mistry *et al*., 1991; Bompart, 1989)

Oxidative stress injury that damage Renal Cells

Oxidative stress is actively involved in the pathogenesis of cisplatin and other chemotherapeutic agents induced kidney injury. There is an important role of free oxygen radicals in the pathophysiology of nephrotoxicity induced by cisplatin. Reactive oxygen species (ROS) such as hydrogen peroxide, hydroxyl radicals or superoxide anion, these are normally generated in renal cells. Reactive oxygen species are immediately detoxified by endogenous antioxidants as they generate inside the cells, e.g. Glutathione (GSH), superoxide dismutase (SOD) or catalase (CAT). Intracellular accumulation of ROS leads to DNA damage and membrane lipid peroxidation. Cisplatin also lead to GSH depletion, thus allowing lipid peroxidation to occur followed by cellular damage (Kuhlmann *et al.*, 1997). Reactive oxygen species (ROS) directly act on cellular components, including proteins, lipids and DNA also destroy their structure.

Mitochondria are reported to be a major site of ROS production under aerobic conditions, according to an endogenous and continuous physiological process. Mitochondrial damage includes reduction in mitochondrial inhibition of Na+/K+-ATPase, Ca^{2+} uptake, lipid peroxidation, and depletion of pyridine nucleotides and collapse of the mitochondrial membrane potential. Cisplatin inhibits the function of respiratory complexes in normal renal tubular cells stimulates ROS production by damaged mitochondria (Kuhlmann *et al.*, 1997).

Oxidative stress is defined by excessive production of reactive oxygen species (ROS) and deficient in antioxidant systems. Oxidative stressed cells are characterized by redox imbalance with the accumulation of depletion of ATP, reduced pyridine

nucleotides, Cytochrome-c release, mitochondrial membrane depolarization and activation of apoptotic and necrotic pathways. In all aerobic cells, mostly ROS are generated as by-products of normal metabolic reactions (Armann *et al*., 2007). ROS include oxygen radicals, such as the hydroxyl radical (OH^{o}) and superoxide anion ($O2^{o-}$) and also some non-radical derivatives of oxygen, like hydrogen peroxide (H_2O_2). About 1–5% of mitochondrial oxygen consumption leads to H_2O_2 production. A reductive homolytic cleavage of H_2O_2 produces the highly cytotoxic hydroxyl radical (OH^{o}). This highly reactive radical is assumed to be directly responsible for most of the oxidative cellular damage leading to the non-physiological necrosis.

In the presence of cisplatin, ROS are produced through defined pathways that are implicated in the pathogenesis of drug induced renal injury. ROS are mainly produced in mitochondria via the xanthine-xanthine oxidase system, and NADPH oxidase in cells (Kawai *et al*., 2006). Cisplatin induces hexokinase activity and glucose-6-phosphate dehydrogenase, which increase free radical production and decrease antioxidant production. When intracellular cisplatin concentration is high mitochondrial damage is observed. In recent studies, damage to renal tubular mitochondria was observed when millimolar concentration of cisplatin administered. Hydrogen peroxide (H_2O_2), Superoxide anion (O_2^{o}) and hydroxyl radical (OH^{o}) are increased in cisplatin and chemotherapeutic agents treated kidneys. These free radicals damage the lipid components of the cell membrane by peroxidation and denature proteins, which further lead to enzymatic inactivation. Cisplatin causes inhibition of antioxidant enzymes and renal activities of superoxide dismutase, glutathione peroxidase, and catalase are significantly decreased (Badary *et al.*, 2005; Kadikoylu *et al.*, 2004; Shino *et al.*, 2003; Durak *et al*., 2002; Davis *et al.*, 2001)

The main targets of ROS in mitochondria are the polyunsaturated fatty acids and the protein components of the membranes. In membranes, ROS affect cysteine residues (sulfhydryl groups), causing intramolecular cross-linking and formation of protein aggregates. The OH^{o} radicals can initiate lipid peroxidation and also generate peroxyl and alkoxyl radical intermediates. Oxidants increase the release of calcium from mitochondria and stimulate calcium-dependent enzymes, such as proteases, phospholipases and nucleases. Because of the absence of mitochondrial DNA-protecting proteins and low-efficiency reparation mechanism and the proximity of the respiration chain, mtDNA is an important target for ROS. The oxidative stress caused due to an excessive accumulation of ROS results in necrotic cell death as a result of massive cellular damages associated to alterations of proteins and nucleic acids and lipid peroxidation (Shoji *et al.*, 1995).

Mammalian cells also feature several antioxidant defence systems that consist of enzymatic oxidants (catalase, superoxide dismutase and peroxidase) and non-enzymatic antioxidants (vitamins, thioredoxin and reduced glutathione) (Adam and Chinopoulos, 2006; Halliwell, 1999; Fridovich, 1998). Mitochondria possess an antioxidant system with the NADH, superoxide dismutase (SOD) and a complete glutathione redox system, which is formed of glutathione reductase, glutathione peroxidase and reduced glutathione (Fleury *et al.*, 2002).

The Regulation of Reactive Nitrogen Species in nephrotoxicity

In eukaryotic cells, mitochondria are the main source of ATP, that produced through the mitochondrial electron-transport chain which is a multicomponent system involved in a series of oxidation-reduction reactions. Several cellular processes produce significant amounts of ROS that is responsible for intracellular oxidative stress.

Alternatively, mitochondrial superoxide may also react with nitric oxide to produce peroxynitrite anion, a potent oxidant which causes irreversible damage to mitochondria components and inhibition of mitochondria respiration (Figure 2). It induces cell death or necrosis in several cell types. Nitric oxide (NO) is potent oxidant can also promote or inhibit apoptosis, according to the cell type, the rate of production and the interaction with other intracellular molecules, it leads to nitrosative stress (Fleury *et al.*, 2002). Reactive nitrogen species includes $OONO^-$, NO_2Cl, and NO_2^o. RNS originates from nitric oxide free radical (NO^o), which are synthesised from family of nitric oxide synthase (NOS) composed of three isoforms: neuronal NOS (nNOS), endothelial (eNOS) and inducible (iNOS). iNOS is inducible isoform, up regulated or activated in response to lipopolysaccharides, cytokines and oxidative stress. The iNOS produces large amount of NO^o that under oxidative stress condition react with O_2^{o-} to form nitric-peroxide free radicals (Chirino *et al*., 2008).

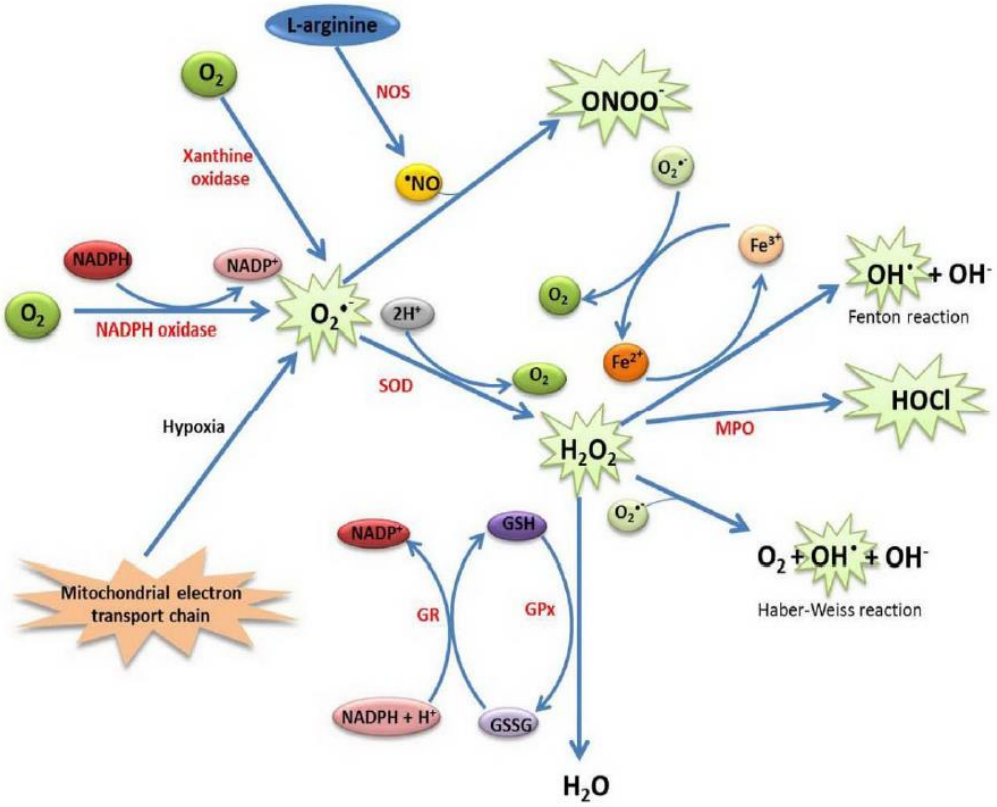

Figure 2: Graphical representation for generation of ROS and RNS (modified from Valko *et al.*, 2007). Note: superoxide anions (O2º-); hydroxyl radical (ºOH); hydrogen peroxide (H_2O_2); hypochlorous acid (HOCl); peroxynitrite ($ONOO^-$); nitric oxide (ºNO); nitric oxide synthase (NOS); superoxide dismutase (SOD); myeloperoxidase (MPO); glutathione peroxidase (GPx); glutathione reductase (GR); reduced glutathione (GSH); oxidized glutathione (GSSG); nicotinamide adenine dinucleotide phosphate (NADPH); ferric (Fe^{3+}); and ferrous (Fe^{2+})

Apoptosis and necrosis are two cisplatin induced model for toxicity. Apoptosis is associated with toxicity-induced by therapeutic doses whereas necrosis has been associated with high doses of cisplatin (Arany and Safirstein, 2003). Several studies indicate that in drug-induced nephrotoxicity is mainly associated with reactive oxygen species (ROS) which leads to the activation of apoptotic pathways. ROS have been shown to induce tumour suppressor protein p53 activation, mainly by causing oxidative DNA damage (Casares *et al.*, 2012; Conklin, 2000; Sahin *et al.*, 2010).

Thus, one may assume that cisplatin increases ROS production, which in turn contributes to p53 activation and ultimately apoptosis of renal cells (Ju *et al.*, 2014).

Inflammatory cytokines in nephrotoxicity

The inflammatory response is not only a local action but it can also be evidenced systemically, as it is accompanied by increases in inflammatory markers including acute phase proteins and adhesion molecules and cytokines. Studies reported that patients with established kidney injury, there was the increase in serum interleukin IL-6, IL-8, IL-10, IL-1β and tumour necrosis factor-α (TNF-α) (Simmons *et al*., 2004). The role of inflammation in renal toxicity has been increasingly appreciated with involvement of the leukocytes, chemokines, adhesion molecules, and cytokines (Safirstein, 2007). TNF-alpha is a potent proinflammatory cytokine and important mediator of inflammation in tissue damage. Recent studies have shown that cisplatin-induced acute renal failure is correlated with proinflammatory cytokines such as Interleukin-6 (IL-6), Tumour necrosis factor-alpha (TNF-α) and Interleukin-1beta (IL-1β) (Gihyun *et al.*, 2012).

The renal tubular epithelium is a major prone site of cell injury and necrosis during renal toxicity and plays a pro-inflammatory role in the injury. Interferon regulatory factor-1 (IRF-1) is a transcription factor known to activate pro-inflammatory genes, including chemokine and interferon. A recent study has demonstrated that IRF-1 is an early evaluative pro-inflammatory signal produced within S3 proximal tubule cells stimulated by reactive oxygen species during chemic injury in-vitro and in-vivo (Wang *et al.*, 2009).

Many cytokines are released by renal tubular cells and leukocytes into the injured kidney and are important components of both the initiation and extension of inflammation during toxicity. Chemokines are a large subgroup of cytokine-like molecules that play a major role in the recruitment of leukocytes and produced inflammation. Chemokines are induced by cytokines (IL-1β and TNF-α), complement activation, NF-κB, reactive oxygen species and Toll-like receptors related pathways (Ali *et al*., 2009).

Cell cycle regulation in nephrotoxicity

Reactive oxygen species are the endogenous cause of DNA damage in kidney cells (Yan *et al*., 2016). It has also been reported, that cells in the kidney enter the cell cycle in-vivo, after cisplatin administration. Researchers suggested that cell cycle inhibition could be one of the important targets of preventing renal toxicity, it allows time and opportunity to damaged DNA to get repaired and complete the regeneration process. The cell cycle inhibitory drug could be an effective therapy for cisplatin nephrotoxicity (Megyesi *et al.*, 1998; Megyesi *et al*., 2002; Hanigan and Devarajan, 2003).

Medicinal plants for nephroprotective activity

Overages of 65 years, more than one-fifth of the world population have some degrees of kidney disease. Kidney problems are caused by various agents. Oxidative stress play prominent role in renal toxicity. Oxidative stress is an important factor contributing to kidney damage by increasing production of oxidants, particularly insufficiency of endogenous antioxidant defence system. Antioxidants properties of medicinal plants are able to ameliorate oxidatively induced kidney damage by

reduction of reduced glutathione and lipid peroxidation and enhancement of scavenging ability of antioxidant defence system. Supplementation of medicinal plants as herbal products might be considered as important remedies to invalidate pathology of oxidative stress induced kidney toxicity. Modern research has shown that a wide range of plants can neutralize or detoxify toxins and protect respiratory, hepatic, urinary and neural systems from the toxic effects of drugs and chemicals (Snafi, 2015). The nephroprotective activities of some medicinal plants are shown in table 1.

Table 1: Medicinal plants with reported nephroprotective activity

S.No.	Plant name	Family	Part used	Nephrotoxicity model	Reference
1	*Kalanchoe pinnata*	*Crassulaceae*	Leaf	Gentamycin	Harlalka *et al.*, 2007
2	*Hemidesmus indicus*	Apocynaceae	Root	Gentamycin	Kotnis *et al.*, 2004
3	*Bauhinia variegata*	Fabaceae	Stems	Gentamicin, cisplatin	Sharma *et al.*, 2011; Pani *et al.*, 2011
4	*Phoenix dactylifera*	Arecaceae	Dates	Gentamycin	Qarawi *et al.*, 2008
5	*Benincasa hispida*	Cucurbitaceae	Fruit	Paracetamol	Varghese *et al.*, 2013
6	*Brassica nigra*	Brassicaceae	Leaves	D-GalN-	Rajamurugan *et al.*, 2012
7	*Bryophyllum calycinum*	Crassulaceae	Leaves	Gentamycin	Kadhim, 2014
8	*Calotropis procera*	Apocynaceae	Flowers	Carbon-tetrachloride	Dahiru *et al.*, 2013

9	*Cassia occidentalis*	Fabaceae	Leaves	Gentamicin	Gowrisri *et al*., 2012
10	*Adhatoda zeylanica*	Acanthaceae	Leaves	Gentamycin	Kumar *et al*., 2012
11	*Aegle marmelos*	Rutacaeae	Leaves	Gentamycin	Kore *et al*., 2011
12	*Aerva javanica*	Amaranthaceae	Fresh roots	Cisplatin	Movaliyaa *et al*., 2011
13	*Aerva lanata*	Amaranthaceae	Whole plant	Cisplatin	Shirwaikar *et al*., 2004
14	*Allium sativum*	Amaryllidaceae	Garlic	Cisplatin	N. Anusuya *et al*., 2013
15	*Aloe barbadensis*	Xanthorrhoeace-ae	Leaves	Cisplatin Gentamycin	Chatterjee *et al*., 2012
16	*Avuri kudineer*	Fabaceae	Roots and Leaves	Cisplatin	Priyadarsini *et al*., 2012
17	*Berberris aristata*	Berberidaceae	Root bark	Cisplatin	Adikay *et al*., 2010
18	*Boerhaavia diffusa*	Nyctaginaceae	Leaves	Cisplatin	Singh *et al*., 1992
19	*Butea monosperma*	Fabaceae	Whole plant	Gentamycin	Sonkar *et al*., 2010
20	*Carica papaya*	Caricaceae	Seeds	Paracetamol	Madinah *et al*., *2015*
21	*Cassia auriculata*	Fabaceae	Root	Gentamycin	Shirwaikar *et al*., 2005
22	*Casuarina equisetifolia*	Casuarinaceae	Dried leaves	Gentamycin	Tantawy *et al*., 2013
23	*Cichoriumintybus*	Asteraceae	Aerial Parts	Cisplatin	Noori and Mahboob, 2012
24	*Clitoria ternatea*	Papilionaceae	Whole plant	APAP-induced	Sarumathy,

					2011
25	*Crataevanurvula*	Capparidaceae	Fruit	Gentamycin	Shelkea *et al*., 2011
26	*Curcuma longa*	Zingeberaceae	Rhizome	Cadmium	Tarasub *et al*., 2011
27	*Dichrostachys cinera*	Mimosaceae	Roots	Cisplatin	Adikay *et al*., 2009
28	*Diospyros lotus*	Ebenaceae	Seeds	Gentamycin	Moghaddam *et al*., 2012
29	*Elephantophus scaber*	Asteraceae	Leaves	Gentamycin	Bhusan *et al*., 2012
30	*Emblica officinalis*	Euphorbiaceae	Fruits	Gentamycin	Pallavi and Balaraman, 2004
31	*Ficus religiosa*	Moraceae	Dried latex	Cisplatin	Yogesh *et al*., 2011
32	*Ficus racemosa*	Moraceae	Stem bark	Gentamycin	Gowda *et al*., 2011
33	*Ginkgo biloba*	Ginkgoceae	Leaves	Gentamycin	Naidu *et al*., 2000
34	*Harungana madagascarienis*	Hypericaceae	Root	Acetaminophen	Adeneye *et al*., 2011
35	*Ichnocarpus frutescens*	Apocynaceae	Whole plants	Cisplatin	Anbu *et al*., 2012
36	*Kalancho epinnata*	Crassulaceae	Leaves	Gentamycin	Harlalka *et al*., 2007
37	*Kigelia africana*	Bignoniaceae	Fruits	Cisplatin	Azu *et al*., 2010
38	*Lantana camara*	Verbenaceae	Roots	Gentamycin	Vyas and Argal, 2012
39	*Mammea africana*	Guttiferae	Stem bark	Acetaminophen	Okokon and

					Michael, 2014
40	*Momordica tuberosa*	Cucurbitaceae	Dried tubers	Cisplatin, Gentamycin & Acetaminophen	Kumar *et al*., 2011
41	*Moringa pterygosperma*	Moringaceae	Leaves	Acetaminophen	Lakshmana *et al*., 2013
42	*Mulberry (Morus Sp.)*	Moraceae	Leaves	Acetaminophen	Salih *et al*., 2015
43	*Nigella sativa*	Ranunculaceae	seeds	Gentamycin and cisplatin	Hosseinian *et al*., 2016; Ali, 2004
44	*Oroxylum indicum*	Bignoniaceae	Whole plant	Gentamycin	Mishra *et al*., 2014
45	*Panax ginseng*	Araliacea	Roots	Cisplatin	Lobina *et al*., 2014
46	Pedalium murex	Pedaliaceae	Dried fruits	Cisplatin& Gentamycin	Sreedevi *et al*., 2011; Shelke *et al*., 2009
47	Phaseolus radiatus	Leguminosae	Seeds	Gentamycin	Chaware, 2012
48	*Phyllanthus amarus*	Euphorbiaceae	Seeds	Gentamycin	Adeneye and Benebo, 2008
49	*Phyllanthus niruri*	Euphorbiaceae	Leaves	Gentamycin	Reddy *et al*., 2015
51	*Pimpinella tirupatiensis*	Apiaceae	Whole plant	Acetaminophen	Palani *et al*., 2009
53	*Piper cubeba*	Piperaceae	Dried berries	Gentamycin	Ahmad *et al*., 2012
54	*Plectranthus amboinicus*	Lamiaceae	Leaves	Acetaminophen	Palani *et al*., 2010
55	*Pongamia pinnata*	Papilionaceae	Flowers	Cisplatin	Shirwaikar *et al*., 2003

56	*Portulaoleracea*	Portulaceae	Leaves Stem	Cisplatin	Sudhakar *et al*., 2010
57	*Rhazyastricta*	Apocynaceae	Leaves	Gentamycin	Ali, 2002
58	*Rubiacardifolia Linn*	Rubiaceae	Root	Ethylene glycol	Divakar *et al*., 2010
59	*Saccharum offcinarum*	Poaceae	Jaggery	Acetaminophen	Samiulla, 2000
60	*Salviae offcinalis*	Lamiaceae	Whole plant	Cisplatin	Dizaye *et al*., 2010
61	*Sida cordifolia*	Malvaceae	Leaves & Root	Gentamycin	Mehul *et al*., 2012
62	*Sida rhomboidea*	Malvaceae	leaves	Gentamycin	Thounaojam *et al*., 2010
63	*Solanum xanthocarpum*	Solanaceae	fruits	Cisplatin& Gentamycin	Hussain *et al*., 2012
64	*Tinospora cardifolia*	Menispermeac-ea	Stem	Cisplatin	Uppuluri *et al*., 2013
65	*Tribulus terrestris*	Zygophyllacea-e	Fruits	Gentamycin	Meher *et al*., 2016
66	*Trichosanthes cucumerina*	Cucurbitaceae	Fruits	Cisplatin	Prasanthi and Adikay, 2017
67	*Vitex negundolinn*	Verbenaceae	Bark	Chemical	Kadir *et al*., 2013
68	*Withania somnifera*	Solanaceae	Roots	Gentamycin	Shimmi *et al*., 2011
69	*Zingiber officinale roscoe*	Zingiberaceae	Ginger Rhizome	Gentamycin	Rodrigues *et al*., 2014

Pathophysiology of Ocular infection

Indian population is vulnerable to infections of the eye by virtue of subtropical climate. Infections are among the most common disease processes that can affect the

eye. They can be mild infections that are essentially self-limiting or severe sight-threatening conditions that require aggressive intervention to preserve sight. Many of the institutions are established with highly advanced microbiology laboratory facilities dedicated to the investigation of eye infections. We must first understand the basic anatomy of the normal eye (Figure 3) (Sharma, 2010; Thielen *et al*., 2000).

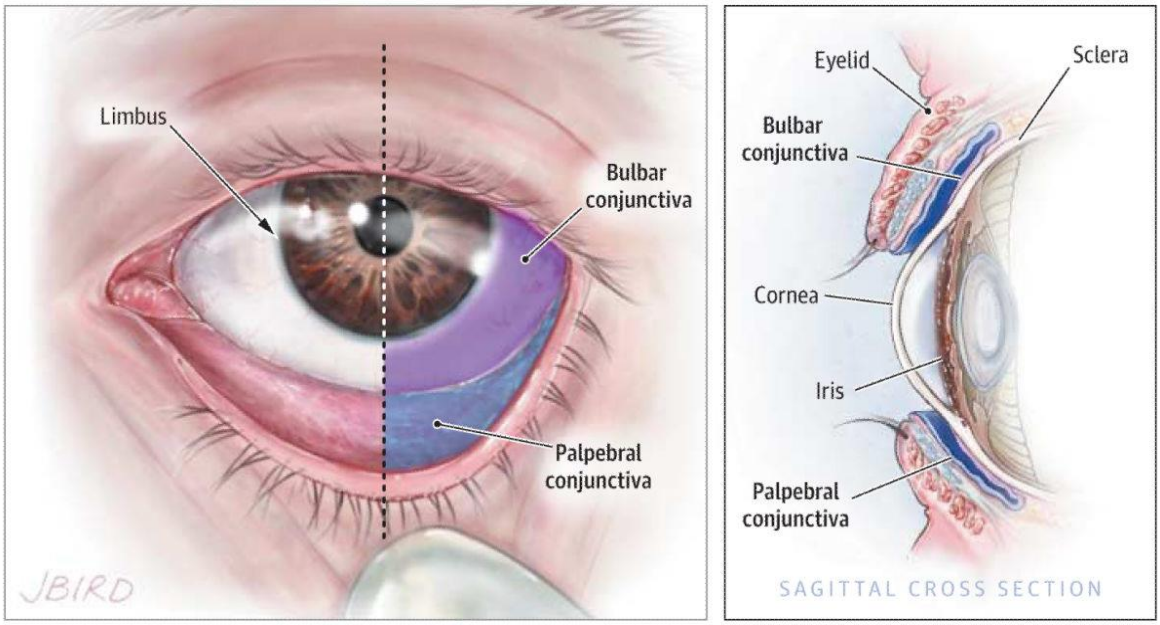

Figure 3: Anatomy of normal eye.

Conjunctivitis

Conjunctiva is a thin, translucent membrane lining the anterior part of the sclera and beneath of the eyelids. It has 2 parts, bulbar and palpebral. The bulbar portion starts at the edge of the cornea and covers the visible part of the sclera. The palpebral part lines the inside of the eyelids. Infection or inflammation of the conjunctiva is known as conjunctivitis and is characterized by dilatation of the conjunctiva vessels, resulting in hyperaemia and oedema of the conjunctiva, typically with associated discharge (Leibowitz, 2000).

The occurrence of conjunctivitis varies according to the underlying cause, which may be influenced by the patient's age, as well as the season of the year. Viral conjunctivitis is the most prominent cause of infectious conjunctivitis both overall and in the adult population and is more prevalent in summer. Bacterial conjunctivitis is the second important cause and is responsible for the majority (50%-75%) of cases in children, these are observed more frequently from December through April. Allergic conjunctivitis is the most frequent cause, affecting 15% to 40% of the population and observed more frequently in spring and summer (Amir *et al*., 2013; Uchio *et al*., 2000; Horven, 1993; Harding *et al*., 1987; Ronnerstam *et al*., 1985; Stenson *et al*., 1982). There are common types of conjunctivitis include bacterial, allergic, toxic and viral. Clinical signs and symptoms vary depending on the aetiology (Table 2).

Bacterial Conjunctivitis

Bacterial conjunctivitis can be divided into three groups: hyper-acute, acute, or chronic (Thielen, 2000).

Hyper-acute Bacterial Conjunctivitis

Hyper-acute bacterial conjunctivitis can occur at any age, but is most often seen in neonates or sexually active adolescents and also in young adults. It is characterized by a purulent discharge, copious, significantly injected conjunctiva and lid oedema. The infection can be unilateral or bilateral and corneal involvement is also prevalent. Perforation and ulceration of the cornea occur in approximately 10% of patients. The most common causative organisms are *Neisseria gonorrhoeae* and *Neisseria meningitides*.

Acute Bacterial Conjunctivitis

Acute bacterial conjunctivitis presents with a purulent discharge, eyelid sticking, foreign body sensation, chemosis, papillae on the tarsal conjunctiva, and conjunctival injection. It can be seen in any age group and can be unilateral or bilateral. In children under six years of age, otitis media (inflammatory disease) can develop as a result of bacterial conjunctivitis. The most common causative agent worldwide is *Staphylococcus aureus*, *Streptococcus pneumonia* and *Haemophilus influenzae.*

Chronic Bacterial Conjunctivitis

Chronic conjunctivitis is a condition that lasts four weeks or longer. The patient may have nonspecific symptoms such as irritation, burning, tearing, sticky eyelids, redness, and foreign body sensation. They may have diffused the conjunctival injection, mild mucous discharge and a papillary or follicular palpebral conjunctival reaction. The most common causative agents are *Moraxella lacunata* and *Staphylococcus aureus.*

Chlamydial Conjunctivitis

Chlamydia is obligate intracellular parasites that require a host cell for its metabolic biosynthesis. *Chlamydia trachomatis* is the species that causes ocular disease in humans.

Inclusion

Inclusion conjunctivitis is found most commonly in adolescents and young adults and is generally a sexually transmitted. Transmitted disease associated with chlamydial urethritis or cervicitis. Patients present with an acute follicular response and mild mucopurulent discharge, which is most commonly unilateral with pre-auricular

lymphadenopathy on the affected side. The cornea may become involved, result in keratitis, infiltrates and superior sub-epithelial opacities. The disease may last from three to 12 months if untreated.

Trachoma

Trachoma is a major cause of preventable blindness worldwide. It is largely found in areas with populations that have poor hygiene and low socioeconomic status. It is endemic in many developing countries. Acute infection is generally seen in children and is characterized by photophobia, bilateral conjunctival infection, tearing and mucopurulent discharge. Conjunctival follicles may form and the cornea often becomes involved with superior epithelial keratitis and pannus.

Fungal Conjunctivitis

Similar to bacterial conjunctivitis but is often delayed in the onset of symptoms. Long-term corticosteroid treatment and extended contact lens wear are also risk factors. Species like *Candida, Aspergillus* and *Fusarium* are the most common pathogens. Fungal ulcers resolve very slowly and antifungal agents are toxic to the epithelium of the cornea. Topical corticosteroids may enhance the spread of fungal ulcers and are therefore contraindicated.

Table 2: Types of Conjunctivitis (Ocular infection) with causing organism and treatment

Infection	**Signs and Symptoms**	**Common Organism(s)**	**Antimicrobial Treatment**
Conjunctivitis			
Hyperacute bacterial Conjunctivitis	Purulent discharge, injected conjunctiva, conjunctival/lid edema	*Neisseria meningitides Neisseria gonorrhoea*	Ceftriaxone
Acute bacterial Conjunctivitis	Purulent discharge, eyelid sticking, conjunctival/injection hyperemia, foreign body sensation, conjunctival edema	*Staphylococcus aureus Streptococcus pneumoniae, Haemophilus influenzae*	Trimethoprim/polymixin B or polymixin B with Bacitracin,or an Aminoglycoside
Chronic bacterial Conjunctivitis	Irritation, foreign body sensation, burning, tearing, redness, mild discharge.	*Staphylococcus aureus, Moraxella lacunata*	Bacitracin or Erythromycin
Chlamydial			
Inclusion	Conjunctival follicles, mild discharge, lymphadenopathy	*Chlamydia trachomatis*	Tetracycline/doxycycline or Erythromycin
Trachoma	Conjunctival injection, tearing, photophobia, mucopurulent discharge	*Chlamydia trachomatis*	Erythromycin
Ophthalmia neonatorium			
Chlamydial	Mucopurulent discharge, lid edema	*Chlamydia trachomatis*	Erythromycin
Gonococcal	Periorbital/conjunctival edema, purulent exudate, chemosis	*Neisseria gonorrhoeae*	Ceftriaxone

Bacterial	Hyperemia, conjunctival/lid edema, purulent discharge	*Staphylococcus aureus*, *Haemophilus influenzae*, *Streptococcus viridans*, *Escherichia coli*	Trimethoprim/polymixin B
Fungal	similar to bacterial (delayed)	*Candida* spp., *Aspergillus* spp., *Fusarium* spp	Natamycin or Amphotericin B

PLANT PROFILE

Exacum (Gentianaceae), also known as German violet and Persian violet is a genus of about 65 species. Genus *Exacum* was introduced by Linnaeus in 1753. Later Genus *Exacum* was revised by Klackenberg in 1985 with 65 species (Klackenberg, 1985). Most of the *Exacum* species are not sufficiently studied because of their restricted and local endemic distribution and collected only a few times in a year (Peter, 2006). India is enriched with 16 species of Genus *Exacum* (John *et al*., 2001). The species of *Exacum* are used by human since ancient time as herbal remedies for fever, eye and skin diseases and urinary disorders by traditional medical practitioners. In the last decades, the use of *Exacum* species in traditional medical practices led to rapid increase in information available on active phytoconstituents present in *Exacum*.

Exacum lawii C.B. Clarke, species of genus *Exacum* is small herb commonly distributed in the Western peninsula, Western coast region of India, Mysore and Coimbatore, Southern part India. It is endemic to Jarandeshwar hill from Satara district, Maharashtra and Western ghat of Karnataka. Plant taxonomy of *Exacum lawii* is shown below. The *Exacum lawii* is annual, glabrous, small erect herb rarely reaching 15 cm tall and flowers are bluish-purple. The whole plant has been used traditionally as the folk remedy for the treatment of kidney disorders, eye diseases and also used as laxative. The common name is Law's Persian violet. It is locally known as Lahan chirayata in Maharashtra, Manali in Malayalam, Marukozhunthu in Tamil. The *Exacum lawii* is annual, glabrous, small erect herb rarely reaching 15 cm tall. Flowers are bluish-purple.

Class: Equisetopsida C. Agardh

Subclass: Magnoliidae Novak ex Takht.

Superorder: Asteranae Takht.

Order: Gentianales Juss. ex Bercht. & J. Presl

Family: Gentianaceae Juss.

Genus: Exacum

Species: Lawii

Since the *Exacum lawii* has traditional medicinal values still, there is no data reported on its quality control profile, pharmacology and phytochemistry. Therefore, the aim of the present study is to develop the scientific standardization monograph with DNA fingerprinting profile and to explore the phytochemistry and pharmacology of whole plant of *Exacum lawii.*

Botanical description and distribution

Exacum is erect annual herb to perennial sub herbs 2 cm to 1m tall. Stems are terete to quadrangular often with four wings or lines/ribs. Leaves are opposite decussate, rarely verticillate or rosulate. The inflorescence is monochasial or dichasial cyme, sometimes umbel-shaped. Flowers are tetra- or penta- merous, actinomorphic to often zygomorphic by having the anthers forming a cone above a bent (enantiostyly). Calyx persistent each lobe furnished with a keel or a wing that might enlarge in fruit, rarely zygomorphic by having two well-developed wings and three reduced ones. Corolla is white to violet up to 7 cm long, tube short and lobes usually spreading, rarely persistent in fruit. Stamens are protruding from corolla tube, anthers usually connivent around or above the style forming a cone, dehiscent by one or two apical pores usually furnished by small papillae on their dorsal side. Testa cells are star-shaped or isodiametric. The ovary is 2 carpellate, 2-locular, placentation axile, style filiform, straight, or curved; stigma small, entire rarely cup-shaped (Merckx *et al*., 2013).

Exacum shows a typical paleotropical distribution (Klackenberg, 2002; Thulin, 2001; Klackenberg, 1985). Gondwana vicariance hypothesis has been suggested on the basis of morphological and anatomical characters, to explain the distribution pattern of *Exacum*, especially for the divergence among the species of Madagascar, India and Socotra-Arabia. The majority of species (38 species) occur in Madagascar. The second most species-rich area (14 species) is Sri Lanka and the Southern tip of the Indian subcontinent (mainly the Western Ghats), three species found in North India and the Himalayas. Southern Arabian Peninsula (Dhofar of Oman and nearby Mahrah of Yemen) and the Island of Socotra shared three species (Figure 5). The African continent has only two species *Exacum oldenlandioides* widespread throughout the entire tropical Africa and *Exacum zombense* endemic to the Shire Highlands in Malawi. Two species, *Exacum hamiltonii* and *Exacum teres*, are restricted to the Himalayas, and two species, *Exacum pteranthum* and *Exacum sutaepense*, are limited to the mountainous regions between Burma and Thailand (Yong *et al*., 2005). *Exacum paucisquamum* is a new record to the Flora of Hong Kong (Paul *et al*., 2012). Madagascar and the area including Southern India and Sri Lanka are the major centers of diversity, and only some of its species occur in Socotra (Arabian Peninsula), Himalayas, Southeast Asia, New Guinea and in the extreme Northern Australia. *Exacum* species have a wide spectrum of habitat preferences. Most of its species occur in lowland and montane rainforest area, they mainly grow in full sun (Klackenberg, 1985; Klackenberg, 1990; Klackenberg, 2002). Some *Exacum* species found in India are shown below (Figure 4). *Exacum* species reported in Indian literature are listed in table 3.

Table 3: Species of Exacum reported in India literature.

Species	Distribution	Threatened species	Reference
Exacum anamallayanum	Western ghats, anamallais and pulney hills		Gamble, 1967
Exacum atropurpureum	Western ghats, hill of Tinnevelly and Travancore, Palghat hill, poonachi, Kanniyakumari		Henry *et al*., 1987; Gamble, 1967
Exacum affine	Socotra, Yemen	Least concern	Gamble, 1967
Exacum arabicum	Arabian Peninsula, Yemen and Dhofar in Southern Oman	Least concern	Patzelt and Knees, 2013
Exacum bicolor	Deccan hills of Mysore and Coimbatore, Western ghats.	Endangered	Henry, 1987; Gamble, 1967
Exacum courtallense	Western ghats, Tinnevelly district, Kanniyakumari		Henry, 1987; Gamble, 1967
Exacum caeruleum	Socotra, Yemen.	Vulnerable	Miller and Morris, 2004
Exacum grande	Coimbatore, Nilgiris and Salem		Henry, 1987
Exacum grcilipes	Dhofar region of southern oman		Miller and Morris, 2004
Exacum lawii	Bababudan hills of mysore, Coimbatore and western coast		Henry, 1987; Gamble 1967; Chopra *et al*., 1956
Exacum pedunculatum	All plains, grass lands and moist places of India		Henry, 1987; Gamble, 1967
Exacum perrotteti,	Deccan hills of Mysore, Western ghats, Nilgiris		Gamble, 1967
Exacum petiolare	Western ghats, Coimbatore, Deccan hills of Mysore, Nilgiris, Anamalais and Cochin		Henry, 1987; Gamble, 1967

Exacum pumilum	Coimbatore, Maharashtra		Henry, 1987
Exacum pusillum	western Mediterranean, Spain, Portugal, France	Near threatened	Belair, 2010
Exacum sessile	Western coast and western ghats, Kanniyakumari, Coimbatore and nilgiris		Henry, 1987, Gamble, 1967
Exacum socotranum	Island of Socotra, Yemen	Critically endangered	Miller and Morris, 2004
Exacum tetragonum	Rampa hills of Godavari, Coimbatore, nilgiri, tirunelveli, Himalayas from Shimla to Bhutan, Bengal and Chota Nagpur to Madhya Pradesh		Henry, 1987; Gamble, 1967; Chopra *et al*., 1956
Exacum travancoricum	Western ghats, hill of Tinnevelly, agastiamalai		Henry, 1987; Gamble, 1967
Exacum wightianum	Western ghats, nilgiri, hills of Travancore		Gamble, 1967

Figure 4: Images of some selected Exacum species found in India a) *Exacum affine*, b) *Exacum bicolor*, c) *Exacum lawii* d) *Exacum pedunculatum*, e) *Exacum tetragonum*, f) *Exacum wightianum*, g) *Exacum paucisquamum*, h) *Exacum pumilum*, i) *Exacum subteres*.

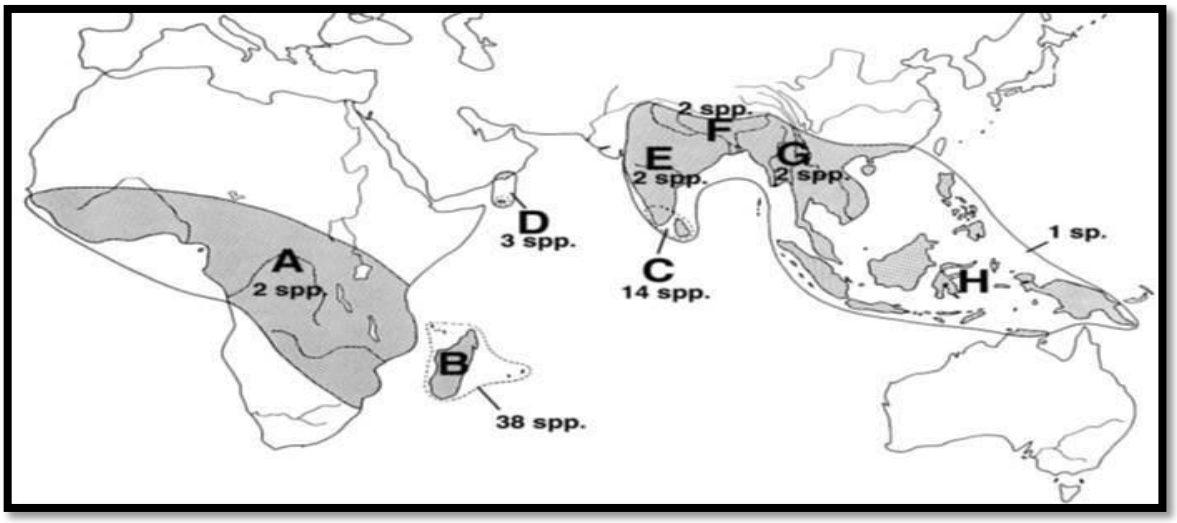

Figure 5: Distribution and relevant numbers of species of *Exacum***.** A: African continent B: Madagascar, C: Srilanka and southern tip of Indian subcontinent, D: Southern Arabian Peninsula, E: India, F: Himalayas, G: Burma and Thailand, H: Indomalesia.

Ethnopharmacological uses

Being bitter in taste, people of Palakkad, Malappuram, Kozhikode, Kannur and Kasaragod districts of Kerala take *Exacum bicolor* as herbal remedy against diabetes and skin disorder. Traditional practitioners prescribe decoction of whole plant in eye diseases, malaria and in urinary disorders (Reddi *et al*., 2005; George, 1952) and it is mentioned as good substitute for Gentian and Swertia for their bitter property (Chopra *et al*., 1956). Traditional healers of Pundra and Bilaspur region of Chhattisgarh use whole plant of *Exacum bicolor* as blood purifier and in treatment of malaria (Megoneitso and Rao, 1983). *Exacum wightianum* was prescribed in maintaining blood glucose level and extensively used as bitter tonic and febrifuge in Ayurvedic system of medicine (Nadkarni, 1982). *Exacum lawii* is given in kidney disorders and applied in eye diseases by boiling in oil (kirtikar and Basu, 1975). *Exacum affine* was

traditionally used in infectious illness like skin and respiratory infection (Mothana *et al*., 2006).

Chemical constituents

The qualitative analysis confirmed the genus *Exacum* is rich in phenols, flavonoids, alkaloids, glycosides, saponins, steroids and terpenoids. Compounds from different species of *Exacum* are listed in table 4.

Table 4: Compounds reported from different species of *Exacum*

S. no.	Species	Compound	References
1	*Exacum affine*	P-coumaric acid	Hegnauer, 1966
2		Affinoside	Kariyone *et al*., 1956
3		Glucopaeonol	Kariyone *et al*., 1956
4		2′-Hydroxy-4′-methoxyacetophenone (Paeonol)	Miwako, 1994
5	*Exacum bicolor*	Ursolic acid	Hegnauer, 1966, Daniel and Sabnis, 1978
6		Apigenin,	Hegnauer, 1966; Daniel and Sabnis, 1978
7		Luteolin	Hegnauer, 1966; Daniel and Sabnis, 1978
8		Chlorogenic acid	Hegnauer, 1966; Daniel and Sabnis, 1978
9		Hydroxybenzoic acid	Hegnauer, 1966; Daniel and Sabnis, 1978
10		Hydroxycinnamic acid	Hegnauer, 1966; Daniel and Sabnis, 1978
11		Vanillic acid	Hegnauer, 1966; Daniel and Sabnis, 1978

12		P-coumaric acid	Hegnauer, 1966; Daniel and Sabnis, 1978
13		Protocatechuic acid	Hegnauer, 1966; Daniel and Sabnis, 1978
14		Polyphenolic group containing 7'-Chloro-3'-(2, 4 dichlorophenyl)-3', 4'-dihydrospiro (1, 3- dioxolane)	Jeeshna and Paulsamy, 2011
15		Polyphenolic group containing a'-D Galactopyranoside, methyl 2,6- bis-0-(trimethylsilyl)- cyclic butyl-boronate	Jeeshna and Paulsamy, 2011
16		Alkaloidal group containing (1, 16- Cyclocorynan-16-carboxylic acid, 17-(acetyloxy)-19, 20-didehydro-10-methoxy-, methyl ester,(16.xi., 19E)	Jeeshna and Paulsamy, 2011
17		Alkaloidal group containing 4 – (4 – Chlorophenyl) - 5 – morpholin - 4 - yl- thiophen -2-carboxylic acid, ethylester)	Jeeshna and Paulsamy, 2011
18		Glycoside group containing a'-D Galactopyranoside, methyl 2,3-bis-0-(trimethylsilyl)- cyclic phenyl-boronate	Jeeshna and Paulsamy, 2011
19		steroid group 9, 19 – Cycloergostan – 3 – ol – 7 – one, 4, 14 – dimethyl	Jeeshna and Paulsamy, 2011
20	*Exacum macranthum*	Linarin	Gunatilaka *et al*., 1983
21	*Exacum pedunculatum*	Luteolin	Daniel and Sabnis *et al*., 1978

22		Diosmetin	Daniel and Sabnis *et al*., 1978
23		Hydroxybenzoic acid	Daniel and Sabnis *et al*., 1978
24		hydroxycinnamic acid	Daniel and Sabnis *et al*., 1978
25		2-hydroxy-4-methoxy benzoic acid	Daniel and Sabnis *et al*., 1978
26	*Exacum quinquenervium*	Gentianine	Delaude, 1984
27	*Exacum tetragonum*	gentiopicroside	Sanjib *et al*., 1984
28		methyl ester of methylgrandifloroside	Sanjib *et al*., 1984

Pharmacological activity

Pharmacological activities of *Exacum* have attracted extensive attention. Traditional uses of *Exacum* genus have been scientifically validated but most of the species have not been completely elucidated.

Anthelmintic activity and antimicrobial activity of *Exacum bicolor*

The anthelmintic activity was performed on adult Indian earthworm, *Pheretima posthuma.* Leaf extract of *Exacum bicolor* was prepared with different solvents hexane, chloroform, ethylacetate, methanol and water. Their anthelmintic activity was investigated by taking approximately equal size earthworms consisting of six in each group were released into 15 ml of each extract (5 mg/ml, 10 mg/ml and 15 mg/ml). Albendazole (10 mg/ml) was used as reference drug (Appaji and Mala, 2014).

Antihyperglycemic activity of *Exacum wightianum*

Exacum wightianum was reported to have anti-hyperglycemic activity. Ethanolic extract of the whole plant of *Exacum wightianum* at the doses of 100 and 200 mg/kg p.o. was administered on streptozotocin induced diabetic rats for 14 days. Treatment with extract at dose of 200 mg/kg p.o. significantly decreased the blood glucose level in streptozotocin induced diabetic rats. Glibenclamide was taken as the standard drug (Thimmayan *et al*., 2012).

Anti-inflammatory activity of *Exacum wightianum*

The crude extract of *Exacum wightianum* was reported to have anti-inflammatory activity. Ethanolic, methanolic and chloroform extract of whole plant of *Exacum wightianum* at the dose of 100 mg/kg p.o. and 200 mg/kg p.o. of each extract were evaluated for anti-inflammatory activity by carrageenan induced rat paw oedema method, indomethacin was taken as the standard drug. The ethanolic, methanolic and chloroform extract of *Exacum wightianum* at the dose of 200 mg/kg p.o. was found to be significant than 100 mg/kg p.o. (Thimmayan *et al*., 2011).

Larvicidal and ovicidal activity against malarial vector of *Exacum pedunculatum*

Exacum pedunculatum showed larvicidal and ovicidal activity against common malarial vector. Hexane, chloroform, petroleum-ether and ethanolic extract of *Exacum pedunculatum* were prepared for testing larvicidal and ovicidal activity against malarial vector mosquito, *Anopheles stephensi* under laboratory condition. The larvicidal activity crude extracts of *Exacum pedunculatum* were tested with different concentrations like 50 ppm, 100 ppm, 150 ppm, 200 ppm and 250 ppm against fourth instar larvae *of Anopheles stephensi*. The LC_{50} (lethal concentration) value of hexane

extract was found to be 127.45 ppm, for chloroform 127.39 ppm, petroleum ether with 151.96 ppm and the least LC_{50} value of 121.24 ppm was recorded with ethanolic extract. Likewise, the LC_{90} values and their LCL-UCL (lower confidence limit- upper confidence limit) concentrations were determined against hexane, chloroform, petroleum ether and ethanol extracts respectively towards the fourth instar larvae of *Anopheles stephensi*. The result showed that 200-300 ppm concentration of the petroleum and ethanol extracts showed strong ovicidal activity (Elangovan *et al*., 2012).

Antiviral activity of *Exacum affine*

Antiviral activity against *Herpes simplex* virus (HSV-1 strain KOS) and *Human influenza* virus A/WSN/33 (H1N1) was shown by methanolic and aqueous extract of the whole plant of *Exacum affine*. Extract of *Exacum affine* was assayed in two types of *in-vitro* viral systems, one used *influenza* virus type A/MDCK cells and other used *Herpes simplex* virus type 1/Vero cells, at non-cytotoxic concentrations. The *Herpes simplex* virus type-1 showed more sensitivity than the influenza virus type A against the extracts investigated. The methanolic extract of *Exacum affine* showed anti-influenza virus type-A activity with 50% inhibition (IC_{50}) concentrations ranging from 0.7 to 12.5 µg/ml and exhibited anti-HSV-1 activity. The antiviral activity of extracts was also observed on a molecular level (Mothana *et al*., 2006).

***In vitro* culture of *Exacum affine* to increase secondary metabolites**

Previously there was no data reported on the elicitation and precursor feeding experiments in the *in-vitro* culture of plants from genus *Exacum*. Ewa *et al.* investigated the influence of precursor L- phenylalanine, elicitor methyl jasmonate

and different concentration of sucrose on the phenolic acids accumulation in the agitated shoot culture of *Exacum affine* (Gentianaceae). HPLC-DAD analysis was used for identification of compounds. Analyzed extracts were found to contain 14 phenolic acids, including 5 cinnamic acid derivatives and 7 benzoic acid derivatives. The total free phenolic acid content was found to be increased by 2.6 times and the total content of the whole phenolic acid (free and bound) was 1.6 times. The increase in the free form accumulation was observed for synapic acid 9.7 folds, *o*-coumaric acid 9.4 folds, caffeic acid 7.2 folds, *p*-coumaric acid 7.2 folds, chlorogenic acid 6.0 folds, vanillic acid 5.8 folds, the increase of the total content (free and bound) was noted for synapic acid 7.7 folds, chlorogenic acid 7.3 folds, 4-hydroxybenzoic acid 3.6 folds (Ewa *et al*., 2014).

Multiple shoot induction and germplasm conservation of *Exacum bicolor*

The efficient and reliable protocol was developed for multiple shoot induction by using Murashige and Skoog medium supplemented with various cytokinins. The cytokinins 6-benzyladenine, 6-furfurylaminopurine, 2-isopentenyladenine and zeatin were used individually or in combination at different concentrations. Regenerated plantlets were transferred medium containing coco peat: perlite mixture and showed a 75% survival rate. Genetic stability studies were done by using random amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSR) markers and comparing with mother plant. All the regenerated plants were genetically identical to their mother plant, no detectable genetic variation in the regenerated plantlets was obtained (Appaji *et al*., 2015).

Role of Chemotaxonomic marker in quality control standardization

Selection of chemical markers is crucial for the quality control of herbal medicines (Songlin *et al*., 2008). Gentianaceae is the family of flowering plants comprising of 70-80 genera and 900-1200 species. The plants of the family are annual or perennial herbs. Plants belonging to Gentianaceae are best known for their bitter taste, which can be related to their content of iridoids. Their medicinal properties are mainly due to presence of iridoids and secoiridoids (hostettmenn-kaldas *et al*., 1981). The iridoids are a group of natural products belonging to the terpenoids. In plants, iridoids are usually found as glucosides. Secoiridoids appears to be present in all the species of family Gentianaceae. The iridoid glycosides (mainly secoiridoid glycosides) serve as important chemotaxonomic markers of family Gentianaceae (Li *et al*., 2015).

Swertiamarin is a secoiridoid glycoside widely distributed in family Gentianaceae (Figure 6). *Enicostemma littorale*, *Enicostemma axillare*, *Swertia chirata*, *Swertia japonica* and *Centaurium minus*. Swertiamarin is a secoiridoid glycoside is a representative constituent of many plants of the Gentianaceae family (Inouye and Nakamura, 1971).

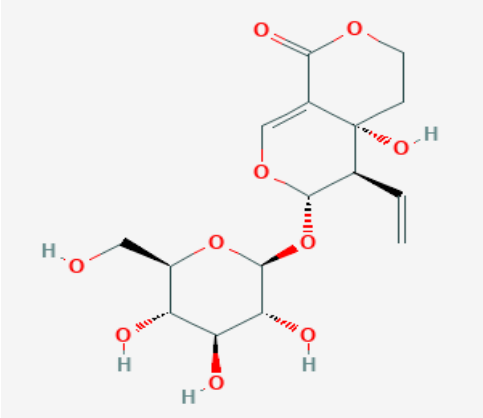

Figure 6: Structure of swertiamerin

White amorphous solid

Molecular Formula: $C_{16}H_{22}O_{10}$

Molecular Weight: 374.342 gm/mol

Recently it has been reported to possess pharmacological activities like hepatoprotective, antiedematogenic/anti-inflammatory, free radical scavenging activity, antispastic activity (Vaijanathappa and Badami, 2009), antidepressant (Dharaniyambigai and Doss, 2013), diabetic nephropathy (Sonawane *et al*., 2010), Antinociceptive activity (Jaishree *et al*., 2009). It has no toxicity and reported to be safe and effective anti-spastic agent. It showed the sedative effect in mice, rabbits and pigeons also induced sleep in mice (Mandal *et al*., 1998). Antibacterial and anticholinergic activities were also reported (Kumarsamy *et al*., 2003; Bhattacharya *et al*., 1976).

Isolation of swertiamerin from *Enicostemma littorale* Blume (Gentianaceae)(0.53 gm in 25 gm of plant material), *Enicostemma axillare* (Gentianaceae) was reported to be done by column chromatography over silica gel along with some other plants of Gentianaceae family (Vishwakarma *et al*., 2004).