PHARMACOGNOSTICAL EVALUATION

In recent scenario, the requirement of medicinal plants has been considerably increased as there is an increase in demands of raw material for pharmaceutical preparations as well as for self-medication in large population in the world (Abou-Arab and Abou Donia, 2001). It is very important to obtain a proper quality control profile for every medicinal plant used in traditional system of medicine. This may be beneficial in minimizing the contamination of medicinally important plants which occurs mainly due to incomplete knowledge regarding the varied associated problems like similar vernacular names, varied geographical conditions, and same morphology of powdered drug. It is also suggested that, correct identification and proper quality assurance of the starting raw material is an essential prerequisite to ensure consistent quality of herbal medicine which contributes to its safety and efficacy (Laloo *et al*., 2013). According to the World Health Organization (WHO), pharmacognostical standards are considered to be the primary step for diagnosis of the herbal drug, which includes macroscopic and microscopic evaluation of whole plant or each plant part to be used. Further, macroscopical examination of a plant or plant parts gives detailed information regarding the qualitative assessment of medicinal plant based on its morphological and organoleptic properties such as colour, size, shape, odour, taste etc. While the evaluation of crude drugs on the basis of histological studies helps to expand the perspective about the type of cells or their arrangement in plant tissues, which in turn is necessary for correct identification of plant or plant parts (Kumar *et al*., 2014; Prasad *et al*., 2013).

Each medicinal plant species has its own nutrient composition besides having pharmacologically important phytochemicals. These nutrients are essential for the physiological functions of human body. Such nutrients and biochemicals like carbohydrates, fats and proteins play an important role in satisfying human needs for energy and life processes (Hoffman *et al*., 1998; Mathews *et al*., 1999; Dingman, 2002). As various medicinal plant species are used either in the form of extract or decoction by the local people in different regions, therefore, evaluating their nutritional significance can help to understand the worth of these plants species in different ecological conditions (Adnan *et al*., 2010). The nutritional content analysis identified *Exacum lawii* as a rich source of vitamin C or ascorbic acid which acts as an antioxidant, which protects the body from oxidative stress and maintains the immune system. In addition, it also prevents damage to lipids, protein and DNA by neutralising free radicals (Wilson, 1999). Vitamin A (retinoic acid), it is a bioactive metabolite and a potent signalling molecule in the brains which modulates neurogenesis, neuronal survival, synaptic plasticity and regulates numerous gene products (Olson and Mello, 2010). Vitamin E is the major component of the cell antioxidant defence system and has numerous important roles in the body due to its antioxidant activity (Saliha *et al.*, 2014). The most abundant macroelements detected were Calcium (Ca), Potassium (K), Iron (Fe) and Sodium (Na). Nutritional analysis of fatty acids can be classified as qualitative and quantitative. Qualitative analysis of fatty acid ensures the fatty acid composition and quantitative analysis is to quantify the actual amount of each fatty acid that is present in the plant material. *Exacum lawii* contains rich concentration of polyunsaturated and monounsaturated fatty acid among which alpha-linolenic acid, linoleic acid and oleic acid were found to be in higher content. Fatty acids can classified as bad and good fatty acids. Bad fatty acids are

saturated and trans-fatty acids which increase total cholesterol and LDL levels. Good fatty acids are monounsaturated and polyunsaturated fatty acids, which can play the role in many therapeutic activities (Nurnadia *et al*., 2013; Zevenbergen *et al*., 2009).

Physicochemical parameters serve as a valuable source of information for the differentiating the plant material from its closely related species. It also provides suitable standards to determine the quality and purity of the plant. Different physicochemical parameters evaluated in the present study include determination of foreign matter which indicates the presence of visible matter other than the crude drug itself. Foreign matter was found to be less than 0.5% w/w. The foreign matter should be as less as possible otherwise it has a huge impact on quality of drug, which can produce error during latter stage of the standardization process. The results showed contain considerably low amount of moisture in whole plant powder. Since higher moisture content in plant material may lead to its deterioration and may lead to hydrolysis of active constituents. The ash values represent inorganic salts occurring naturally or deliberately added to crude drug as a form of adulterant. Total ash in a plant material includes both physiological as well as non-physiological ash. Acid-insoluble ash and water soluble ash values quantify the inorganic material and silica respectively. From the results, it was found that the *Exacum lawii* showed the presence of higher quantity of water-soluble ash compared to acid insoluble ash. Swelling index of a plant material is pharmaceutical value which denotes the presence of gums, mucilage, pectin and hemicelluloses. Presence of saponins in medicinal plants can be detected by their ability to form the persistent foam when the plant material is shaken in water which is measured in terms of foaming index (Prasad *et*

al., 2012), we found low saponin content present in plant. All the physicochemical parameters were standardised and documented with their estimated limits present.

The alcohol-soluble extractive was found to be more compared to water-soluble extractive. Extractive value is the amount of active chemical constituents present in plant material extracted through different solvents (Anonymous, 2002b).

Another critical issue associated with the use of herbal medicines is presence of heavy metals intentional or accidental more than the permissible range set by the regulatory authorities (Sahoo *et al*., 2010). Lead, mercury, arsenic and cadmium are the main contaminants. Heavy metal poisoning from intake of herbal medicinal products has caused several health hazards like liver and kidney toxicity and even death. In order to prevent heavy metal toxicity quality control and requisite regulatory measures of medicinal plants is essential (Street, 2012). Heavy metals like Pb, Cd, Zn and Hg in the whole plants of *Exacum lawii* were found to be within the permissible range as proposed by WHO. Plants are prone to deteriorate either by insects or by infection therefore, pesticides are used as a protective measure (Abou-Arab and Abou Donia, 2001). The presence of pesticide residue may be as a result of either environmental contamination or agricultural practice. Pesticide residue includes their secondary metabolite and/or their degradation products which remain in plant parts or in the soil. Chlorinated and organophosphorus pesticides are the most harmful pesticides among others, and exhibit adverse health issues (Zhang *et al*., 2012). The presence of pesticide residue in herbal medicine seriously affects development and the process of commercialization of traditional herbal medicine (Sahoo *et al*., 2010). Hence, WHO and other organizations have established standards to limit pesticide residue content in herbal material. The whole plants of *Exacum lawii* was estimated for the presence of

chlorinated and phosphate pesticides residue which were found to be within the prescribed limits as per WHO.

Fluorescence drug analysis has an immense value in the qualitative analysis of phytoconstituents in crude herbal drug, since some phytochemical constituents of plant exhibit fluorescence in the visible range in day light while some produce fluorescence in long and short ultra violet range (e.g. alkaloids like berberine) (Prasad *et al*., 2012).

DNA markers are more authentic for identification as the genetic information of each species is its unique characteristic and is independent of environmental factors, physiological conditions and other subjective errors like age. Different types of DNA-based markers viz., RAPD, RFLP (Restriction Fragment Length Polymorphism), ISSR (Inter Simple Sequence Repeat), AFLP (Amplified Fragment Length Polymorphism), SSR (Simple Sequence Repeat) etc., are employed for identification of plant species along with other methods of plant identification mainly taxonomy, embryology and physiology. The authentication of medicinal plant and discrimination of adulterants from genuine medicinal herbs are essential for both pharmaceutical companies as well as public health (Kiran *et al*., 2010). In the RAPD method, random short synthetic oligonucleotide primers are used to amplify the genomic DNA through polymerase chain reaction under low annealing temperature up to 28° C- 38° C. The DNA fragments generated are separated on agarose gels based on sizes and compared with marker (Showkat *et al*., 2015) The DNA fingerprinting analysis of *Exacum lawii* has been performed for the first time. In the present study 2 gene combination of ribulose-1, 5-bisphosphate carboxylase oxygenase large subunit (rbcL) and maturase K (matK) as the standard plant barcode were chosen (CBOL, 2009). These two loci of

chloroplast DNA were taken due to their efficient recovery of good quality sequences and high levels of species discrimination (Burgess *et al*., 2011).

PHYTOCHEMICAL EVALUATION

The bioactive constituents of medicinal plants like alkaloids, tannins, steroids, terpenoids and phenolics are responsible for their therapeutic activity (Paul *et al.*, 2015). Flavonoids have been found to associate in treatment of various cardiovascular diseases due to their antioxidant potential and anti-inflammatory properties (Crespy *et al*., 2002; Gabor, 1979). Phenols can neutralize oxygen derived free radicals by donating an electron or hydrogen atom to the free radicals. Therefore, they are considered as strong antioxidant and free radical scavengers possess anticarcinogenic, antibacterial, anti-inflammatory activities and are also used in coronary heart disease and coronary artery disease (Yildiz *et al*., 2011; Shukla *et al*., 2012). Tannins have a strong astringent action and are reported to have anti-oxidant activities, anti-bacterial, anti-inflammatory and anti-viral (Prasad *et al*., 2012; Kapu *et al*., 2001; Schulz *et al*., 2002). Alkaloids have been reported to possess wide range of therapeutic importance in the treatment of cancer, malaria, inflammation, pain, parkinsonism, hypertension and number of neuronal disorder (Rathbone and Bruce, 2002). Preliminary phytochemical analysis performed in the study gives information about the chemical identity of the active constituents present in that extract. Ethanolic extract of *Exacum lawii* as well as its successive fractions (petroleum ether fraction, chloroform fraction, ethylacetate fraction and aqueous fraction) were subjected to phytochemical screening. Ethanolic extract and its fractions showed presence of alkaloids, terpenoids, flavonoids, steroids, phenols, tannins, protein, amino acid and coumarins.

Preliminary phytochemical screening and TLC analysis confirm that the ethanolic extract of *Exacum lawii* quantifies higher number of phytocompounds in comparison to its fractions.

The iridoids are a group of natural products belonging to the terpenoids. Iridoids are exclusively distributed in family Gentianaceae. The iridoid glucosides of family Gentianaceae are usually secoiridoids. Swertiamarin is a secoiridoid glycoside is a representative constituent of many plants belonging to family Gentianaceae (Inouye and Nakamura, 1971). Swertiamerin, is bitter secoiridoid glycoside found in various species of family gentianaceae and have been reported to possess anti-edematogenic, antioxidant, hepatoprotective, anti-inflammatory, anti-nociceptive, anti-hyperlipidemic and analgesic (Jaishree and Badami, 2010; Jaishree and Badami, 2009; Jaishree *et al*., 2008; Jaishree *et al.*, 2009; Vaidya *et al.*, 2009; Lei, 1982).

Chemotaxonomic marker swertiamerin was firstly confirmed with co-chromatography with standard swertiamerin and then quantified by using HPLC. Swertiamerin is found to be major bioactive molecule in whole plant of *Exacum lawii* and also quantified by HPLC. The swertiamerin was successfully isolated from the ethanolic extract of *Exacum lawii*. Isolated compound was identified and confirmed as swertiamerin on the basis of results from various analytical techniques, the swertiamerin was successfully isolated. The yield of the swertiamarin can also be enhanced by repeating the protocol using similar experimental conditions. The method has been found to be simple, rapid and efficient method for isolation of swertiamerin and removing other impurities from the crude ethanolic extract which interferes in its crystallization. The melting point and UV absorption maxima of swertiamerin obtained was compared with literature found to be 113ºC to 114ºC and

238 nm. FTIR spectra was recorded and compared with reported spectra. The structure was elucidated by H-1 NMR, C-13 NMR and ESI-MS data. Spectra were recorded and compared with literature secoiridoid moiety appeared at δ 5.019-5.009 (d, 1H, C6- pyranopyrone) appeared at δ 96.455 (C6- pyranopyrone) in 13C NMR, which further showed the correlation with C atom, appeared at δ 98.288 (C2-tetrahydropyran). Electrospray ionization-mass spectrometry (ESI-MS) analysis of swertiamarin showed that the molecular ion peaks 397.50 [$M+Na^+$], 771.27 [$2M+Na^+$] which confirm the molecular weight of the compound is 374.34 and mass spectrum also confirmed the molecular formula as $C_{16}H_{22}O_{10}$. *Exacum lawii* could be the new source of swertiamerin (secoiridoid glycoside). Swertiamerin can be efficiently isolated by using standard protocol from the *exacum lawii*.

GC-MS is an analytical technique used to separate and identify compounds that can be vaporised without decomposition. In present study, GC-MS analysis of ELE revealed 22 compounds with their molecular weight, peak area and retention time. A compound found with highest peak area percentage (18.31) was 2H-Tetrazol-5-amine, 2-(phenylmethyl). Tetrazole and tetrazole derivative have diverse biological activities like hypotensive, antimicrobial, antiviral, antiallergic, cytostatic, nootropic, and other biological activities (Ostrovskii *et al.*, 2012). Ascorbic acid 2, 6-dihexadecanoate was found as next dominant component with peak area percentage 14.31 and has been reported to have antioxidant, anti-inflammatory and anti-nociceptive properties (Akinmoladun *et al.*, 2007; Okwu and Emenike, 2006). Astaxanthin, a naturally occurring terpenoid compound with peak area percentage 7.17 was also detected. Echitamine a monoterpene indole alkaloid (peak area percentage 1.04) was confirmed

along with some polyunsaturated fatty acids (Sasmita, 2014). The compounds identified may play an essential role in diverse therapeutic activity.

Different *in vitro* antioxidant model were performed in the present study that demonstrated the potent antioxidant potential of ELE and its fractions. The ethanolic extract exhibited the potent in vitro antioxidant activity followed by its fractions ethanolic extract, ethyl acetate fraction, chloroform fraction, aqueous fraction and hexane fraction in descending order. Antioxidants are considered are used as important nutraceuticals on account of many health benefits. Majority of the diseases or disorders are mainly linked to oxidative stress due to free radicals. Free radicals or reactive oxygen species (ROS) are highly reactive molecules with an unpaired electron and are produced by radiation or as by–products of metabolic processes. The most common reactive oxygen species (ROS) include superoxide (O_2^{o-}), hydrogen peroxide (H_2O_2), proxy (ROO^-) radicals and reactive hydroxyl (OH^o) radicals. These radical initiate chain reactions which lead to disintegration of cell membranes and cellular components including lipids, proteins and nucleic acids, thereby leading to the generation of various disease ailments (Devi *et al*., 2008). Oxidative stress condition is a result of excessive free radical production and reduction in antioxidant enzymes which overcome cellular antioxidant defences this in turn leads to progression of several degenerative diseases such as cancer, aging related disease, cardiovascular diseases, diabetes mellitus and various neurodegenerative disease, via DNA mutation/damage, protein oxidation and/or lipid peroxidation. Thus antioxidants play vital role either by preventing or delaying the oxidative damage caused by free radicals in various mechanism and hence medicinal plants having antioxidant potential have attained extensive relevance in curing such chronic diseases (Amessis-

Ouchemoukha *et al*., 2014; Chiang *et al*., 2015). Recently, interest has been developed in medicinal plants containing antioxidants as active phytochemical, such as phenol compounds, vitamins and terpenoids for their potential use as food additives and/or nutraceuticals in the prevention of many diseases also for boosting the immune system (Craig, 1999). Dietary polyphenols are thought to be beneficial for human health by exerting various biological effects such as free-radical scavenging, metal chelation, alteration of signal transduction pathways and modulation of enzymatic activity (Sato *et al*., 2011). From the overall observation, it can be suggested that the potent in vitro antioxidant activity of the *Exacum lawii* may be attributed due to phenolics, terpenoids and flavonoids which were found to be present in considerable rich amount (Souza *et al*., 2008).

There are evidences suggested the role of nitrosative stess in kidney toxicity. Nitrosative stess involves the reactive nitrogen species (RNS). RNS originates from nitric oxide (NO^o), which is synthesised by family Nitric oxide synthase which is localised in kidney. iNOS (inducible nitric oxide synthase) is up-regulated in response to lipopolysaccharides, cytokines and oxidative stress (Fleury *et al*., 2002). The iNOS is the enzyme responsible for synthesis of nitric oxide free radical which further generates reactive nitrogen species. In addition, isolated swertiamerin was investigated virtually for their binding interactions within the active site of iNOS (PDB ID: 3NQS) using the molecular docking software AutoDock4.2 and the docked conformations showed the binding energy of -6.38 Kcal/mol. Swertiamerin exhibited Inhibition constant with an Ki of 21.17 mM. Docking simulations were performed to predict the binding mode of swertiamerin in human iNOS active sites and evaluated for its binding affinity. This study could be beneficial for further scientific validation

of traditional medicinal uses of *Exacum lawii* involve in different pathological processes, like cell apoptosis, tissue damage, inflammation or ischemia.

PHARMACOLOGICAL EVALUATIONS

Cisplatin (cisplatinum or *cis*-diamminedichloroplatinum (II)) is one of the most effective and widely used chemotherapeutic agents. Nephrotoxicity was reported in the initial clinical trials of cisplatin chemotherapy (Hill and Speer, 1982). It is the major dose limiting side effect of cisplatin because it accumulates in tubular epithelial cells five times more than in serum (Chirinoa and Pedraza, 2009; Kuhlmann *et al*., 1997). The disproportionate accumulation of cisplatin in kidney tissues may contribute to cisplatin-induced nephrotoxicity. Its mechanism of renal toxicity is different from killing tumour cells in the kidney (Hanigen and Devarajan, 2003). The quiescent proximal tubule cells are also selectively damaged by cisplatin. The in-vivo pathogenesis of cisplatin-induced nephrotoxicity is complex and involves inflammation, oxidative stress and apoptosis (Yao *et al*., 2007). Cisplatin administration involved in oxidative stress also leads to nitrosative stress which implicates reactive nitrogen species (RNS). It has been demonstrated that iNOS mRNA level gets increased in cisplatin administered kidney (Chirino *et al.*, 2008).

Abnormal elevation in intracellular ROS level in kidney cells leads to the oxidative stress phenomenon, which further leads to a disturbance in the normal redox level inside the renal cell (Pabla and Dong, 2008; Das *et al*., 2012). ROS produced through the pathways mainly by xanthine-xanthine oxidase system, mitochondria, and NADPH oxidase in renal cells and are implicated in the pathogenesis of acute cisplatin-induced renal injury. Cisplatin in millimolar concentration causes depletion of GSH and protein-SH inside the renal cells. The consequences resulting in reduced

enzymatic activities (SOD and CAT), mitochondrial Ca^{2+} uptake, inhibition of Na+/K+-ATPase, depletion of pyridine nucleotides, lipid peroxidation and collapse of the mitochondrial membrane potential. It stimulates the ROS production by damaged mitochondria and inhibited the antioxidant enzymes. DCFDA dye is non-fluorescent but in the presence of ROS, gets oxidized and produces green fluorescent. These highly reactive radical is assumed to be directly responsible for DNA damage and necrosis (Davis *et al*., 2001; Kawai *et al*., 2006). Reactive oxygen species are the endogenous cause of DNA damage in kidney cells (Yan *et al*., 2016). Cisplatin causes normal renal cells to enter into cell cycle for uncontrolled proliferation and fate to necrosis and apoptosis. Cell cycle inhibition could be the important target of preventing renal toxicity, it allows time and opportunity damaged DNA to get repaired and complete the regeneration and replacement process. The cell cycle inhibitory drug could be an effective therapy for cisplatin nephrotoxicity. (Megyesi *et al*., 1998; Hanigan and Devarajan, 2003)

The role of inflammation in kidney toxicity has been increasingly appreciated with the involvement of leukocytes, adhesion molecules, chemokines, and cytokines (Safirstein, 2007). Many cytokines are released by leukocytes and renal tubular cells into the injured kidney and are important components of both the initiation and extension of inflammation in nephrotoxicity. Cisplatin-induced kidney toxicity extensively depends on TNF-α, as TNF-α-deficient mice and TNF-α antibody-treated wild-type mice reported to be resistant to cisplatin-induced kidney damage. It has been reported to increase the renal expression of proinflammatory cytokines such as Interleukin-6 (IL-6) and Interleukin- 1beta (IL-1β) (Sugiyama *et al*., 1989; Ramesh and Reeves, 2005; Ramesh and Reeves, 2002; Zhang *et al*., 2007).

A single dose of cisplatin (6mg/kg, i.p.) administration causes marked elevation in serum urea, creatinine and other biochemical parameters in experimental rats. The cisplatin (4 mg/kg, i.p.) administration does not cause significant rise in serum urea and creatinine. Hence, cisplatin (6mg/kg, i.p.) was selected for drug treatment protocol for evaluating nephroprotective activity of *Exacum lawii* and swertiamerin in rats.

Toxicity studies were performed to determine the toxicity profile of *Exacum lawii* and selecting the dose for evaluating nephroprotective activity in rats. During Acute toxicity study the single dose of 2000 mg/kg p.o. showed no toxic symptoms throughout the observation period of 14 days. In repeated dose 28-days oral toxicity study, animals were observed throughout the period of 28 days of study, there were no signs abnormal preclinical condition like loss of consciousness, tremors, convulsions, irregular breathing, lethargy, aggression, diaphragmatic breathing, gait, licking and piloerection were observed. It was observed that the rats in each group were survived with a normal weight gain pattern until the termination of experiment. ELE (1000 mg/kg, p.o., 2000 mg/kg, p.o. and 4000 mg/kg, p.o. per day) administration for 28 days causes no significant difference body weight, vital organ weight, biochemical parameters and haematological when compared with the control group. Acute toxicity study and repeated dose 28-days oral toxicity study established that the ELE is non-toxic and safe up to 4000 mg/kg, p.o. Therefore, according to OECD guidelines, 1/10th of the safe dose of the extract (400 mg/kg, p.o.) was selected as a median pharmacological dose for further study, while the dose level of 100 mg/kg and 200 mg/kg were taken as to be lower and higher limits.

Single dose of cisplatin (6mg/kg, i.p.) administration causes marked elevation in serum urea, creatinine and other biochemical parameters. The ELE (400 mg/kg, p.o.) treatment more significantly ($p < 0.05$) balanced the serum urea and creatinine concentration along with other biochemical parameters than swertiamerin (20 mg/kg, p.o.). It was observed that SOD, CAT and GSH got depleted while LPO level elevated in cisplatin treated renal tissues. These anti-oxidant parameters were restored significantly by administration of ELE (400 mg/kg, p.o.) and swertiamerin (20 mg/kg, p.o.). The results obtained from flowcytometry analysis of ROS in kidney cells of cisplatin treated rats, indicated that ROS production plays important role in cisplatin-induced renal toxicity. Geometrical mean (GeoMean) values of the fluorescence intensities were found to be higher in cisplatin treated renal cells compared with control group due to ROS production. ROS production was found to get reduced in renal cells treated with ELE (400 mg/kg, p.o.) significantly than swertiamerin (20 mg/kg, p.o.) treatment. DNA fragmentation assay was performed to determine the DNA damage as cellular response to oxidative stress produced by cisplatin accumulation. ELE 400 mg/kg, p.o. and swertiamerin 20 mg/kg, p.o. treatment prevented the oxidative DNA damage or repaired the damaged DNA which can be visible by reduced fragmentation in gel electrophoresis.

The results supported the finding, as the treatment with ELE 400 mg/kg, p.o. reduces the level of proinflammatory cytokines including IL-1β, IL-6 and TNF-α in cisplatin treated rats significantly than swertiamerin 20 mg/kg, p.o. treatment alone. The histological architecture of kidney tissues exposed to cisplatin revealed the glomerular changes, collagen deposition around renal glomeruli, dilations in distal convoluted tubules, hemolysis and necrosis in tissues and cytoplasmic vacuolisation in their

lining epithelial cells. Treatment with ELE 400mg/kg, p.o. and swertiamerin 20 mg/kg, p.o. showed normal histological architecture reappearance comparable to tissue histology control group.

From the study performed on HEK-293 cells for optimizing the nephroprotective activity of ethanolic extract of *Exacum lawii* (ELE) and swertiamerin. Our results manifest that in MTT cell viability assay, cisplatin treatment found to decimate 50% of HEK-293 at the dose of 2.261µg/ml. ELE and swertiamerin treatment were found to be safe and non-toxic to HEK-293. Cisplatin produces nitrosative stress in HEK-293 cells by enhancing the iNOS expression. ELE and swertiamerin selectively inhibit iNOS expression as shown by western blot analysis. TNF-α and IL-6 cytokines level measured as inflammatory biomarkers were upregulated in cisplatin treated HEK-293 and found to get significantly more reduced by treating with ELE than swertiamerin. ROS level increased in cisplatin treatment was reduced significantly by ELE and swertiamerin treatment. ELE was observed to be more effective antioxidant than swertiamerin. DNA fragmentation was observed through gel electrophoresis in cisplatin treated cells. DNA fragmentation represents the DNA damage by action of reactive oxygen species. Distribution of cells was observed in various stages of the mitotic phase M, the DNA synthetic phase S, and the pre- and post-DNA synthetic phases, G1 and G2, respectively. Increased cell frequency in G2/M phase (DNA damage checkpoint) in cisplatin treated cells showed damage DNA content. ELE and swertiamerin treatment repaired DNA content with enhanced cell number in G1 phase. Cell cycle inhibition could be the important target of preventing renal toxicity, it allows time and opportunity damaged DNA to get repaired and complete the regeneration and replacement process. The cell cycle inhibitory drug could be an

effective therapy for cisplatin nephrotoxicity (Megyesi *et al*., 1998; Hanigan and Devarajan, 2003).

It has been already reported that whole extract produces better effect than single active ingredient (Weerapreeyakul *et al*., 2016) and ELE was found to be rich source of bioactive constituents along with swertiamerin mainly phenols, flavonoids (Quercetin), terpenoids (Ursolic acid), Vitamin C and Vitamin E. The phenolic compounds are reported to possess potent antioxidant activity and capable to cure nephrotoxicity (kumar *et al.*, 2013) also Quercetin (Annie *et al.*, 2005), Ascorbic acid (Vitamin C) (Huang *et al.*, 2000) and Vitamin E (Bursell *et al.*, 1999) scientifically reported to have nephroprotective potential. Hence it could be the reason behind better nephroprotective effect of ELE than treatment with swertiamerin alone in cisplatin induced nephrotoxicity. The pathogenesis involved in the nephroprotective potential of *Exacum lawii* extract and swertiamerin may be due to their antioxidant and inflammatory property.

The aromatic oil extracted from the wide range of plants is reported to possess antimicrobial activity (Koutsaviti *et al.*, 2011; Stefanello *et al.*, 2011). Since, the folklore medicinal plant, *Exacum lawii* has been traditionally used for eye problems. In the present study, the antimicrobial activity of volatile oil and extracts of *Exacum lawii* against pathogenic strains of bacteria and fungi was determined by measuring the diameter of the inhibition zones around the discs. The MBCs and MFCs were also calculated by micro dilution method. The study showed that whole plant of *Exacum lawii* has a broad spectrum antimicrobial activity against pathogenic bacteria and fungi causing ocular infection along with some other pathogenic bacteria and fungi. The MBCs and MFCs for volatile oil is found to be lower than ethanolic extract and

petroleum ether extract. The study justified the traditional use for eye problems by showing antimicrobial activity against pathogens causing ocular infection. The *Exacum lawii* extract is rich in phytoconstituents. The most of the secondary metabolites like phenols, alkaloids, flavonoids possess antimicrobial activity (Hassan *et al.*, 2004). GCMS results confirmed that ethanolic extract contains polyunsaturated fatty acids, indole alkaloids also tetrazole derivative which are reported to have antimicrobial activity (Ostrovskii *et al.*, 2012). They all may play very essential role in diverse therapeutic activity. The results obtained from the present study confirmed that *Exacum lawii* is rich in phytoconstituents that are able to combat the microbial defences. Henceforth, the results justify that further investigation should be performed to investigate the composition of volatile oil extracted from *Exacum lawii*. It will strengthen its potential of *Exacum lawii* as a novel antimicrobial agent.