5. DISCUSSION

Medicinal plants have been used by human beings since early ages in traditional medicine due to their therapeutic potential and the search on medicinal plants has led to the discovery of novel drug candidates used against diverse diseases. Higher plants as sources of bioactive compounds continue to play a dominant role in the maintenance of human health (Sermakkani and Thangapandian, 2012).

Plants are the rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with variety of structural arrangements and properties.

Pharmacognostic studies

Medicinal plants are playing very important role in a long history of traditional medicine. Ethic knowledge on herbal plants and their application by indigenous culture is helpful in the conservation of traditional culture and drug development (Farnsworth,1983). The usage of medicinal plants play a vital role in several kind of human disease. Since the practice of "Medicinal remedies" validating the scientific application, orthodox medicine resembles herbal medicine as an alternative medicine (Sarkar *et al*., 2015). Pharmacognostic steps and processes are useful in identification, standardization and authentication of the plant sample (Ahmad *et al*., 2006; Odugbemi, 2008; Wachtel-Galor *et al*., 2011; Liu *et al*., 2015). Despite the recent modern techniques, identification and analysis of herbal drugs by pharmacognostic studies is still more reliable, accurate and inexpensive (Singh and Sharma, 2010). According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken.

The standardization of a crude drug is an integral part of establishment for its correct identity. Before any crude drug can be included in an herbal pharmacopoeia, pharmacognostic parameters and standards must be established. The pharmacognostic standards for leaf and stem bark of *Chionanthus mala-elengi* were carried out for the first time in our study.

Morphological characteristics

Morphological characters of *Chionanthus mala-elengi* (Dennst.) P.S. Green show it is a small, evergreen tree with the height of 8 meter tall grown in forest areas. The stem bark is greyish brown, smooth, covered with corky lenticels and coriaceous. The leaves are simple, opposite, decussate, petiole 0.5-1.2 cm long, elliptic-obovate with a pointed tip wedged shaped at the base. Flowers are bisexual, petals are yellowish white in colour, fragrant, sessile. Fruit is drupe (10×5 mm), ellipsoid, slightly curved, acute and ridged. According to the recent report by (Lombardi (2006), *C. chrysopetalous* (Oleaceae) is a tree (or) shrub known from two regions in Peru Cusco and Ucayali. Leaves are opposite, exstipulate, pulvinate at base. Flowers are tetramerous linear, gamosepalous, superior ovary. Fruits and seeds are unknown. *C. gigas* is a rare endemic species of Malaysia, it is well known for its large coriaceous leaves and it has condensed inflorescence of sessile flowers with long corolla lobes and large unridged fruits (Kiew, 1998).

The identification of the species was also authenticated by BSI Coimbatore Circle, vide the letter dated on 4.10.2018, reference Number: BSI/SRC/5/23/2018/Tech/1850.

Microscopical characteristics

Microscopic evaluation is one of the simplest and cheapest methods for the correct identification of the source of the material. Importance of epidermal characters in general is widely recognized in taxonomic considerations of angiosperms (Stace, 1965 a,b; Rao and Ramayya, 1987). Microscopic studies of *C. mala-elengi* leaf indicated the presence of epidermis, palisade, spongy parenchyma cells and collateral vascular bundle having xylem and phloem. Filiform sclereids are scattered on the mesophyll cells. Similar microscopical studies were reported in leaf of *Jasminum sambac* showed single layer of epidermis, stomata were present in lower epidermis. The midrib region has collenchymatous cells. Vascular bundles were present composed of xylem and phloem (Gowdhami and Rajalakshmi, 2015). Similar results has been reported in certain species of *Jasminum*, anomocytic type of stomata, absence of subsidiary cells, surrounded the guard cells, most of the species contain hypostomatic leaf (Ali and sasa, 2015).

J. mesyni belongs to Oleaceae also showed the similar microscopic features such as the presence of epidermis, palisade, xylem, phloem and collenchyma (Bhushan *et al.,* 2015). Thus, the microscopic character of the leaf of *C. mala-elengi* contain filiform sclereids on the mesophyll cells, it is one of the specific character of this studied species.

Analysis of various types of tissue and other microscopic techniques such as linear measurements has been studied. Evaluation of leaf constants and quantitative microscopy are the initial step in identification of plant sample and for drug determination in pharmacognosy (Jarald and Jarald, 2007). The stomatal type of *C. mala-elengi* is anomocytic and its stomatal index was 26.11±3.89 and stomatal number was 30.33±1.20 (Table 1 & Plate-3). Similar type of stomata was reported in leaf of *Nyctanthes arbor-tristis* and its stomatal index was 14.78 (Biswas and Mukherjee, 2011). Similar type of studies were also reported in *J. sambac* (Oleaceae) and its stomatal index was 12.26 (Sabharwal *et al*., 2011).

Organoleptic evaluation

Sensory evaluation plays a key role in determining the suitability or denunciation of a crude drug. Organoleptic testing of a crude drug is mainly for qualitative evaluation based on the observation of morphological and sensory profile. The appearance of colour, touch, taste and odour of dry plant sample is given in Table 2 & Plate-4. These characters were helpful in identifying the purity of *C. mala-elengi* to ensure quality of a particular drug.

Based on the (Bhushan *et al*., 2015) *J. mesyni* the leaves showed green colour, bitter taste, characteristic odour, elliptical shape, smooth and shining texture and these kind of properties could be helpful in identification and authentication of herbal drug obtained from leaves. These properties are major important for identification of powdered plant drug where in most of the morphological diagnostic features are lost (Serrano *et al*., 2010).

Powder study

C. mala-elengi leaf powder showed the presence of pitted vessels, rosette crystals, stone cells, uniseriate trichomes, calcium oxalate crystals and parenchyma cells. The stem bark powder showed the presence of calcium oxalate primes, macroscleride, periderm and starch grains (Plate - 5, 6).

Trichome features are also play a very important role in proper identification of the plants and considered as one of the valuable taxonomic marker now (Leelavathi and Ramayya,

1983). Presence of unicellular trichomes, collenchyma cells with palisade cells, spongy parenchyma cells and vascular bundle of midrib was crescent shaped.

Physicochemical analysis

The physical constant evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs. The ash values are particularly important to find out the presence or absence of foreign inorganic matter such as metallic salts or silica (earthy matter). Acid insoluble ash provides information about non-physiological ash produced due to adherence of inorganic dirt, dust to the crude drug. Increased acid insoluble ash indicates the adulteration due to dirt, sand or soil (Nayak and Patel, 2010). The extractive values are primarily useful for the determination of exhausted or adulterated drug and helpful in the detection of adulteration (Pimple *et al*., 2012). Moisture content of the plant drug is responsible for its decomposition because of microbial attack and physical changes. High amount of moisture content in a plant drug, at relatively high temperature, will lead to the activation of enzymes and provide favourable conditions for the proliferation of microorganisms (Arora *et al*., 2013).

In case of crude drug evaluation, physico chemical analysis play a very important role which includes percentage of total ash, water soluble ash and acid insoluble ash. Ash value gives a marker character for identification of crude drugs obtained from the investigated taxa. Here total ash value of *C. mala-elengi* leaf is 10.38±0.09 and stem bark is 9.67±0.06 which is very distinct and could be used in evaluation of drug quality of the plant. The water soluble ash, acid insoluble ash, sulphated ash and extractive value of leaf are more comparable to stem bark. The moisture content of leaf (9.03±0.37) is more than stem bark moisture content (7.53±0.55) and they are significant at $p<0.05$ level (Table 3). Similar results have been reported in the plant *J. mesyni.* The total ash 6.2%, water soluble ash 2.4% and acid insoluble ash 4.6%, Ethanol and aqueous extract, extractive values are found to be 9.6% and 10.4% respectively (Bhushan *et al*., 2015).

Total ash value of *J. auriculatum* was 19%, acid insoluble ash was 15%, water soluble ash was 18% and moisture content was 14% (Sucharitha *et al*., 2018). Total ash value of *J. sambac* was 13.5%, water soluble ash was 6.7%, acid insoluble ash was 8.2%

and moisture content was 6.19%, alcohol soluble extractive value was 30% and water soluble extractive value was 11.8% (Gowdhami and Rajalakshmi, 2015). These characteristics could be useful as hallmark for ascertaining the purity of the sample.

Extractive value

On analysis, the extractive value of *C. mala-elengi* leaf in ethanol was 41.52±0.48%, water - 18.04±0.36%, ethyl acetate - 5.56±0.23%, chloroform - 3.53±0.25% and petroleum ether - 2.42±0.19%. In stem bark, extractive value in ethanol was 32.73±0.84%, chloroform - 17.33±0.88%, ethyl acetate-16.79±0.21%, water-14.33±0.33% and petroleum ether - 1.48±0.24% (Table 4). Ethanol leaf and stem bark extract of *C. mala-elengi* showed higher result of extractive value and they are significant at $p<0.05$ level.

Fluorescence analysis

Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Some constituents show fluorescence in the visible range in day light. The ultra violet light produces fluorescence in many natural products (eg. alkaloids like berberine), which do not visibly fluorescence in daylight. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents, hence some crude drugs are often accessed qualitatively in this way it is an important parameter of pharmacognostical evaluation (Ansari, 2006; Gupta *et al.,* 2006). Fluorescence analysis is an important parameter for standardization of herbal drug. This process was revealed by different biochemical compounds present in the plant sample (Ahmed and Urooj, 2011; Suresh and Arunachalam, 2012).

Similarly fluorescence characters of the crude drugs are considered very important marker in making distinction among the drugs. The fluorescence analysis of leaf and stem bark powder of *C. mala-elengi* was done and results were given in Table 5 and 6. The powder was treated with various reagents and the mixture was observed under day light and UV 254 nm to observe the type of fluorescence. These datas were helpful in identifying and ascertaining the quality of the collected crude drug. Similar study has been determined in leaves of *J. sambac* (Sabharwal *et al*., 2011) and leaves of *Nyctanthes arbor-tristis* (Shivani *et al*., 2015). Although our present investigation of fluorescence analysis of *C. mala-elengi* can be used a character for identification.

Phytochemical analysis

Phytochemical evaluation and molecular characterization of plants is an important task in medicinal botany and drug discovery (Patel and Zaveri, 2011). Phytochemicals and chemical compounds which are non-nutritive occur naturally in plants with diverse medicinal properties (Meenakshi *et al.,* 2014). Secondary metabolites like alkaloids, flavonoids, phenols, tannins and coumarins etc., have numerous curative properties against several human ailments. Analysing phytochemicals will provide scientist insight for the effectiveness of the plants which can lead to development of new pharmaceuticals with fewer side effects.

Qualitative phytochemical analysis

For preliminary qualitative phytochemical analysis in leaf and stem bark of *C. mala-elengi* various solvents such as petroleum ether, chloroform, ethyl acetate, ethanol and water were used. Qualitative phytochemical analysis of leaf extract of *C. mala-elengi* showed the presence of glycosides in all the extracts tested. The chloroform, ethyl acetate, ethanol and water extracts showed the presence of tannins, phenolic compounds, steroids, sterols, triterpenoids, flavonoids and balsams. Alkaloids, phlobatannins and resins were present in ethyl acetate, ethanol and water extracts. Proteins, amino acids and volatile oils were present in petroleum ether, ethyl acetate and ethanol extracts. Saponins were present in petroleum ether, ethyl acetate, ethanol and water extracts. Carbohydrates were present in chloroform, ethanol and water extracts (Table 7). These results indicated that among the different solvents used in the extraction, maximum number of bioactive compounds were present in ethanol extract of leaf.

Preliminary qualitative analysis of stem bark extract of *C. mala-elengi* revealed that steroids and sterols and glycosides were present in all the tested extracts. Volatile oils were present in petroleum ether, chloroform, ethyl acetate and ethanol extracts except water extract. Saponins, tannins and phenolic compounds were present in petroleum ether, ethyl acetate, ethanol and water extracts. Carbohydrates, proteins and amino acids were present in petroleum ether, chloroform and ethyl acetate extracts. Alkaloids, triterpenoids and flavonoids were present in ethyl acetate, ethanol and water extract. Phlobatannins, balsams

and resins were present in ethanol and water extract (Table 8). These results indicated among the different solvents used in the extraction, the maximum number of bioactive compounds were present in ethanol extract of stem bark.

Similar results were found in methanol stem bark extract of *C. zeylanica* which has most of the phytochemicals such as flavonoids, steroids, terpenoids, tannins etc. than the n-hexane and ethyl acetate extracts (Rao *et al*., 2013). Preliminary qualitative analysis of aqueous and ethanol leaf extracts of *Olea europaea* revealed the presence of tannins, saponins, phlobatannins, flavonoids, terpenoids and cardiac glycosides (Edrah and kumar, 2014). Phytochemical investigation of ethanol leaf extract of *J. sambac* exhibits the presence of glycosides, flavonoids, tannins, alkaloids and reducing sugars (Rahman *et al.,* 2011). Oleuropein is the secoiridoids compound majority present in fruits of *Olea europaea* (Hashmi *et al.,* 2015).

Harborne and Green, 1980 reported that *C. retusus* contain flavone glycosides such as luteolin 7-glucoside; 2, luteolin 7-rutinoside; apigenin 7-glucoside and 7-rutinoside. Leaf of *C. retusus* also having the capability to store the flavanone derivatives in their leaves. The leaf of *Fraxinus chinensis* possess various biological compounds like oleuropein, neooleuropein, cichoriin and frachinoside (Inouye *et al*., 1975; Kikuchi *et al*., 1987; Steinegger and Brantschen, 1959; Khan *et al*., 1968).

In the present study, the above qualitative phytochemical screening revealed that the maximum number of phytochemicals were present in ethanol leaf extract when compared to the other solvent extracts of leaf and stem bark. Minimum number of phytochemicals were found in petroleum ether leaf extract. Methanol and ethanol extracts were generally more potent than the other extracts probably because the active principles in the plant dissolved more readily in these solvents than other solvents. These studies indicate the medicinal values of the plant studied in the present investigation.

Quantitative phytochemical analysis

The main active components of *C. mala-elengi* have been identified as phenols, alkaloids, flavonoids, saponins, polysaccharides and proteins. All of these components have been reported to be closely associated with the health enhancing effects (Sheng *et al.,* 2007).

In quantitative analysis, among the ten different extracts, ethanol leaf and stem bark extracts of *C. mala-elengi* showed higher level of total phenol content (leaf-318.33±1.64 mg GA/gm and stem bark 297.33±1.87 mg GA/gm) and they are significant at $p<0.05$ level (Table 9). The highest amount of phenol is important in regulation of plant growth development and disease resistance. *J. multiflorum* ethanol leaf extract exhibited higher total phenol and flavonoid content (31.58±1.61 mg/g and 25.98±1.32 mg/g respectively) than the flower extract (Kumaresan *et al.,* 2019).

The abundance of flavonoids in the ethanol leaf and stem bark extracts are also indicative of its potent antioxidant effect, which suggests that the plant may be very useful as an anti-bacterial, anti-inflammatory, anti-allergic, anti-viral, anti-thrombotic, anti-mutagenic and vasodilatory compounds as reported by Allan and Miller (1996). The flavonoid content in ethanolic leaf extract of *C. mala-elengi* is 162.37±1.41 mg QE/gm while in ethanolic stem bark extract showed 133.48±2.85 mg QE/gm and they are significant at $p<0.05$ level (Table 10). Leaf of *C. virginicus* having flavonoid such as rutin; kaempferol-3-glucoside; kaempferol-3-rutinoside and quercetin triglycosides. It also contains triterpenoid compound like ursolic acid. Stem and root barks contain lignin and phillyrin (Pourra *et al.*, 1954; Steinegger and Jacober, 1959; Harborne and Green, 1980).

Total tannins are generally defined as naturally occurring polyphenol compounds of high molecular weight to form complexes with the proteins. Tannins are the important source of protein in animals and their effects on animals ranges from beneficial to toxicity and death. Dietary supplementation of these compounds reduces the oxidative damage to cell membrane, lipid, protein and nucleic acid due to strong quenching property of free radicals (Verma *et al*., 2011; Han *et al.,* 2007). The total tannin content of ethanol leaf extract of *C. mala-elengi* was higher 445±2.17 mg TA/gm than ethyl acetate stem bark extract 387.5±1.91 mg TA/gm and they are significant at $p<0.05$ level (Table 11).

Alkaloids which are one of the largest group of phytochemicals in plants have amazing effects on humans and this has led to the development of powerful pain killer medications (Kam and Liew, 2002), also studies have shown that alkaloid is capable of reducing headache associated with hypertension (Ayitey Smith and Addae-Mensah,1977).

The alkaloid content of *C. mala-elengi* ethanol leaf extract was higher (28.76±0.28 mg AE/gm) than ethyl acetate stem bark extract (26.04±0.6 mg AE/gm) and they are significant at $p<0.05$ level (Table 12).

Saponins, although non-toxic, can generate adverse physiological responses in animals that consume them. Akindahunsi and Salawu (2005) pointed out clearly that saponins have tumour-inhibiting effect in animals. Asl and Hosseinzadeh (2008) also reported that there is evidence of saponin in traditional medicine preparations, where oral administration might be expected to lead to hydrolysis of glycoside from terpenoids. Their abundance in the north is for traditional treatment of ailments (Soladoye and Chukwuma, 2012). The saponins in *C. mala-elengi* ethanol leaf extract was higher (442±1.15 mg DE/gm) than the ethanol stem bark extract (394.44±1.01 mg DE/gm) and they are significant at $p<0.05$ level Table 13.

Nyctanthes arbor-tristis ethanol extract has total steroids, phenols, alkaloids, saponins, tannins and flavonoids with values of 0.53 mg/g, 0.36 mg/g, 0.24 mg/g, 0.34 mg/g, 0.20 mg/g and 0.42 mg/g respectively (Ramachandran *et al.,* 2014). Methanol leaf extract of *J. matthewii* contained total phenolic content of 8.25±0.12 mg GAE/g (Sharmin *et al.,* 2017).

Antioxidant activity

The antioxidant properties of phytochemicals are due to several different mechanisms, such as scavenging of free radicals that prevent human body against free radicals that produce various diseases. Antioxidants are having resistance against oxidative stress by scavenging free radicals. It is responsible for inhibiting lipid peroxidation and by other mechanism (Miller and Rice-Evans, 1997; Potterat, 1997; Mahakunakorn *et al*., 2003). Reports revealed that the pathogenesis of various ailments such as cardiovascular disorder, cancer, ageing, inflammation and brain disfunction is caused by the generation of free radicals promote oxidative stress (Kris-Etherton *et al*., 2002).

Antioxidant property is useful to observe the potential use of medicinal plants. Recently, usable synthetic antioxidants have been reported to cause or stimulate negative health effects. So, there is a need to substitute them with natural antioxidants. Currently there has been a rapid of interest in the disease curing potentials of herbal plants, as antioxidants in reducing such free radical produced tissue injury (Wittschier *et al*., 2009).

Indeed, because of various antioxidant activities of different compounds, the antioxidant potential of extract strongly depends on the extraction solvent (Jang *et al*., 2007). Antioxidant activities of the sample were determined in terms of low IC_{50} value which indicates to a high antioxidant capacity (Dhanani *et al*., 2017).

DPPH free radical scavenging activity

DPPH is a stable free radical, which when proton donors like antioxidants, the radicals are quenched and absorbance get decreased. It has been extensively used for the measurement of the scavenging ability of antioxidants (Wu *et al*., 2003). In the ethanol leaf extract of *C. mala-elengi* exhibit the percentage of inhibition increased from 40.61±0.23% at 10 µg/ml to 84.04±0.28% at 50 µg/ml (Table 14). Ethanol extract exhibited highest antioxidant activity (IC_{50} value - 21±1.15 µg/ml). IC_{50} value of positive control (ascorbic acid) was 18±1.15 µg/ml. To conclude, the DPPH scavenging activity showed effective activity present in ethanol leaf extract than the other extracts (Table 15).

The ethanol stem bark extract of *C. mala-elengi* was found effective in DPPH scavenging activity exhibited the percentage of inhibition increased from 36.78±0.38% at 10 µg/ml to 66.58±0.43% at 50 µg/ml (Table 16). Ethanol extract exhibited the highest antioxidant activity (IC_{50} value-33±1.73 µg/ml). IC_{50} value of positive control ascorbic acid was 18±1.15 µg/ml. To conclude, the DPPH radical scavenging activity showed effective in ethanol leaf extract than the other extracts and they are significant at $p<0.05$ level (Table 17).

Ethyl acetate extract of *C. zeylanica* stem bark showed significant DPPH radical scavenging activity than the methanol extracts (Rao *et al*., 2013). DPPH radical scavenging activity of ethyl acetate dried root bark extract of *C. virginicus* showed significant activity than the methanol extract (Gulcin *et al*., 2007). DPPH radical scavenging activity of methanol extract of *O. ferruginea* showed higher radical scavenging activity (Mehmood and Murtaza, 2018).

Nitric oxide scavenging activity

Nitric oxide is a chemical mediator of physiological functions such as smooth muscle relaxant, neuronal signalling, inhibition of platelet accumulation and regulation of cell mediated toxicity. It is a diffusible free radical that regulates numerous roles as an effectors molecule in various biological system as well as neuronal messenger vasodilation, antimicrobial and antitumour function (Miller *et al*., 1993). Excess amount of nitric oxide concentration is related with numerous illnesses. DNA fragments cell damage and neuronal cell deaths are associated with over production of NO (Dawson *et al*., 1992; Ialenti *et al*., 1992).

In the present study, nitric oxide scavenging activity of ethanol leaf extract of *C. mala-elengi* showed the percentage of inhibition increased from 40.16±0.09% at 10 µg/ml to 77.59±0.29% at 50 µg/ml (Table 18). Ethanol extract exhibited the highest antioxidant activity 18.33±0.88 µg/ml. IC_{50} values of positive control (ascorbic acid) was 10±0.58 µg/ml. To conclude, the nitric oxide scavenging was effective in ethanol leaf extract than other extract (Table 19).

The ethanol stem bark extract of *C. mala-elengi* showed the percentage of inhibition increased from 37.83±0.12% to 77.41±0.19%. IC_{50} value found 27.67±0.33 µg/ml than ascorbic acid was 10±0.58 µg/ml and they are significant at $p<0.05$ level (Table 20, 21). *In vitro* nitric oxide scavenging activity of olive leaf extract (*O. europaea*) was showed better antioxidant activity (Lins *et al*., 2018).

Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity is a weak oxidising agent, non-radical Reactive Oxygen Species (ROS). Though, it is not toxic by itself, but can be converted to another even most dangerous radical such as OH by fenton reaction or hypochlorous acid by the enzyme myeloperoxide (Bandyopadhyay *et al*., 1999; Droge, 2002). Hydrogen peroxide is present inside the cell which can cross the cell membrane rapidly, H_2O_2 can react with Fe^{2+} and Cu^{2+} ions to hydroxyl give rise to hydroxyl radicals and this may be the beginning of numerous toxic effects (Miller *et al*., 1993). Hydrogen peroxide activity of acetone-soluble fractions of its ethyl acetate extract revealed significant activity (Rathee *et al*., 2007).

The present study hydrogen peroxide scavenging activity of ethanol leaf extract of *C. mala-elengi* was found effective. In ethanol leaf extract, the percentage of inhibition increased from 49.12±0.08% at 10 µg/ml to 79.63±0.18% at 50 µg/ml (Table 22). The positive control of ascorbic acid was 78.06±0.14% at 50 µg/ml. IC_{50} values showed that the ethanol leaf extract exhibited the highest antioxidant activity 17.33±0.33. IC_{50} values of positive control ascorbic acid was 16.67±0.88 µg/ml. To conclude, the hydrogen peroxide scavenging activity was effective in ethanol leaf extract than other extracts (Table 23).

The ethanol stem bark extract of *C. mala-elengi* was found effective in hydrogen peroxide scavenging activity and showed the percentage of inhibition increased from 45.58±0.08% at 10 µg/ml to 79.63±0.18% at 50 µg/ml (Table 24). IC_{50} values showed that ethanol extract exhibited the highest antioxidant activity 18±1.15 µg/ml. IC_{50} value of positive control ascorbic acid was 16.67±0.88 µg/ml and they are significant at $p<0.05$ level. To conclude, the hydrogen peroxide scavenging activity was effective in ethanol leaf extract than the other extracts (Table 25).

The ethanol extract of extra virgin olive oil is one of the important primary source of phenolic compounds. It showed significant hydrogen peroxide scavenging activity (Lee *et al*., 2008). Hydrogen peroxide scavenging activity of *J. sambac* cultivar variety showed the maximum antioxidant property (Shekhar and Prasad, 2015). Excessive concentration of hydrogen peroxide (H_2O_2) that can be responsible for many pathological diseases (Werns and Lucchesi, 1989).

Superoxide radical scavenging activity

The superoxide radical scavenging activity is ubiquitous in aerobic cells. Even though they are reactive towards biological molecules (Aust *et al*., 1985; Babbs, 1985; Deby and Goutier, 1990). The human cells are capable of maintaining the balance of production and inactivation of the superoxide radicals (Sies, 1997). Therefore, the superoxide is excessively generated in inflammation. If not inactivated by chemical or biochemical defenses, this excess superoxide can damage cells (Aust, 1985; Tien *et al*., 1982; Deby and Goutier, 1990). The advantage of plant extracts in inflammation has long been recommended and that excess superoxide was eliminated by flavonoids (Sichel *et al*., 1991).

In the present study superoxide radical scavenging activity was effective in ethanol leaf extract of *C. mala-elengi* which was found effective, percentage of inhibition increased from 56.59±0.15% at 10 µg/ml to 85.56±0.13% at 50 µg/ml (Table 26). IC_{50} values showed that the ethanol extract exhibited the highest antioxidant activity 15.67±1.20 µg/ml. IC_{50} values of positive control ascorbic acid was 9.67±0.67 µg/ml. To conclude, the superoxide scavenging activity was effective in ethanol leaf extract than the other extracts (Table 27).

The ethanol stem bark extract of *C. mala-elengi* was found effective in superoxide scavenging activity showed the percentage of inhibition increased from 42.02±0.14% at 10 µg/ml to 83.16±0.07% at 50 µg/ml (Table 28). IC_{50} values showed that the ethanol extract exhibited the highest antioxidant activity 27.0±0.58 µg/ml. IC_{50} value of positive control ascorbic acid was 9.67±0.67 µg/ml and they are significant at $p<0.05$ level. To conclude, the superoxide scavenging activity was effective in ethanol leaf extract than other extract (Table 29).

The plant *C. virginicus* was reported in folk medicine. It is used for cholagogue, diuretic and tonic. In homeopathy medicine, root bark is used for hepatitis and icterus. Ethyl acetate dried root bark extract of *C. virginicus* showed significant activity in different antioxidant assay (Duke and Wain 1981; Guermonprez *et al*., 1997; Gulcin *et al*., 2007).

Total antioxidant capacity

Total antioxidant activity can be described as the ability of a compound to inhibit the oxidative degradation of lipid. Lipid peroxidation process involves oxidative deterioration of lipids with unsaturation. This peroxidation known the initiative mechanism starts with the production of conjugated dienes and trienes, called as primary oxidation products due to the abstraction of a hydrogen molecule. This leads to the formation of new radical which triggers a number of various diseases in the mankind (Chaiyasit *et al*., 2007; Etim *et al*., 2015).

In the present study, total antioxidant activity of ethanol leaf extract of *C. mala-elengi* was found effective, the percentage of inhibition increased from 47.32±0.19% at 10 µg/ml to 79.40±0.19% at 50 µg/ml (Table 30). IC_{50} values showed that the ethanol extract exhibited the highest antioxidant activity 18.67±0.67 µg/ml. IC_{50} value of positive

control ascorbic acid was 8.67±0.67 µg/ml. To conclude, the total antioxidant activity showed significant result in ethanol leaf extract than the other extracts (Table 31).

The ethanol stem bark extract of *C. mala-elengi* was found effective in total antioxidant activity, the percentage of inhibition increased from 38.98±0.25% at 10 µg/ml to 74.27±0.39% at 50 µg/ml (Table 32). IC_{50} values showed that the ethanol stem bark extract exhibited the highest antioxidant activity 23.67±1.20. IC_{50} value of positive control ascorbic acid was 8.67±0.67 µg/ml. To conclude, the total antioxidant activity was effective in ethanol leaf extract than the other extracts and they are significant at $p<0.05$ level (Table 33).

Total antioxidant activity of the ethanol bark extract of the *O. dioica* revealed good antioxidant activity due to the presence of the pure compound. Benzene, ethanol, 4-hydroxyl-alcohol (Ashwathanarayana and Naika, 2017). Thangavelu and Thomas, 2010 studied total antioxidant activity of ethanoliic leaf extract of *N. arbor-tristis*.

Antimicrobial activity

Plants provide sophisticated traditional medicinal systems that have been in existence for thousands of years and continue to serve mankind with newer remedies (Prabhu *et al,* 2011). Plants are the important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity (Tona *et al*., 1998).

Medicinal plants have been used for various purposes which are curing disease and reducing the symptoms (Shinde and Mulay, 2015). It also contains a wide range of biologically active compounds which can be used to cure infectious diseases (Kiruba *et al.,* 2011; Raja *et al*., 2011; Tirupathi *et al*., 2011). Traditionally, many plants are having the properties to treat the disease caused by microbial pathogens. Recent reports have revealed that the crude plant extract has the higher antibacterial activities against gram positive bacteria than gram negative bacteria. This has been associated to structural variation observed in envelop of the bacteria (including those of cytoplasmic membrane and cell wall components) between gram positive and gram negative bacteria (Silhavy *et al*., 2010; Voon *et al*., 2012). The evaluation of bioactive compounds from natural source has always

been of tremendous interest to researches looking for novel sources of new drugs for curing infectious disease caused by bacteria, fungi and virus etc (Okeke *et al*., 2005).

The present antimicrobial study revealed that the plant extracts such as leaf and stem bark of *C. mala-elengi* was treated against two gram positive bacteria, two gram negative bacteria and three fungal strains. Different solvents such as petroleum ether, chloroform, ethyl acetate, ethanol and water extracts were used for this study.

Ethanol leaf extract of *C. mala-elengi* showed the maximum inhibition against *Bacillus cereus* (18±1.53 mm), *Staphylococcus aureus* (17.33±0.98 mm), *Citrobacter freundii* (17±1.15 mm), *Klebsiella pneumoniae* (16.33±1.86 mm), *Aspergillus niger* (30±1.15 mm) *Aspergillus flavus* (14.33±0.88 mm) and *Candida albicans* (15±1.73 mm) than the other tested stem bark extracts and they are significant at $p<0.05$ level (Table 34).

The antimicrobial activities are therefore attributed to the presence of bioactive molecules such as saponins, tannins, alkaloids and phenolic compounds which leads to therapeutic usage (Javid *et al.,* 2015).

FT-IR analysis

Fourier Transform Infrared Spectroscopy reflecting objectively the panorama of chemical constituents in complex system is the most credible method to validate and identify the mix-substance system such as traditional medicine and herbal medicine (Mariswamy *et al*., 2012). FTIR technique is used to detect the kind of organic and inorganic compound present in traditional plants. It is one of the powerful technique for identifying the type of chemical bonds or functional group present in the phytochemicals. The wavelength of light absorbed and the characteristics of the chemical bonds can be revealed in the annotated spectrum (Nagarajan and Kumar., 2017).

FTIR analysis of the crude powders of leaf and stem bark of *C. mala-elengi* showed the presence of different kind of functional groups such as alcohol, phenol, H-bonded alcohol, alkanes, alkenes, alkyl halides, nitro compounds, carboxylic acid, ethers, esters and amines. In addition to the above mentioned functional groups, the stem bark powder showed H-bonded carboxylic acid, transition metal carboxyl alkenes (Table-36, 37 & Figure-7,8).

The result of present study confirmed the presence of number of functional groups in leaf and stem bark extracts which are responsible for various medicinal properties. The ethanol stem bark extract of the *C. mala-elengi* has more functional group than the ethanol leaf extract and our study developed novel phytochemical marker to identify the medicinally important plant. FT-IR spectroscopic studies of leaf extract in hexane extract *J. azoricum* done by Hari and Nair, 2018.

GC-MS analysis

GC-MS analysis is the main research tool commonly employed to determine the composition of plant volatiles can identify pure compounds present at less than one nanogram level. In the last few years, Gas Chromatography Mass Spectrometry has become firmly established as a key technological platform for metabolite profiling in both plant and non-plant species (Fiehn, 2002; Summer *et al*., 2003; Fernie *et al*., 2004; Kell *et al*., 2005; Robertson, 2005). Recently only a limited number of plant research laboratories have access to GC-MS instrumentation, however such machines are increasingly nowadays.

Extraction is the essential step to separate the medicinally active portion of plant tissue with the help of selective solvents. It is the major process for isolating the individual chemical entities (Handa *et al*., 2008). Gas chromatography is the most commonly used technique for the quantification and identification of bioactive compounds. The unknown organic compound in complex mixture can be detected by interpretation and also by matching the spectra with reference spectra. It was established as a key technological tool for bioactive compounds profiling in medicinal plant species (Fernie *et al*., 2004; Kell *et al*., 2005; Robertson, 2005).

GC-MS analysis of ethanol leaf extract of *C. mala-elengi* confirmed the presence of 17 different chemical compounds. The identified compounds possess many biological properties. The major compound present in leaf of *C. mala-elengi* was Cuparophenol which was found at retention time at 15.589 and peak area 19.26% and stem bark of *C. mala-elengi* at retention time at 15.896 and peak area 24.87% revealed 3-(Dodecanoylamino) benzoic acid.

Ethanol leaf extract of *C. mala-elengi* exhibited the presence of 17 different bioactive compounds (Table 38 & Figure-10). In terms of percentage amount Cuparophenol (19.26%), Ethyl-p-methoxycinnamate (13.92%), Phytol (8.50%), Hexadecanoic acid (7.70%) and 2H-Benzocyclohepten-2-one,3,4,4A,5,6,7,8,9-octahydro (7.45%) are predominant in the leaf extract.

Ethanol stem bark extract of *C. mala-elengi* confirmed the presence of 16 various bioactive compounds (Table 40 and Figure-12). In terms of percentage amount 3-(Dodecanoylamino) benzoic acid (24.87%), 1H-Inden-1-one,3A,4,5,6,7,7A-hexahydro-5,5-dimethyl-,CIS-(14.73%),2(3H)-Naphthalenone,4,4A,5,6,7,8,-Hexahydro-4A,7,7-trimethyl-, (R)- (14.27%), Phenol,2-methyl-5-(1,2,2-trimethylcyclopentyl)-, (S)- (12.26%) and Alpha-asarone (5.05%) are predominant in the stem bark extract.

The above mentioned major constituents have some vital medicinal activity in upcoming drug discovery system such as cuparophenol (sesquiterpene) shows antimicrobial activity (Agger *et al*., 2009). On the other hand, Ethyl-p-methoxycinnamate (Aromatic ester) having anti-inflammatory effect, analgesic effect and anti angio genic effect (Umar *et al*., 2014). It possesses antimicrobial, anti-inflammatory, anticancer, diuretic, resistant gonorrhoea, joint dislocation, headache, hernia and antimalarial properties (Tyagi and Agarwal, 2017). It is also used for tuberculosis (Saikia *et al*., 2010), Antispasmodic (Pongprayoon *et al*., 1992), antinociceptive and antioxidant (Santos *et al*., 2013). Hexadecanoic acid (fatty acid) shows antioxidant, hypocholesterolemic, antiandrogenic and hemolytic effects. It has various activities such as nematicide, pesticide, lubricant, flavouring agent and also act as alpha reductase inhibitor and hemolytic-5 alpha reductase inhibitor.2(3H)-Naphthalenone4,4A,5,6,7,8-hexahydro-4A-methyl-(Ketone) has antiinflamatory activity (Murugesan *et al*., 2014; Kavitha *et al*., 2012). P-vinylguaiacol (Phenolic compound) revealed the antioxidant, antimicrobial and antiinflammatory activity (Ravikumar *et al*., 2012). Phytol (diterpene alcohol) (a precursor of synthetic Vitamin E and Vitamin K was proven to be cytotoxic against breast cancer cell lines (MCF7) (Ogunlesi *et al.,* 2009; Satyal *et al*., 2012).

Among the identified bioactive compounds, Isoeugenol is a phenyl propene, occur in the essential oil of *Cananga odorata* (Ylang ylang) and is synthesized from the eugenol. It is used in manufacturing of perfumeries, flavours, essential oil and vanillin. It has medicinal properties such as antiseptic and analgesic (Vijay *et al*., 2017). 2,4 –di-tert – butyl phenol (2,4, DTBP) is a volatile phenolic compound. It has been reported to be present in fruits and seeds. It possess antifungal, antioxidant, antimalarial activities and has cytotoxic activity against mammalian cancer cell lines such as H9C2, Hela and MCF-7, (Varsha *et al*., 2015; Kusch *et al*., 2011). It is also used in pharmaceutical industries and manufacturing fragrances (Choi *et al*., 2013).

7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6-9-diene-2,8-dione has antioxidant property (Pandit *et al*., 2012; Grover and Patni, 2013). Ar-tumerone is effective in treating neurogenerative disease and stroke. It is also responsible for the anti-tumor properties, anti-inflammatory properties, apoptosis, inhibition of tumour cell invasion, parkinson disease, neurological disorder and alzheimer disease (Lee, 2009; Hirsch and Hunot, 2009; Perrin *et al*., 2009; Schroeter *et al*., 2009; Ladecola and Anrather, 2011; Park *et al*., 2008). It has cytotoxic activity to HC-60, K-562, L-1210, Hela-U-937 and RBL 2H3 cells (Baik *et al*., 1993; Ji *et al*., 2004; Schmidt *et al*., 2015).

Pharmacological study

***In vitro* antidiabetic activity**

Diabetes mellitus is a vital chronic metabolic disorder that cause metabolism of carbohydrate, fat and protein. It is a group of disease such as hyperglycemia, heart disease, stroke, dysfunction and failure of various organs (Keerthana *et al*., 2013). α-glucosidase is the enzyme which is responsible for the breakdown of oligo or disaccharides to monosaccharides (Groop *et al*., 1997; Perfetti *et al*., 1998). The inhibitory action of these enzyme leads to the lowering of blood sugar level. Another significant method to control diabetes is to inhibit the activity of α-amylase enzyme that plays the role in breakdown of starch into simple sugars (Alexander, 1992). This is attributed by α-amylase inhibitor that delays the glucose absorbtion rate. Thus, maintaining the blood glucose level in hyperglycemic people (Dineshkumar *et al*., 2010). Traditionally, many herbs have been used for the treatment of *Diabetic mellitus* (Ghorbani, 2013). Medicinal plants possess anti-

diabetic properties and also they are cost effective. Around 47 species that belongs to 29 plant families act as the source of alpha glucosidase inhibitors (Benalla *et al*., 2010).

The present study illustrates the ethanol leaf extract of *C. mala-elengi* possesses significant inhibitory activity on α-amylase at the concentration of 25 µg/ml showed 14.5±0.69% and at the concentration of 200 µg/ml it exhibited 67.22±1.40% and the IC_{50} value was found to be 134±0.58 µg/ml. In case of inhibitory activity on α-glucosidase at the concentration of 25 µg/ml showed 17.32±0.56% and at the concentration of 200 µg/ml it showed 69.28±0.98% . The IC_{50} value was found to be 129.33±0.33 µg/ml (Table 42-45). Our present study results revealed that *C. mala-elengi* efficiently inhibit α-amylase and α-glucosidase enzyme and the results were significant at $p<0.05$ level. *In vitro* actions of *C. mala-elengi* leaf can also be attributed the intestinal α-amylase and α-glucosidase inhibitory activity.

According to Ali, 2014 in his study hypoglycemic activity of olive leaf extract showed a significant reduction of blood glucose of diabetic animals (350.5±0.03 mg/dl). Jasmine oil inhibited the activity of α-amylase in a dose dependent manner at the concentration 10 µl, it showed 13.1% and 40 µl concentration, showed 48% of inhibition activity (Kaviya *et al*., 2019). Hypoglycemic activity of methanol extract of *N. arbor-tristis* root was studied by Sharma *et al*., 2011.

***In vitro* cytotoxicity assessment (MTT assay)**

Medicinal plants play a significant role in novel drug research. The search for few anticancer agents is a long term goal followed by isolation, identification of bioactive compounds. Screenings of natural bioactive components from plants are considered as an excellent anticancer agent. The biological investigations of cytotoxicity using plant extract are essential to determine the aptitude of the compound. The WHO promoted the use of medicinal plants for their efficacy, affordability and safety. More than 50% of drugs extracted from natural products were in clinical trials for anticancer activity.

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium bromide) colorimetric assay is widely used in determination of cytotoxicity, cell viability and proliferation studies in cell biology (Ferrari *et al.,* 1990; Van de Loosdrecht *et al*., 1991). MTT is cleaved by mitochondrial enzyme dehydrogenase of viable cells, resulting a quantifiable purple

product formazan which can be counted by means of a computer based evaluation system. In this method, the number of cell is quantifiable using spectrophotometer as the concentration of the formazan product (Rubinstein *et al*., 1990; Takimoto, 2003; Covell *et al*., 2007; Madhuri and Pandey, 2009). In the present study, the ethanol leaf extract of *C. mala-elengi* was investigated for cytotoxic studies. The ethanol leaf extract of *C. mala-elengi* showed significant inhibitory activity in 10 µg/ml, it exhibited 94±0.29% and at the concentration of 50 µg/ml it exhibited 28±0.46%. The concentration required for 50% inhibition (IC_{50}) was found to be 22±1.0 µg/ml.

The observation strongly suggested that the test extract *C. mala-elengi* possess significant ($p<0.05$) anticancer activity against HepG2 cell lines and further drug designing is needed to increase its potential (Table 46, 47). Wild olive leaves extract has significant cytotoxic activity (Fu *et al*., 2010). According to the report of Makowska-was *et al*., (2017) Wild olive leaves extract having cytotoxic activity against a numerous cancer cells namely skin cells (melanomas HTB-140, Wistar melanoma 793 (WM 793), Normal skin fibroblasts BJ) and Prostate epithelial cells with additionally Hepato carcinoma cell lines HepG2.

Around 35,000 plant samples from 20 countries has screened about 1,14,000 extracts have been screened for anticancer activity (Samuelsson and Bohlin, 1999). About 3,00,000 plant species with antitumour property have been reported (Krishnamoorthi, 2007). Recent literature survey suggested that India is one of the leading country in cancer related scientific literature on ethno medicinal, phytochemistry and pharmacological aspects. Around 42 major anticancer plants were reported from India because of having strong traditional system (Mathur and Joshi 2013).

Hela cells were treated with ethanol flower extract of *Nyctanthes arbor-tristis*, there was a concentration dependent effect. It showed the potential anticancer activity. The extract showed the percentage of viability from 82% at 10 µg/ml to 41% at 50 µg/ml (Timsina and Nadumane, 2016).

Anticancerous compounds observed by GC-MS analysis (Table 39, 41) could be responsible for this property. Anticancer activity of ethanol leaf extract of *C. mala-elengi* studied in the present work suggests us the possibility of using such extracts against cancer cell lines.

In vivo pharmacological study

Acute toxicity study

Phytotherapy has more significant ethnomedicinal use and insist safety evaluation of herbal medicines. The current study was to assess the toxicity of plants which is determined by acute toxicity method for screening mortality or safe use of drugs. The present study undertaken to prove the safe use of *C. mala-elengi* medicinal plant extract in mice.

Herbal medicines are the source of drugs. They are still not approved by regulatory drug agencies to be marketed. Hence their efficacy and potential toxicity is not evaluated. According to the traditional medicines, there is a common opinion that these products are safe and harmless (Rasmussen, 2012).

The therapeutic potential of herbal plants used by indigenous people may be due to the presence of one or more compounds of the plant species. Some of these compounds may be toxic. However the plant having them, when consumed they may cause some toxicity to the individual (Humphrey and Mckenna, 1998). In herbal medicinal preparation, there is a growing demand for toxicity assessment of the different indigenous preparations used in the treatment of disease (Yakubu *et al*., 2005).

The present investigation was to evaluate the toxicity of therapeutic plants which is determined by acute toxicity study method for analyse the lethality or safe utilization of *C. mala-elengi* curative plant extract in mice.

In the ethanol leaf extract of *C. mala-elengi,* the result of acute toxicity at the concentration of 2000 mg/kg was observed to be safe according to OECD-423 guidelines. On oral administration of ethanol leaf extract of *C. mala-elengi,* no lethality or mortality was recorded after 24 hours and 72 hours. This study concluded that the above mentioned leaf extract was recommended to be safe for mankind consumption (Table 48 & 49).

A single dose (2000 mg/kg) of *O. europaea* leaf extract causes no death for the period of 14 days assessment. Thus, it can be considered as the safe dose (Dekanski *et al*., 2014).

Wound healing activity

Wound healing activity is a series of complex process which include clotting, inflammation, epithelisation, collagen synthesis and tissue remodelling. Through this process

the damaged tissues are restored. Majority of wound contraction rely on the type and extend of damage. Wound is granulated majorly by the collagen, fibroblast, edema and new small blood vessels (Nayak and Mohan, 2007). Many herbal plants play a vital role in the process of wound healing. Herbal plants are promising healers, because they improve the healing mechanism in natural way (Sabharwal *et al*., 2012).

In the present study, the percentage of wound contraction of control animals were showed 57.3±1.6% when compared to positive control 78.2±3.2%. The ethanol leaf extract of *C. mala-elengi* treated groups showed a significant $p<0.01$ wound contraction (72.5±1.2%) compared to control group. The complete healing of wound was observed on 18^{th} day in 20% ointment treated group on the 17^{th} day in positive control (Table 50).

J. grandiflorum flower extract showed significant wound contraction (100%) than the positive control (99.3%). Oleuropein has been reported to having wound healing properties (Koca *et al*., 2011). Oleuropein has promoted wound healing potential was studied by AL-Basher and AL-Otibi, 2018.

Hepatoprotective activity

Liver is the organ regulating homeostasis in the body. It is playing a vital role in biochemical pathway related to growth, fight against disease, nutrient supply and reproduction (Ward and Daly, 1999). Liver disease is a serious health problem in world wide. Conventional drugs used in curing the liver disease causes serious adverse effects. Thus, there is a need to search for alternative drugs for the treatment of hepatic disease (Ozbek *et al*., 2004). Medicinal plants and their formulations are used for liver complaints in ethno medicinal practices additionally in traditional system of medicines in India (Subramoniam *et al*., 1998). Plants possess phytochemical compounds such as triterpenoid and flavonoids are well known for their antioxidant and hepatoprotective activities (Alex *et al*., 2004).

Hepatoprotective activity was evaluated against paracetamol induced hepatic damage. The group of six treatment with control, negative control, positive control, 250 mg, 500 mg of ethanol leaf extract showed a significant effect against paracetamol induced liver damage in rats (Table 51).

Administration of ethanol leaf extract of *C. mala-elengi* extract at concentration of 250 mg/kg and 500 mg/kg for 14 days resulted in a significant reduction of paracetamol induced elevation of serum enzyme markers. Our test extract comparable to the effect of positive control silymarin used. Silymarin is one of the hepato protective compounds which are protecting the plasma membrane of hepatocytes (Ramellini and Meldolesi, 1976).

Hepatic toxicity induced by paracetamol causes elevated level of liver enzymes such as SGOT, SGPT, ALP, Bilirubin, Urea and Creatinine. Treatment at the concentration of 250 mg/kg and 500 mg/kg ethanol leaf extract of *C. mala-elengi* revealed a comparable activity with the reference standard silymarin, an effective hepatoprotective drug. Administration of paracetamol at the concentration of 2 g/kg to the wistar rats caused an increasing level of lipid peroxidation and decreased the activity of enzymatic antioxidants.

In our present study, results of cytotoxicity against $HepG_2$ cell line was one of the evidence for the test extract *C. mala-elengi* having the ability to cure the hepatic related disease.

Histopathological studies

Histopathology is used to visualize various component of the tissue. It gives more detailed view of diseases and its effect on tissue. The diagnosis from the histopathological images can be considered as a "gold standard" in the medicinal field (Rubin *et al*., 2008). Hepatoprotective activity of *C. mala-elengi* was further confirmed by histopathological studies of the liver, which effectively supported the result from the serum assay.

Histopathological studies of the control group liver showed normal hepatic architecture normal hepatocytes sinusoid and bile ducts. Paracetamol treated rats showed binucleation and interface hepatitis which leads to the inflammation and damage of liver cells. The maximum protection against hepatic injury was achieved with ethanol leaf extract of *C. mala-elengi* extract at a dose of 500 mg/kg.

The histopathological observation of the liver of rats administrated with *C. mala-elengi* showed the regeneration of more number of hepatocytes and cytoplasmic vascularization. The present study confirmed that the test extract possess potent hepatoprotective activity (Plate-13).

N. arbor-tristis possess a significant hepatoprotective activity. It has been proved by the histopathological analysis where the flower extract at the dose of 200 mg/kg showed significant protection as it was evident by the absence of necrosis, tissue damage and vacuoles (Wagh *et al*., 2010).