4. EXPERIMENTAL RESULTS

Experiment No-1

4.1 Pharmacognostic studies

Herbal medicines play a significant role in drug discovery. Plant based drug plays a vital role in conventional medicines for curing different health ailments. They are always considered as inexpensive, potent and safe. The advantage of plant drug is its easy availability, economic and fewer or no side effects. They also have been extensively used in indigenous medicine practices. Despite extensive increase in synthetic medicine, several other diseases have developed due to their harmful side effects after prolonged usage. The misuse of plant medicine or natural products start with wrong identification. In common, the potent drugs are always either adulterated are substituted based on its morphological properties or biological activity. Although modern medicine is applicable, plant medicines occupy their image for historical and cultural reasons. Pharmacognostic study is the primary step in standardization of herbal drug. The Pharmacognostic characterizations are important reliable and inexpensive criteria for confirmation of the crude drugs. Standardization is the initial step in quality assurance of raw material and also to determine the authenticity of the specific species for the intended use. The modern standardization technique contains valuable information about the morphology, microscopical and physical nature of herbal drugs.

For the process of standardization we can apply various techniques and methodology to attain our goal in these manner. These steps and processes are utilized in identification and standardization of the plant drug. Accurate characterization and quality assurance of starting material is an crucial step to ensure reproducible quality of herbal drug which will help us to confirm its safety and efficacy. In such cases, there is a chance of selecting incorrect raw adulterants. However, an extensive pharmacognostic study is necessary for each crude drug used in formulation to avoid any ambiguity and such a study will emphasize as a reference for further studies.

Despite taxonomic identification, pharmacognostic investigation contain parameters that aid identifying adulteration in dry powder form. It is essential because once the plant is dried and made into fine powder it loses its morphological identity and becomes easily prone to adulteration.

The quantitative determination of certain pharmacognostic parameters aid in setting standards for herbal drugs. In the present study, leaf and stem bark of *Chionanthus mala-elengi* (Dennst.) P.S. Green was collected from Nilgiri Biosphere Nature Park, Anaikatti, Coimbatore, Tamilnadu (Figure-1). The Plant was undertaken to characterize the various pharmacognostic parameters for estimate the standards and specifications for their curative uses. The two parts (Leaf and stem bark) have been analyzed based on the following studies.

I. Macroscopic characters

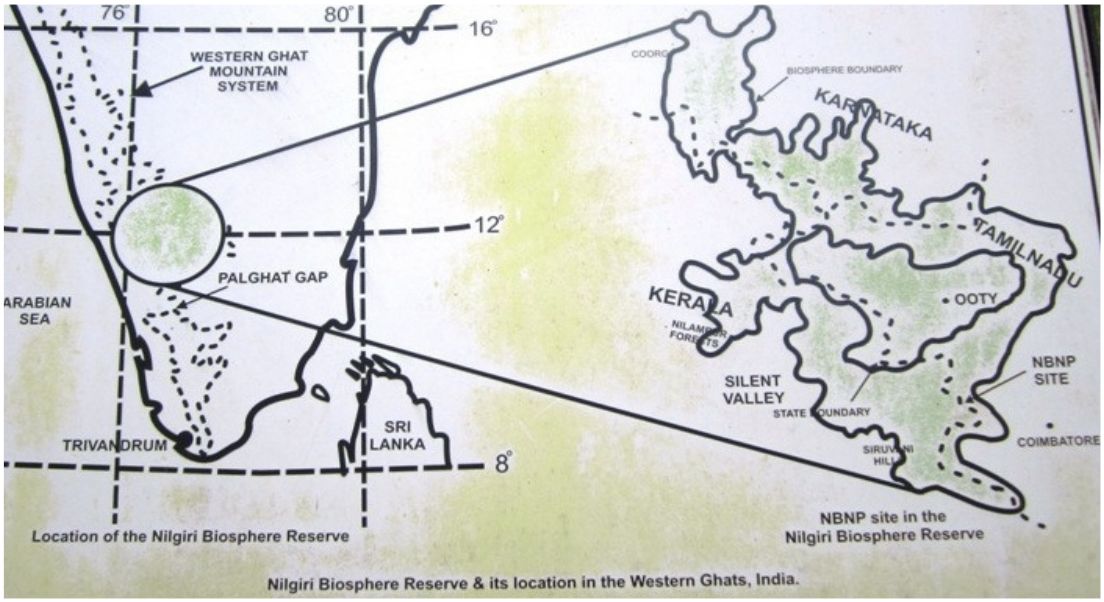
II. Microscopic characters

III. Organoleptic characters

IV. Powder study

Figure-1

Location map of the plant collection at Nilgiri Biosphere Nature Park



4.1.1 Morphological characteristics

The Macroscopic study is the morphological description of the plant parts which are seen by naked eye or magnifying lens. Hence to identify the plant *Chionanthus mala-elengi* (Dennst.) P.S. Green through naked eye the following characteristic features were recorded (Plate-1). Identification of plant was authorized by Botanical Survey of India, Coimbatore, Tamilnadu, India**. (Annexure-I)**

Botanical name: *Chionanthus mala-elengi* (Dennst.) P.S. Green

Common name: Malabar fringe Tree **(Tamil-Sorkili)**

Family: Oleaceae

Habit: It is a small ever green medium sized tree with the height of 8 meter tall. It grows on the moist slope, especially along ravines, streams and forest areas

Root: The root system consist of a long, vigorous tap root

Stem Bark: Dark brown in colour smooth shining, covered with raised corky lenticels, glabrous, coriaceous and the blaze is dull yellow or orange in colour

Leaves: The leaves are simple, opposite, decussate, petiole 0.5-1.2 cm long, glabrous, leaf blade 5-14.5 x 2.5-5 cm, elliptic-obovate, exstipulate, apex obtuse, margin entire, slender, intercostal reticulate venation

Inflorescence: Flowers are cymes or on axillary fascicles, slender, peduncles

Flowers: Flowers bisexual, yellowish white in colour, fragrant, sessile, 6 mm long in axillary cluster, Peduncle 1.5 cm long, bearing fascicles of 1-5 stalkless flowers at apex. Style is short, Ovary pubescent, densely hairy

Calyx: Gamosepalous, tube is 4 toothed, lobes are 1.5 mm, ovate, puberculous, thick, grey-pubescent

Corolla: Gamopetalous, 4-10 lobes attached in pair linear acuminate, glabrous, white

Androecium: Stamens are 2 in number, inserted on the corolla-tube, sessile

Gynoecium: Bicarpellary, syncarpous, style short, stigma terminal

Fruit: Drupe (10×5 mm), ellipsoid, slightly curved, acute, ridged

4.1.2 Microscopical characteristics

Microscopical evaluation is one of the cheapest method to identify the selected species and raw material accurately. Microscopic characterization of the leaf of *C. mala-elengi* was done with the help of transverse section of the leaves under observation.

The transverse section of *C. mala-elengi* leaf through the midrib showed the following tissue systems (Plate-2).

T.S of leaf contains a single layer of adaxial and abaxial sides. The adaxial epidermis is single layered and made up of small transversely elongated cells covered with a thin cuticle. Mesophyll is differentiated into palisade and spongy parenchyma. Palisade tissue is compactly arranged and composed of a single layer of closely arranged cells, 4-6 layers of spongy parenchyma cells located in the central part of leaf. It is small, loosely arranged oval to round shaped parenchymatous cells. Rosette of calcium oxalate is present in throughout the mesophyll cells, filiform sclerides were also scattered on the mesophyll cells. The vascular bundle of midrib is crescent shaped. The bundle exhibits collateral with xylem facing adaxial side and phloem facing towards abaxial side. Single layered epidermis is present in the lower surface.

4.1.2.1 Determination of stomatal number and stomatal index

It is one of the quantitative method to determine the quality standard of the species. This study is helpful to ensuring standardization of herbal drug under observation. Leaf constants such as stomatal index and stomatal number were measured. Details of measurements are represented in Materials and Methods (3.1.2.1). The stomatal index was 26.11±3.89, stomatal number was 30.33±1.20. The stomata is anomocytic type. The results are showed in Table 1 & (Plate-3).

Table 1

Leaf constants of *Chionanthus mala-elengi*

S.No	Parameters	Units
1	Stomata index	26.11±3.89
2	Stomata number	30.33±1.20
T test		0.831^{ns}

Values are mean expressed by ± S.E

Ns – Not significant

4.1.3 Organoleptic evaluation

Organoleptic evaluation is a technique of qualitative evaluation based on the study of morphological and sensory profiles of whole drugs. This provide the simplest as well as quickest means to establish the identity and purity of *C. mala-elengi.* Drugs characters such as colour, odour, texture, taste and touch were studied. The details of methodology are given in Materials and Methods (3.1.3). Leaf and stem bark powder were studied for this purpose.

The leaf was opposite decussate, dorsiventral, elliptic-obovate, apex obtuse, margin entire. Stem bark was grey, smooth shining covered with raised corky lenticels.

The organoleptic characteristics of *C. mala-elengi* leaf and stem bark powder was coarse. The colour of leaf was green and stem bark was dark brown (Plate-4). The taste of leaf was bitter and stem bark was astringent. The leaf has characteristic odour and stem bark has no characteristic odour. The results are shown in the Table 2 & Plate-4.

Table 2

Organoleptic characters of dry powder of leaf and stem bark of *C. mala-elengi*

S. No.	Parameters	Leaf	Stem bark
1	Touch	Coarse	Coarse
2	Colour	Green	Dark brown
3	Taste	Bitter	Astringent
4	Odour	Characteristic	No Characteristic

4.1.4 Powder study

For powder study, dried powder was taken to detect the adulterant in crude drug. It is essential because once the plant parts are dried and made into fine powder form, it loses its morphological nature. Thus, the present powder study is carried out in *C. mala-elengi* to ensure reproducible quality of herbal products which will lead towards safety and efficacy of natural products. The details are represented in Materials and Method (3.1.4).

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

Experiment No - 2

4.2 Physicochemical analysis

Medicinal plants have promising scope over synthetic drugs with least or minimum side effects. Herbal formulations involves the use of fresh or dried parts of plants. The efficacy of herbal medicine depends on the aspect of preparation, safety and correct knowledge of crude drugs. The process of standardization ensure maximum therapeutic effects. Ash values are used to determine quality, purity and authenticity of crude drug. It indicates the presence of various impurities such as carbonate, oxalate and silicate. The water soluble ash is used to estimate the amount of inorganic compound present in drugs. The acid insoluble ash consists mainly of silica and indicates contamination with earthy material. The sulphated ash test is to determine the amount of residual substance not volatilized from a drug. It is used to estimate the content of inorganic impurities in drug. Moisture content (Loss on drying) of drugs should be at minimum level to discourage the growth of bacteria, yeast or fungi during storage. It plays an indigenous role for the standardization of crude drugs in the plants under investigations are explained in Materials and Methods (3.2).

The mean values of physicochemical analysis (n=3) of % w/w ± SEM (Standard Error of the Mean) of total ash, acid insoluble ash, water soluble ash, sulphated ash and moisture content (Loss on drying) are represented in Table 3.

The analytical results showed that total ash value content, water soluble ash, acid insoluble ash, sulphated ash and moisture content (Loss on drying) of the *C. mala-elengi* leaf has 10.38±0.09%, 4.28±0.18%, 2.38±0.27%, 20.20±1.24% and 9.03±0.37%. The stem bark showed 9.67±0.06%, 3.19±0.09%, 1.47±0.18%, 19.14±0.19% and 7.53±0.55% w/w respectively. The moisture content or loss on drying at 105^0C in leaf was found to be (9.03±0.37%) more than stem bark (7.53±0.55%) they are significantly different at $p<0.05$ level.

Table 3

Physicochemical analysis of leaf and stem bark powder of *C. mala-elengi*

S. No	Parameters	Plant parts (%w/w)	
		Leaf	Stem bark
1	Total ash	10.38±0.09[b]	9.67±0.06[b]
2	Water soluble ash	4.28±0.18[c]	3.19±0.09[d]
3	Acid insoluble ash	2.38±0.27[d]	1.47±0.18[e]
4	Sulphated ash	20.20±1.24[a]	19.14±0.19[a]
5	Moisture content (Loss of drying)	9.03±0.37[b]	7.53±0.55[c]
SED Cd (p<0.05)		0.8465 1.8862***	0.2673 0.5955***

[#]Mean ± S.E

Mean in a column followed by a same letter (s) are not significantly (P > 0.05) different according to Duncan's Multiple Range Test

,*Significant at P < 0.01, P < 0.001 respectively; ns - non significant

4.2.1 Determination of extractive value

Leaf and stem bark powders of *C. mala-elengi* were extracted with petroleum ether, chloroform, ethyl acetate, ethanol and water. The percentage yield of each is shown in Table-4. Ethanol showed higher results than the other solvents.

The extractive value of leaf powder in petroleum ether was 2.42±0.19, chloroform- 3.53±0.25%, ethyl acetate- 5.56±0.23%, ethanol- 41.52±0.48% and water- 18.04±0.36%.

The extractive value of stem bark powder petroleum ether - 1.48±0.24%, chloroform- 17.33±0.88%, ethyl acetate-16.79±0.21%, ethanol- 32.73±0.84% and water- 14.33±0.33%. The results are significantly different at p<0.05 level (Table 4).

Table 4

Extractive values of leaf and stem bark powder of *C. mala-elengi*

S. No.	Extracts	% Yield	
		Leaf	Stem bark
1	Petroleum ether	2.42±0.19[e]	1.48±0.24[e]
2	Chloroform	3.53±0.25[d]	17.33±0.88[c]
3	Ethyl acetate	5.56±0.23[c]	16.79±0.21[b]
4	Ethanol	41.52±0.48[a]	32.73±0.84[a]
5	Water	18.04±0.36[b]	14.33±0.33[d]
SED Cd (p<0.05)		0.4521 1.0073***	0.8244 1.8369***

[#]Mean ± S.E

Mean in a column followed by a same letter (s) are not significantly (P > 0.05) different according to Duncan's Multiple Range Test

,*Significant at P < 0.01, P < 0.001 respectively; ns - non significant

4.2.2. Fluorescence analysis

Fluorescence analysis is another technique used to identify the adulteration of crude drug powder. Drug powder treated with different chemical reagents gives characteristic colourations when seen under UV light and it is compared with the colourations observed under visible light. In some cases, there are marked differences in colour, which is an important tool in identification of adulteration in drug powders. The details are explained in Materials and Methods (3.2.2.1) and the results are presented in Table 5 & 6.

The leaf powder of *C. mala-elengi* under day light was blackish green and under UV 254 nm light it was light green. Powder + Concentrated HNO_3 under day light was pista green and UV light it was yellowish green. Powder + Concentrated HCl and Powder + Iodine under day light was dark green and UV 254 nm it was light green. Powder + Concentrared H_2SO_4 under day light was blackish red under UV 254 nm it was red. Powder + Ammonia solution under day light was dark blackish green, under UV 254 nm it was bottle green. Powder + $FeCl_3$ under day light was black, under UV 254 nm it was dark green. Powder + Acetic acid under day light was parrot green and under UV 254 nm it was

pinkish green. Powder + NaOH under day light was brick red and under UV 254 nm it was bottle green. Powder + Distilled water under day light showed blackish green and UV 254 nm it was greenish brown. Powder + Chloroform under day light showed blackish green and UV 254 nm it was reddish green.

Table 5

Fluorescence analysis of *C. mala-elengi* leaf

S.No	Treatment	Visible/Day light	UV light (254 nm)
1	Powder as such	Blackish green	Light green
2	Powder + Concentrated HNO_3	Pista green	Yellowish green
3	Powder + Concentrated HCl	Dark green	Light green
4	Powder + Concentratd H_2SO_4	Blackish red	Red
5	Powder + Ammonia solution	Dark blackish green	Bottle green
6	Powder + $FeCl_3$	Black	Dark green
7	Powder +Acetic acid	Parrot green	Pinkish green
8	Powder + NaOH	Brick red	Bottle green
9	Powder + Distilled water	Blackish green	Greenish brown
10	Powder + Chloroform	Blackish green	Reddish green
11	Powder + Iodine	Dark green	Light green

The stem bark of *C. mala-elengi* under day light was brown and UV 254 nm light it was light brown. Powder + Concentrated HNO_3 under day light was yellowish brown and UV 254 nm showed black. Powder + Concentrated HCl under day light was red and UV 254 nm light it was blackish brown. Powder + Concentrated H_2SO_4, Powder + Ammonia solution, Powder + $FeCl_3$ and Powder and Iodine under day light was dark brown and UV 254 nm it was black, red light brown and black. Powder + Acetic acid under day light was dark brown and UV 254 nm it was light brown. Powder + NaOH under day light was blackish brown and UV 254 nm light it was dark red. Powder + Distilled water and Powder + Chloroform under day light was light brown and UV 254 nm light it was greenish brown and brown.

Table 6

Fluorescence analysis of *C. mala-elengi* stem bark

S.No	Treatment	Visible / Day light	UV light (254 nm)
1	Powder as such	Brown	Light brown
2	Powder + Concentrated HNO_3	Yellowish brown	Black
3	Powder + Concentrated HCl	Red	Blackish brown
4	Powder + Concentrated H_2SO_4	Dark brown	Black
5	Powder + Ammonia solution	Dark brown	Red
6	Powder + $FeCl_3$	Dark brown	Light brown
7	Powder + Acetic acid	Dark black	Dark brown
8	Powder + NaOH	Blackish brown	Dark red
9	Powder + Distilled H_2O	Light brown	Greenish brown
10	Powder + Chloroform	Light brown	Brown
11	Powder + Iodine	Dark brown	Black

Experiment No - 3

4.3 Preliminary phytochemical screening

Phytocompounds derived from plant parts have been utilized in drug production over the years, thus indicating that any part of a plant may contain active components. Recently, there has been continual revival of interest in the use of medicinal plants in many developing countries. Since herbal medicines have been reported as safe and without adverse side effects as compared to synthetic drugs.

Phytochemical analysis is important step in identifying new source of therapeutically and industrially valuable compounds. Therefore to understand and identify the suitable solvent for the extraction of crude drugs, the following experiments were conducted. Details of estimation is given in Materials and Methods (3.3).

Qualitative phytochemical analysis

Medicines are derived from medicinal plants having various therapeutic properties due to the presence of secondary metabolites such as alkaloid, flavonoid, phenol, saponin, glycoside etc. Hence, these bioactive compounds were estimated using different solvents such as petroleum ether, chloroform, ethyl acetate, ethanol and water extract. The details of qualitative estimation are explained in Materials and Methods (3.3.2).

4.3.1 Qualitative phytochemical analysis of leaf extract of *C. mala-elengi*

Glycosides are present in all the extracts tested. The chloroform, ethyl acetate, ethanol and water extracts showed the presence of tannins and phenolic compounds, steroids, sterols, triterpenoids, flavonoids and balsams. Alkaloids, phlobatannins and resins were present in ethyl acetate, ethanol and water extracts. Protein, amino acids and volatile oils are present in petroleum ether, ethyl acetate and ethanol. Saponins were present in Petroleum ether, ethyl acetate, ethanol and water extracts. Carbohydrates are present in chloroform, ethanol and water extracts (Table 7). These results revealed that among the different solvents, used in the extraction maximum number of bio active compounds present in ethanol extract of leaf.

Table 7

Qualitative phytochemical analysis of leaf extract of *C. mala-elengi*

S. No	Phytochemicals	Test Performed	Various alcoholic and water extract of leaf of *C. mala-elengi*				
			Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Water
1	Alkaloids	Mayer's test	-	-	+	+	+
		Dragendorff's test	-	-	+	+	+
		Wagner's test	-	-	+	+	+
2	Carbohydrates	Molisch's test	-	+	-	+	+
		Fehling's test	-	+	-	+	+
		Benedict's test	-	+	-	+	+
3	Proteins & aminoacids	Biuret test	+	-	+	+	-
		Ninhydrin test	+	-	+	+	-
		Xanthoproteic test	+	-	+	+	-
		Millon's test	+	-	+	+	-
4	Tannins and Phenolic compounds	Ferric chloride test	-	+	+	+	+
5	Steroids & Sterols	Libermann-burchard test	-	+	+	+	+
		Salkowski's test	-	+	+	+	+

S. No	Phytochemicals	Test Performed	Various alcoholic and water extract of leaf of *C. mala-elengi*				
			Petroleum ether	**Chloroform**	**Ethyl acetate**	**Ethanol**	**Water**
6	Triterpenoids	Libermann-burchard test	-	+	+	+	+
7	Saponins	Foam test	+	-	+	+	+
8	Flavonoids	Shinoda test	-	+	+	+	+
		Ferric chloride test	-	+	+	+	+
		Lead acetate test	-	+	+	+	+
9	Glycosides	Borntrager's test	+	+	+	+	+
		Keller-Killiani test	+	+	+	+	+
10	Phlobatannins	Hydrochloric acid test	-	-	+	+	+
11	Balsams	Alcoholic $FeCl_3$ test	-	+	+	+	+
12	Volatile oils	Sodium hydroxide test	+	-	+	+	-
13	Resins	Acetone-water test	-	-	+	+	+

(+) - Present; (-) - Absent

4.3.2 Qualitative phytochemical analysis of stem bark extract of *C. mala-elengi*

Preliminary qualitative analysis of stem bark extract of *C. mala-elengi* revealed that steroids, sterols and glycosides were present in all the extracts tested. 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 extracts. Pholobatannins, balsams and resins were present in ethanol and water extracts (Table 8). These results indicated that among the different solvents used in the extraction and the maximum number of bio active compounds present in ethanol extract of stem bark.

Table 8

Qualitative phytochemical analysis of stem bark extract of *C. mala-elengi*

S. No	Phytochemicals	Test Performed	Various alcoholic and water extracts of stem bark of *C. mala-elengi*				
			Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Water
1	Alkaloids	Mayer's test	-	-	+	+	+
		Dragendorff's test	-	-	+	+	+
		Wagner's test	-	-	+	+	+
2	Carbohydrates	Molisch's test	+	+	+	-	-
		Fehling's test	+	+	+	-	-
		Benedict's test	+	+	+	-	-
3	Proteins & aminoacids	Biuret test	+	+	+	-	-
		Ninhydrin test	+	+	+	-	-
		Xanthoproteic test	+	+	+	-	-
		Millon's test	+	+	+	-	-
4	Tannins and Phenolic compounds	Ferric chloride test	+	-	+	+	+
5	Steroids & Sterols	Libermann-burchard test	+	+	+	+	+
		Salkowski's test	+	+	+	+	+

S. No	Phytochemicals	Test Performed	Various alcoholic and water extracts of stem bark of *C. mala-elengi*				
			Petroleum ether	**Chloroform**	**Ethyl acetate**	**Ethanol**	**Water**
6	Triterpenoids	Libermann-burchard test	-	-	+	+	+
7	Saponins	Foam test	+	-	+	+	+
8	Flavonoids	Shinoda test	-	-	+	+	+
		Ferric chloride test	-	-	+	+	+
		Lead acetate test	-	-	+	+	+
9	Glycosides	Borntrager's test	+	+	+	+	+
		Keller-Killiani test	+	+	+	+	+
10	Phlobatannins	Hydrochloric acid test	-	-	-	+	+
11	Balsams	Alcoholic $FeCl_3$ test	-	-	-	+	+
12	Volatile oils	Sodium hydroxide test	+	+	+	+	-
13	Resins	Acetone-water test	-	-	-	+	+

(+) - Present; (-) - Absent

4.3.3 Quantitative phytochemical analysis

Phytochemical constituents are mainly responsible for the medicinal properties of the plants. Bioactive compounds were estimated using different solvents mentioned in the previous experiment to find out which solvent has better quantity of individual secondary metabolites. The amount of phytochemicals that are detected in the plant extract was quantitatively estimated by standard protocols as explained in Materials and Methods (3.3.3).

4.3.3.1 Estimation of total phenolic content

Phenolic compound is one of the largest and most ubiquitous group of plant metabolites. They possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammatory, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiosperms and cell proliferation activities. The antioxidant activity of phenolic compound have the capability of quenching oxygen derived free radicals donating chelate metalcations. Genistein, daidzein, isorhamnetin, apigenin are some of the phenolic compounds which has wide benefits on human for curing various diseases.

The results of total phenolic content are shown in Table 9 & Figure-2. The standard calibration curve is done using Gallic acid. The total phenolic content was found in different extracts of the leaf, the maximum amount was found in ethanol leaf extract (318.33±1.64 mg GA/gm) and the minimum amount was found in petroleum ether extract (21.17±1.2 mg GA/gm). The following order was found among the leaf extract ethanol > ethyl acetate > water > chloroform > petroleum ether.

In stem bark extract, maximum phenolic content was found to be in ethanol extract (297.33±1.87 mg GA/gm) and minimum amount found to be in chloroform extract (52.83±1.45 mg GA/gm). The following order was found among the stem bark extract ethanol > ethyl acetate > water > petroleum ether > chloroform and they are significantly different at $p<0.05$ level.

Table 9

Estimation of total phenolic content of leaf and stem bark extract of *C. mala-elengi*

Extracts	Total phenolic content (mg GA/gm)	
	Leaf	Stem bark
Petroleum ether	21.17±1.2^{e}	80.83±2.19^{d}
Chloroform	72.83±1.64^{d}	52.83±1.45^{e}
Ethyl acetate	260.83±2.52^{b}	244 ±0.87^{b}
Ethanol	318.33±1.64^{a}	297.33±1.87^{a}
Water	163±1.73^{c}	107.17±1.69^{c}
SED Cd (p<0.05)	0.0051 0.0113***	0.0047 0.0106***

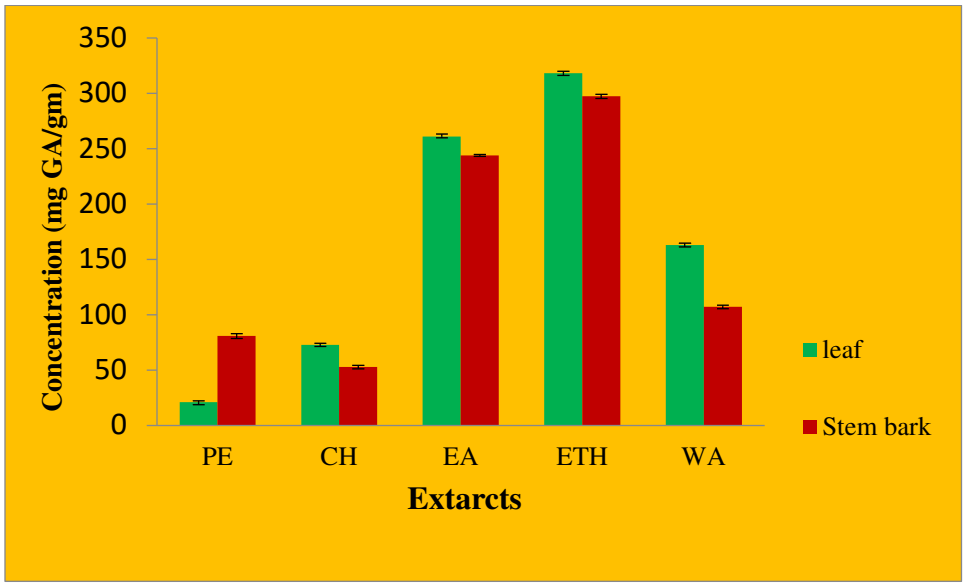
$^{\#}$Mean ± S.E

Mean in a column followed by a same letter (s) are not significantly (P > 0.05) different according to Duncan's Multiple Range Test

,*Significant at P < 0.01, P < 0.001 respectively; ns- non significant.

Figure 2

Estimation of total phenolic content



PE-Petroleum ether, CH-Chloroform, EA-Ethyl acetate, ETH-Ethanol, WA-Water

4.3.3.2 Estimation of total flavonoid content

Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms *in vitro*. Their activity is probably due to the ability to complex with extracellular, soluble proteins and to complex with bacterial cell wall. They are an effective antioxidant and show strong anticancer activities. Some flavonoids possess hormones which can protect against various chronic disease like neuroprotective effect on the brain.

The results of total flavonoid content of different extracts from the leaf showed maximum amount in ethanol leaf extract (162.37±1.41 mg QE/gm) and the minimum amount was found to be in petroleum ether extract (61.48±0.65 mg QE/gm).The following order was found among the leaf extract ethanol > chloroform > ethyl acetate > water > petroleum ether.

In stem bark extract, the maximum total flavonoid content was found to be in ethanol extract (133.48±2.85 mg QE/gm) and the minimum amount was present in petroleum ether extract (24.22±0.38 mg QE/gm). The following order was found among the stem bark extract, it was ethanol > ethyl acetate > water > chloroform > petroleum ether. The standard calibration curve was done using quercetin and the results are significantly different at $p<0.05$ level. The results are represented in Table 10 and Figure-3.

Table 10

Estimation of total flavonoid content of leaf and stem bark extract of *C. mala-elengi*

Extracts	Total flavonoid content (mg QE/gm)	
	Leaf	Stem bark
Petroleum ether	61.48±0.65^{e}	24.22±0.38^{e}
Chloroform	133.78±1.72^{b}	45.48±1.95^{d}
Ethyl acetate	110.15±0.91^{c}	108.81±1.12^{b}
Ethanol	162.37±1.41^{a}	133.48±2.85^{a}
Water	94.52±2.16^{d}	72.81±1.53^{c}
SED Cd (p<0.05)	0.0094 0.0209***	0.0113 0.0251***

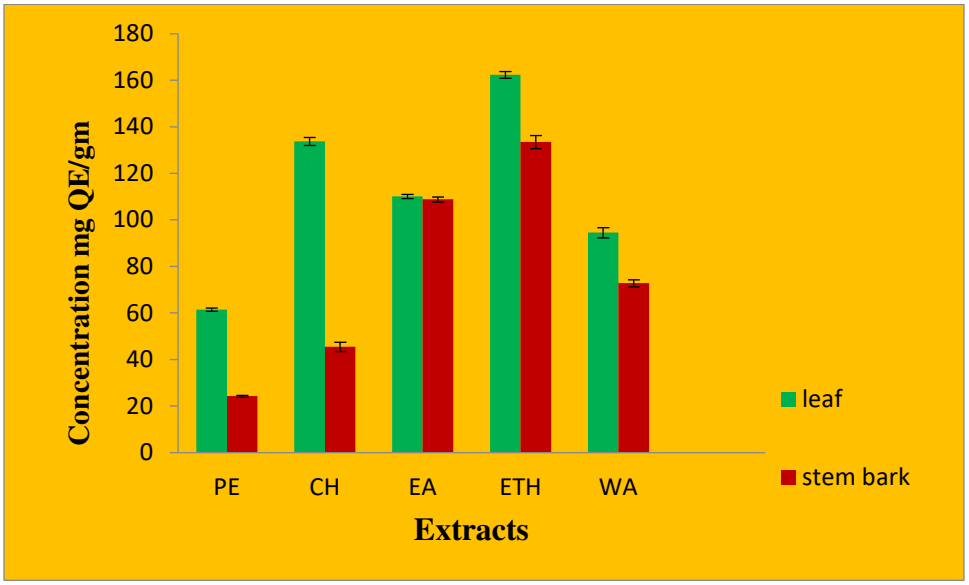
[#]Mean ± S.E

Mean in a column followed by a same letter (s) are not significantly (P > 0.05) different according to Duncan's Multiple Range Test

,*Significant at P < 0.01, P < 0.001 respectively; ns- non significant.

Figure 3

Estimation of total flavonoid content



PE-Petroleum ether, CH-Chloroform, EA-Ethyl acetate, ETH-Ethanol, WA-Water

4.3.3.3 Estimation of total tannin content

Tannins are amorphous, astringent substances occurring widely in the bark, wood, leaf and resinous exudation of plants. Tannins are potential biological antioxidants used against oxidative damage, which has been implicated in a wide range of diseases including cancer, cardiovascular disease, arthritis and ageing. Tannins also exhibits anti nutritional properties.

The tannin content of different extracts from the leaf showed maximum amount in ethanol extract (445±2.17 mg TA/gm) and the minimum in the water extract (147.5±1.25 mg TA/gm). The following order was found among the leaf, it was ethanol > petroleum ether > ethyl acetate > chloroform > water.

In stem bark extract, the maximum amount of tannin was found to be in ethyl acetate extract (387.5±1.91 mg TA/gm) and the minimum amount in chloroform extract (70±2.61 mg TA/gm). The following order was found among the stem bark, it was ethyl acetate > petroleum ether > ethanol > water > chloroform. The standard calibration curve was done using tannic acid and they are significantly different at $p<0.05$ level. The results are represented in Table 11 & Figure-4.

Table 11

Estimation of total tannin content of leaf and stem bark extract of *C. mala-elengi*

Extracts	Total tannin content (mg TA/gm)	
	Leaf	Stem bark
Petroleum ether	420.59±1.68[b]	305.83±1.5[b]
Chloroform	308.33±1.82[d]	70.0 ±2.61[e]
Ethyl acetate	383.75±2.16[c]	387.5±1.91[a]
Ethanol	445±2.17[a]	269.17±2.32[c]
Water	147.5±1.25[e]	207.5±2.17[d]
SED Cd (p<0.05)	0.0021 0.0047***	0.0024 0.0054***

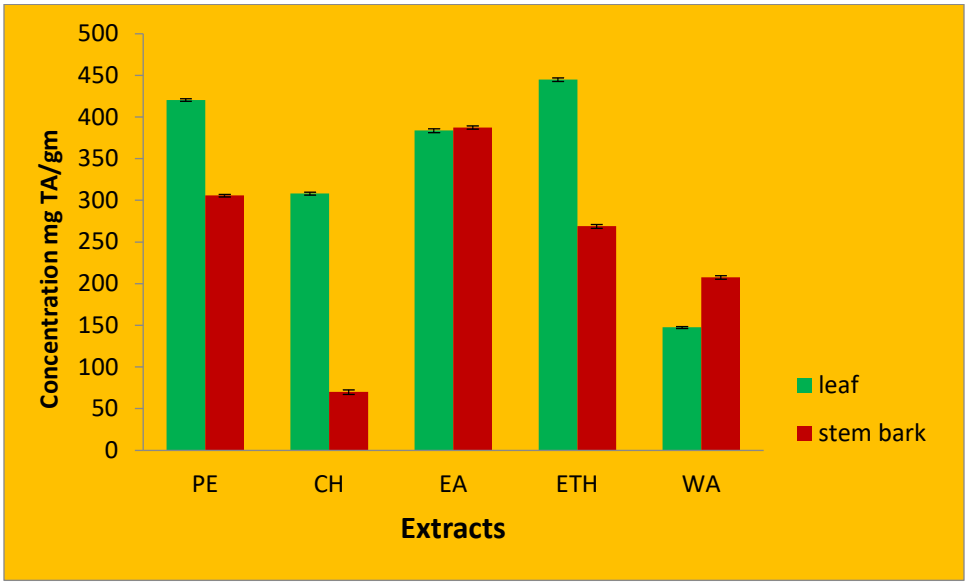
[#]Mean ± S.E

Mean in a column followed by a same letter (s) are not significantly (P > 0.05) different according to Duncan's Multiple Range Test

,*Significant at P < 0.01, P < 0.001 respectively; ns- non significant.

Figure 4

Estimation of total tannin content



PE-Petroleum ether, CH-Chloroform, EA-Ethyl acetate, ETH-Ethanol, WA-Water

4.3.3.4 Estimation of total alkaloid content

Alkaloids have diverse and important physiological effects on human and animals. Well-known alkaloids include codeine, brucine, morphine, ephedrine, quinine and scopolamine. Alkaloids have the various pharmacological properties such as analgesic, antibacterial, antihypotensive, antipyretic, anticholinergic, antitumour, antimalarial activities.

The results of total alkaloids were shown in the Table 12 and Figure-5. The standard calibration curve is done using atropine. Total alkaloid content of different extracts of the leaf showed maximum amount in ethanol leaf extract (28.76±0.28 mg AE/gm) and minimum amount in petroleum ether extract (12.41±0.35 mg AE/gm). The following order was found among the leaf extract ethanol > water > ethyl acetate > chloroform > petroleum ether.

In stem bark extract, maximum alkaloid content was found to be in ethyl acetate extract (26.04±0.6 mg AE/gm) and the minimum amount in chloroform extract (14.3±0.72 mg AE/gm). The following order was found among the stem bark extract, it was ethyl acetate > water > petroleum ether > ethanol > chloroform and they are significantly different at $p<0.05$ level.

Table 12

Estimation of total alkaloid content of leaf and stem bark extract of *C. mala-elengi*

Extracts	Total alkaloid content (mg AE/gm)	
	Leaf	**Stem bark**
Petroleum ether	12.41±0.35^{e}	19.64±0.21^{c}
Chloroform	19.76±0.18^{d}	14.3±0.72^{e}
Ethyl acetate	22.91±0.19^{c}	26.04±0.6^{a}
Ethanol	28.76±0.28^{a}	19.22±0.18^{d}
Water	23.89±0.08^{b}	23.57±0.26^{b}
SED Cd (p<0.05)	0.0063 0.0141***	0.0110 0.0245***

[#]Mean ± S.E

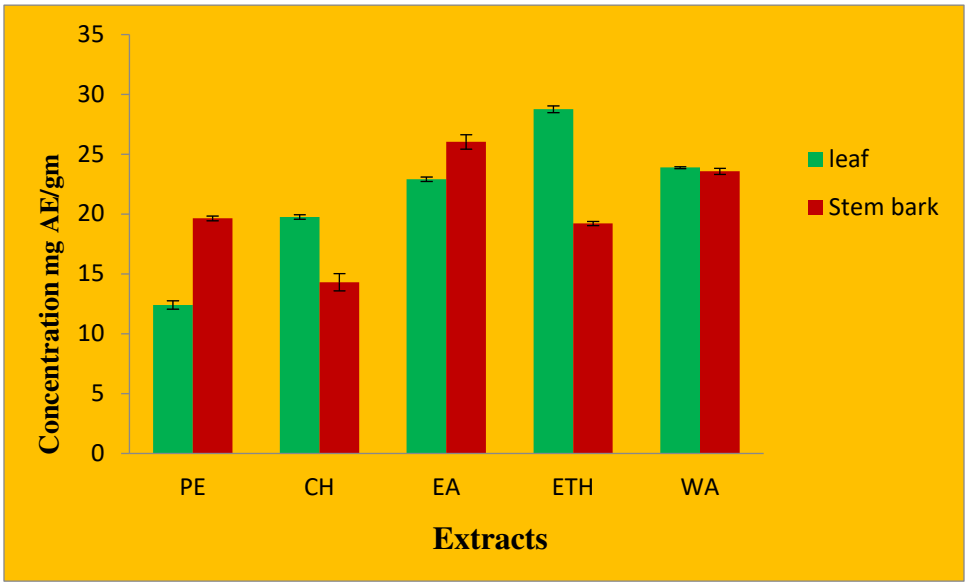
[#]Mean ± S.E

Mean in a column followed by a same letter (s) are not significantly (P > 0.05) different according to Duncan's Multiple Range Test

,*Significant at P < 0.01, P < 0.001 respectively; ns- non significant.

Figure 5

Estimation of total alkaloid content



PE-Petroleum ether, CH-Chloroform, EA-Ethyl acetate, ETH-Ethanol, WA-Water

4.3.3.5 Estimation of total saponin content

Saponins produce inhibitory effect on inflammation. Saponins has the property of precipitating and coagulating red blood cells. Saponins are the heterogeneous group of active glycosides, they are characterized by their foam forming properties in aqueous solution. They are bitter in taste. Steroidal saponins are similar to body hormones which is the important source of production of steroidal hormones and drugs. Diosgenin is one of the steroidal saponin and its aglycone diosgenin have been used for the antitumor effect by cell cycle arrest and apoptosis.

The total saponin content of different solvent extracts of the leaf showed maximum amount in ethanol leaf extract (442±1.15 mg DE/gm) and the minimum amount in chloroform extract (161.78±1.35 mg DE/gm). The following order was found among the leaf, it was ethanol > ethyl acetate > petroleum ether > water > chloroform.

In stem bark extract, maximum amount of saponin found to be in ethanol extract (394±1.01 mg DE/gm) and the minimum amount was found in petroleum ether extract (143.56±1.23 mg DE/gm). The following order was found among the stem bark, it was ethanol > ethyl acetate > water > chloroform > petroleum ether. The standard calibration curve is done using diosgenin and they are significantly different at $p<0.05$ level. The results are represented in Table 13 and Figure-6.

Therefore, from the present quantitative analysis, among the different solvents used in the extraction of leaf and stem bark of *C. mala-elengi* elucidate that ethanol leaf extract has the highest amount of phenols, flavonoids, tannins, alkaloids and saponins compounds.

Table 13

Estimation of total saponin content of leaf and stem bark extract of *C. mala-elengi*

Extracts	Total saponins content (mg DE/gm)	
	Leaf	Stem bark
Petroleum ether	217.78±2.12^{c}	143.56±1.23^{e}
Chloroform	161.78±1.35^{e}	176.67±0.77^{d}
Ethyl acetate	369.78±1.46^{b}	297.56±0.59^{b}
Ethanol	442 ±1.15^{a}	394.0 ±1.01^{a}
Water	194.89±2.94^{d}	268.67±1.39^{c}
SED Cd (p<0.05)	0.0041 0.0091***	0.0021 0.0046***

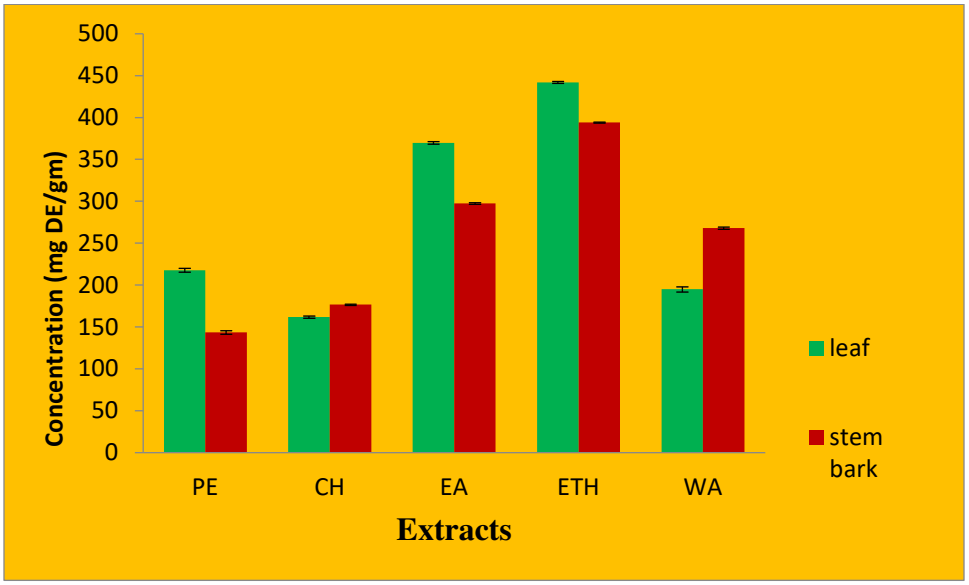
$^{\#}$Mean ± S.E

Mean in a column followed by a same letter (s) are not significantly (P > 0.05) different according to Duncan's Multiple Range Test

,*Significant at P < 0.01, P < 0.001 respectively; ns- non significant.

Figure 6

Estimation of total saponin content



PE-Petroleum ether, CH-Chloroform, EA-Ethyl acetate, ETH-Ethanol, WA-Water

Experiment No-4

In vitro pharmacological studies

Based on the results of qualitative and quantitative phytochemical analysis of leaf and stem bark extract of *C. mala-elengi* from petroleum ether, chloroform, ethyl acetate, ethanol and water extracts were used as antioxidant activities.

4.4 Antioxidant activity

The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Herbal plants considered as good antioxidant since ancient times. In view of the above, we designed the study to determine the antioxidant potential in leaf and stem bark of *C. mala-elengi*. Details of evaluation properties is given in Materials and Methods (3.4).

4.4.1 DPPH free radical scavenging activity

In the present study, five varying concentrations (10, 20, 30, 40 and 50 µg/ml) of different solvent (petroleum ether, chloroform, ethyl acetate, ethanol and water) of leaf extracts of *C. mala-elengi* proved different percentage of inhibition. The percentage of scavenging activity on the DPPH radical was concomitantly increased with the increase in the different range of 10-50 µg/ml concentration. The 50 µg/ml of leaf extracts showed significant antioxidant activity. The percentage of inhibition was increased from petroleum ether leaf extract (52.49±0.18%) to ethanol leaf extract (84.04±0.28%) at 50 µg/ml. 42.84±0.18% at 50 µg/ml for petroleum ether stem bark extract to 66.58±0.43% at 50 µg/ml for ethanol stem bark extract. The results are represented in Table 14 & 16.

Low IC_{50} value of the plant extract indicates the high range of antioxidant activity. The leaf extract of *C. mala-elengi* revealed the IC_{50} value of petroleum ether, chloroform, ethyl acetate, ethanol and water leaf extract found to be 48±0.58 µg/ml, 40.67±0.33 µg/ml, 30.33±0.88 µg/ml, 21±1.15 µg/ml and 43.67±1.76 µg/ml. Based on the IC_{50} value, the leaf extracts were effective in the order ethanol > ethyl acetate > chloroform > water > petroleum ether. IC_{50} value of petroleum ether, chloroform, ethyl acetate, ethanol and water

stem bark extract found to be 57.33±0.33 µg/ml, 38.33±0.88 µg/ml, 37±1 µg/ml, 33±1.73 µg/ml and 42±0.58 µg/ml. Based on the IC_{50} values, the stem bark extracts were effective in the order ethanol > ethyl acetate > chloroform > water > petroleum ether while in IC_{50} value of ascorbic acid is 18±1.15 µg/ml and they are significantly different at $p<0.05$ level. The results are represented in Table 15 & 17.

Table 14

DPPH free radical scavenging activity of *C. mala-elengi* leaf

Concentration (µg/ml)	Percentage of inhibition (%)					
	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Water	Ascorbic acid
10	14.01±0.20^{e}	23.77±0.30^{e}	24.94±0.18^{e}	40.61±0.23^{e}	21.37±0.15^{e}	48.83±0.16^{e}
20	23.48±0.18^{d}	24.80±0.31^{d}	40.53±0.23^{c}	48.63±0.21^{d}	31.05±0.10^{d}	57.40±0.08^{d}
30	32.95±0.17^{c}	42.11±0.20^{c}	52.72±0.28^{d}	60.29±0.43^{c}	35.61±0.15^{c}	66.46±0.31^{c}
40	40.03±0.25^{b}	52.43±0.41^{b}	58.83±0.11^{b}	66.17±0.18^{b}	47.98±0.23^{b}	74.94±0.18^{b}
50	52.49±0.18^{a}	57.51±0.21^{a}	66.46±0.23^{a}	84.04±0.28^{a}	59.01±0.48^{a}	78.16±0.67^{a}
SED	0.00229					
Cd ($p<0.05$)	0.00458***					

[#]Mean ± S.E

Mean in a column followed by a same letter (s) are not significantly ($P > 0.05$) different according to Duncan's Multiple Range Test

,*Significant at $P < 0.01$, $P < 0.001$ respectively; ns- non significant.

Table 15

IC_{50} values of DPPH free radical scavenging activity of *C. mala-elengi* leaf

IC_{50} (µg/ml)						
S.No	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Water	Ascorbicacid
1	48±0.58[a]	40.67±0.33[b]	30.33±0.88[c]	21±1.15[d]	43.67±1.76[b]	18±1.15[d]
SED Cd (p<0.05)	1.5275 3.3282***					

[#]Mean ± S.E

Mean in a column followed by a same letter (s) are not significantly (P > 0.05) different according to Duncan's Multiple Range Test

,*Significant at P < 0.01, P < 0.001 respectively; ns- non significant.

Table 16

DPPH free radical scavenging activity of *C. mala-elengi* stem bark

Concentration (µg/ml)	Stem bark (%)					
	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Water	Ascorbic acid
10	15.20±0.19[e]	22.60±0.46[e]	27.63±0.10[e]	36.78±0.38[e]	25.91±0.18[e]	48.83±0.16[e]
20	18.10±0.23[d]	27.87±0.31[d]	31.73±0.28[d]	40.76±0.13[d]	36.46±0.34[d]	57.40±0.08[d]
30	27.08±0.42[c]	39.71±0.24[c]	40.88±0.18[c]	49.88±0.39[c]	46.11±0.23[c]	66.46±0.31[c]
40	35.61±0.23$_b$	53.74±0.25[b]	53.74±0.20[b]	59.59±0.29[b]	48.65±0.15[b]	74.94±0.18[b]
50	42.84±0.18[a]	57.16±0.20[a]	58.39±0.18[a]	66.58±0.43[a]	53.13±0.21[a]	78.16±0.67[a]
SED Cd (p<0.05)	0.00390 0.00780***					

[#]Mean ± S.E

Mean in a column followed by a same letter (s) are not significantly (P > 0.05) different according to Duncan's Multiple Range Test

,*Significant at P < 0.01, P < 0.001 respectively; ns- non significant.

Table 17

IC_{50} values of DPPH free radical scavenging activity of *C. mala-elengi* stem bark

IC_{50} (µg/ml)						
S. No	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Water	Ascorbic acid
1	57.33±0.33[a]	38.33±0.88[c]	37±1[c]	33±1.73[d]	42±0.58[b]	18±1.15[e]
SED Cd ($p<0.05$)	1.4011 3.0527***					

[#]Mean ± S.E

Mean in a column followed by a same letter (s) are not significantly ($P > 0.05$) different according to Duncan's Multiple Range Test

,*Significant at $P < 0.01$, $P < 0.001$ respectively; ns- non significant.

4.4.2 Nitric oxide scavenging activity

In the present study, the percentage of Nitric oxide scavenging activity was performed with five solvents of leaf and stem bark extract of *C. mala-elengi* and ascorbic acid as standard. The activity was increased with the increase in the concentration of different solvents of both part extracts. The 50 µg/ml of leaf and stem bark extracts showed best antioxidant activity. The percentage of inhibition was increased from petroleum ether (41.88±0.17%) to ethanol (77.59±0.29%) at 50 µg/ml for leaf extract, 43.39±0.12% at 50 µg/ml for petroleum ether to 77.41±0.19% at 50 µg/ml for ethanol extract of stem bark. The results are represented in Table 18 & 20.

The IC_{50} values were calculated for leaf, stem bark extracts of *C. mala-elengi* and ascorbic acid. The IC_{50} values increased from ethanol (18.33±0.88 µg/ml) to petroleum ether (53.67±0.67 µg/ml) at 50 µg/ml for leaf extracts. Based on the IC_{50} values the leaf extracts were effective in the order ethanol > ethyl acetate > chloroform > water > petroleum ether. 27.67±0.33 µg/ml for ethanol increased to petroleum ether 58±0.58 µg/ml for stem bark extract. IC_{50} value of stem bark extracts were effective in the order ethanol

> water > ethyl acetate > chloroform > petroleum ether while in ascorbic acid it was found to be 10±0.58 µg/ml. It was confirmed that the ethanol extract showed highest antioxidant activity than the other extracts and they are significantly different at $p<0.05$ level. The results are represented in Table 19 & 21.

Table 18

Nitric oxide scavenging activity of *C. mala-elengi* leaf

Concentration (µg/ml)	Percentage of inhibition (%)					
	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Water	Ascorbic acid
10	21.98±0.09^{e}	37.83±0.12^{e}	45.66±0.12^{e}	40.16±0.09^{e}	37.91±0.07^{e}	50.48±0.17^{e}
20	22.65±0.14^{d}	45.84±0.19^{d}	51.42±0.30^{d}	52.99±0.07^{d}	38.78±0.29^{d}	53.60±0.12^{d}
30	30.63±0.09^{c}	55.29±0.15^{c}	54.52±0.21^{c}	61.59±0.14^{c}	45.74±0.14^{c}	57.54±0.18^{c}
40	37.72±0.19^{b}	61.53±0.17^{b}	62.51±0.05^{b}	71.19±0.17^{b}	50.37±0.24^{b}	63.20±0.14^{b}
50	41.88±0.17^{a}	74.76±0.21^{a}	69.55±0.14^{a}	77.59±0.29^{a}	53.60±0.14^{a}	74.95±0.16^{a}
SED	0.00124					
Cd ($p<0.05$)	0.00247***					

$^{\#}$Mean ± S.E

Mean in a column followed by a same letter (s) are not significantly ($P > 0.05$) different according to Duncan's Multiple Range Test

,*Significant at $P < 0.01$, $P < 0.001$ respectively; ns- non significant.

Table 19

IC_{50} values of Nitric oxide scavenging activity of *C. mala-elengi* leaf

IC_{50} (µg/ml)						
S.No	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Water	Ascorbic acid
1	53.67±0.67[a]	27.67±1.20[c]	19±0.58[d]	18.33±0.88[d]	40.67±0.33[b]	10±0.58[e]
SED	1.0715					
Cd (p<0.05)	2.3347**					

[#]Mean ± S.E

Mean in a column followed by a same letter (s) are not significantly (P > 0.05) different according to Duncan's Multiple Range Test

,*Significant at P < 0.01, P < 0.001 respectively; ns- non significant.

Table 20

Nitric oxide scavenging activity of *C. mala-elengi* stem bark

Concentration (µg/ml)	Percentage of inhibition (%)					
	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Water	Ascorbic acid
10	23.04±0.23[e]	41.75±0.21[e]	31.38±0.12[e]	37.83±0.12[e]	37.67±0.15[e]	50.48±0.17[e]
20	30.69±0.07[d]	42.38±0.09[d]	37.70±0.17[d]	45.79±0.09[d]	46.43±0.18[d]	53.60±0.12[d]
30	37.67±0.16[c]	45.66±0.17[c]	49.87±0.19[c]	55.19±0.19[c]	52.75±0.12[c]	57.54±0.18[c]
40	41.75±0.14[b]	55.29±0.15[b]	54.44±0.17[b]	68.65±0.14[b]	74.97±0.14[b]	63.20±0.14[b]
50	43.39±0.12[a]	58.31±0.12[a]	69.52±0.09[a]	77.35±0.15[a]	77.41±0.19[a]	74.95±0.16[a]
SED	0.00108					
Cd (p<0.05)	0.00216***					

[#]Mean ± S.E

Mean in a column followed by a same letter (s) are not significantly (P > 0.05) different according to Duncan's Multiple Range Test

,*Significant at P < 0.01, P < 0.001 respectively; ns- non significant.

Table 21

IC_{50} values of Nitric oxide scavenging activity of *C. mala-elengi* stem bark

IC_{50} (µg/ml)						
S.No	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Water	Ascorbic acid
1	58±0.58[a]	36.33±0.88[b]	30.67±0.67[c]	27.67±0.33[d]	28±1.15[d]	10±0.58[e]
SED	1.0541					
Cd(p<0.05)	2.2967***					

[#]Mean ± S.E

Mean in a column followed by a same letter (s) are not significantly (P > 0.05) different according to Duncan's Multiple Range Test

,*Significant at P < 0.01, P < 0.001 respectively; ns- non significant.

4.4.3 Hydrogen peroxide scavenging activity

The results showed that all the extracts has potent H_2O_2 scavenging activity which may be due to the antioxidant compounds. As the antioxidant components present in the extracts are good electron donors, they may accelerate the conversion of hydrogen peroxide.

In the present study, the percentage of Hydrogen peroxide scavenging activity was performed with five solvents of leaf and stem bark extract of *C. mala-elengi* and ascorbic acid as standard. The activity was increased with the increase in the concentration of different solvents of both part extracts. The percentage of inhibition was increased from petroleum ether (57.36±0.12%) to ethanol leaf extract (79.63±0.18%), 65.60±0.12% at 50 µg/ml for petroleum ether stem bark extract to 77.63±0.18% at 50 µg/ml for ethanol stem bark extract. The results are represented in Table 22 & 24.

The leaf extract of *C. mala-elengi* revealed the IC_{50} values of petroleum ether, chloroform, ethyl acetate, ethanol and water extracts were found to be 44.67±0.67 µg/ml, 31±0.58 µg/ml, 26±2.08 µg/ml, 17.33±0.33 µg/ml and 29±1.15 µg/ml respectively. Based

on the IC_{50} value, the leaf extracts were effective in the order ethanol > ethyl acetate > water > chloroform > petroleum ether. The results are represented in Table 23.

The stem bark extract of *C. mala-elengi* revealed the IC_{50} values of petroleum ether, chloroform, ethyl acetate, ethanol and water extract found to be 32.67±1.20 µg/ml, 28.67±0.67 µg/ml, 25±0.58 µg/ml, 18±1.15 µg/ml and 27.33±0.88 µg/ml. Based on the IC_{50} values, the stem bark extract were effective in the order ethanol > ethyl acetate > water > chloroform > petroleum ether, while in IC_{50} value of ascorbic acid is 16.67±0.88 µg/ml and they are significantly different at $p<0.05$ level. The results are represented in Table 25.

Table-22

Hydrogen peroxide scavenging activity of C. *mala-elengi* leaf

Concentration (µg/ml)	Percentage of inhibition (%)					
	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Water	Ascorbic acid
10	30.97±0.14^{e}	42.64±0.20^{e}	50.02±0.33^{e}	49.12±0.08^{e}	35.83±0.14^{e}	52.5±0.14^{e}
20	35.67±0.08^{d}	45.58±0.15^{d}	52.52±0.12^{d}	53.63±0.16^{d}	37.92±0.14^{d}	59.56±0.18^{d}
30	38.01±0.06^{c}	49.70±0.14^{c}	56.69±0.15^{c}	63.77±0.12^{c}	51.90±0.06^{c}	64.24±0.08^{c}
40	43.43±0.08^{b}	52.43±0.14^{b}	59.44±0.17^{b}	66.18±0.17^{b}	70.42±0.11^{b}	66.39±0.17^{b}
50	57.36±0.12^{a}	62.20±0.17^{a}	64.24±0.14^{a}	79.63±0.18^{a}	72.59±0.12^{a}	78.06±0.14^{a}
SED	0.00948					
Cd ($p<0.05$)	0.01896***					

$^{\#}$Mean ± S.E

Mean in a column followed by a same letter (s) are not significantly (P > 0.05) different according to Duncan's Multiple Range Test

,*Significant at P < 0.01, P < 0.001 respectively; ns- non significant.

Table 23

IC_{50} values of Hydrogen peroxide scavenging activity of *C. mala-elengi* leaf

IC_{50} (µg/ml)						
S.NO	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Water	Ascorbic acid
1	44.67±0.67[a]	31±0.58[b]	26±2.08[c]	17.33±0.33[d]	29±1.15[c]	16.67±0.88[e]
SED Cd (p<0.05)	1.5635 3.4065***					

[#]Mean ± S.E

Mean in a column followed by a same letter (s) are not significantly (P > 0.05) different according to Duncan's Multiple Range Test

,*Significant at P < 0.01, P < 0.001 respectively; ns- non significant.

Table 24

Hydrogen peroxide scavenging activity of *C. mala-elengi* stem bark

Concentration (µg/ml)	Percentage of inhibition (%)					
	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Water	Ascorbic acid
10	30.94±0.15[e]	40.76±0.11[e]	45.67±0.14[e]	45.58±0.08[e]	39.28±0.08[e]	52.5±0.14[e]
20	45.63±0.18[d]	47.62±0.17[d]	47.52±0.14[d]	52.63±0.04[d]	45.65±0.06[d]	59.56±0.18[d]
30	47.01±0.08[c]	52.48±0.10[c]	52.66±0.10[c]	60.85±0.12[c]	59.33±0.13[c]	64.24±0.08[c]
40	64.17±0.11[b]	62.45±0.10[b]	62.99±0.21[b]	65.90±0.33[b]	73.33±0.39[b]	66.39±0.17[b]
50	65.60±0.12[a]	69.75±0.13[a]	66.41±0.20[a]	77.63±0.18[a]	72.54±0.18[a]	78.06±0.14[a]
SED Cd (p<0.05)	0.00131 0.00262***					

[#]Mean ± S.E

Mean in a column followed by a same letter (s) are not significantly (P > 0.05) different according to Duncan's Multiple Range Test

,*Significant at P < 0.01, P < 0.001 respectively; ns- non significant.

Table 25

IC_{50} values of Hydrogen peroxide scavenging activity of *C. mala-elengi* stem bark

IC_{50} (µg/ml)						
S.NO	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Water	Ascorbic acid
1	32.67±1.20[a]	28.67±0.67[b]	25±0.58[c]	18±1.15[d]	27.33±0.88[b]	16.67±0.88[d]
SED	1.3053					
Cd (p<0.05)	2.8439***					

[#]Mean ± S.E

Mean in a column followed by a same letter (s) are not significantly (P > 0.05) different according to Duncan's Multiple Range Test

,*Significant at P < 0.01, P < 0.001 respectively; ns- non significant.

4.4.4 Superoxide radical scavenging activity

Super oxide free radical scavenging activity was performed with five different solvents and ascorbic acid (standard). In the present study, five varying concentrations (10, 20, 30, 40 and 50 µg/ml) of different solvents (petroleum ether, chloroform, ethyl acetate, ethanol and water) of leaf and stem bark extracts of *C. mala-elengi* proved different percentage of inhibition. The percentage of scavenging activity on the super oxide radical was simultaneously increased with the increase in the different range of 10-50 µg/ml concentration. The 50 µg/ml of leaf and stem bark extracts showed best antioxidant activity. The percentage of inhibition was increased from petroleum ether leaf extract (49.58±0.30%) to ethanol leaf extract (85.56±0.13%). 60.57±0.07% at 50 µg/ml for chloroform stem bark to 83.16±0.07% at 50 µg/ml for ethanol stem bark extract. The results are represented in Table 26 & 28.

The IC_{50} was measured for different solvents and standard. The IC_{50} value of petroleum ether, chloroform, ethyl acetate, ethanol and water leaf extracts were found to be 52.33±0.88 µg/ml, 30.67±1.76 µg/ml, 24.67±0.76 µg/ml, 15.67±1.20 µg/ml and 35±1.15 µg/ml. Based on the IC_{50} value, the leaf extracts were effective in the order ethanol leaf extract > ethyl acetate leaf extract > chloroform leaf extract > water leaf extract > petroleum ether leaf extract.

IC_{50} value of petroleum ether, chloroform, ethyl acetate, ethanol and water stem bark extract found to be 34±0.58 µg/ml, 31.67±1.20 µg/ml, 29.33±0.88 µg/ml, 27±0.58 µg/ml and 32.33±0.33 µg/ml. Based on the IC_{50} value the stem bark extracts were effective in the order ethanol stem bark extract > ethyl acetate stem bark extract > chloroform stem bark extract > water stem bark extract > petroleum ether stem bark extract while in IC_{50} value of Ascorbic acid is 9.67±0.67 µg/ml and they are significantly different at $p<0.05$ level. The results are represented in Table 27 & 29.

It was observed that as concentration increases, the percentage of scavenging also increases for all solvents, revealed by the regression analysis.

Table 26

Superoxide radical scavenging activity of *C. mala-elengi* leaf

Concentration (µg/ml)	Percentage of inhibitions (%)					
	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Water	Ascorbic acid
10	27.14±0.16^{e}	35.33±0.11^{e}	42.02±0.30^{e}	56.59±0.15^{e}	35.51±0.19^{e}	56.63±0.16^{e}
20	35.14±0.15^{d}	41.90±0.18^{d}	49.11±0.11^{d}	60.44±0.13^{d}	41.73±0.09^{d}	60.62±0.09^{d}
30	36.89±0.17^{c}	50.05±0.14^{c}	56.74±0.19^{c}	64.91±0.16^{c}	46.35±0.31^{c}	64.12±0.18^{c}
40	46.62±0.37^{b}	58.37±0.20^{b}	64.79±0.13^{b}	68.59±0.15^{b}	58.20±0.11^{b}	71.48±0.15^{b}
50	49.58±0.30^{a}	64.00±0.15^{a}	68.67±0.19^{a}	85.56±0.13^{a}	61.04±0.26^{a}	80.10±0.09^{a}
SED	0.00156					
Cd ($p<0.05$)	0.00311***					

[#]Mean ± S.E

Mean in a column followed by a same letter (s) are not significantly ($P > 0.05$) different according to Duncan's Multiple Range Test

,*Significant at $P < 0.01$, $P < 0.001$ respectively; ns- non significant.

Table 27

IC_{50} value of Superoxide radical scavenging activity of *C. mala-elengi* leaf

IC_{50} (µg/ml)						
S. NO	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Water	Ascorbic acid
1	52.33±0.88[a]	30.67±1.76[c]	24.67±0.76[d]	15.67±1.20[e]	35±1.15[b]	9.67±0.67[f]
SED Cd (p<0.05)	1.8459 4.0219***					

[#]Mean ± S.E

Mean in a column followed by a same letter (s) are not significantly (P > 0.05) different according to Duncan's Multiple Range Test

,*Significant at P < 0.01, P < 0.001 respectively; ns- non significant.

Table 28

Superoxide radical scavenging activity of *C. mala-elengi* stem bark

Concentration (µg/ml)	Percentage of inhibition (%)					
	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Water	Ascorbic acid
10	41.93±0.19[e]	35.28±0.09[e]	33.78±0.13[c]	42.02±0.14[e]	56.63±0.16[e]	56.63±0.16[e]
20	45.78±0.38[d]	41.83±0.15[d]	42.00±0.09[d]	64.96±0.09[d]	60.62±0.09[d]	60.62±0.09[d]
30	52.47±0.13[c]	49.51±0.32[c]	51.70±0.04[c]	72.86±0.16[c]	64.12±0.18[c]	64.12±0.18[c]
40	56.72±0.18[b]	57.48±0.15[b]	56.69±0.17[b]	80.81±0.23[b]	71.48±0.15[b]	71.48±0.15[b]
50	62.10±0.05[a]	60.57±0.07[a]	65.48±0.15[a]	83.16±0.07[a]	75.23±0.15[a]	80.10±0.09[a]
SED Cd (p<0.05)	0.08537 0.17076***					

[#]Mean ± S.E

Mean in a column followed by a same letter (s) are not significantly (P > 0.05) different according to Duncan's Multiple Range Test

,*Significant at P < 0.01, P < 0.001 respectively; ns- non significant.

Table 29

IC_{50} values of Superoxide radical scavenging activity of *C. mala-elengi* stem bark

IC_{50} (µg/ml)						
S.No	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Water	Ascorbic acid
1	34±0.58[a]	31.67±1.20[b]	29.33±0.88[c]	27±0.58[c]	32.33±0.33[b]	9.67±0.67[d]
SED	1.0715					
Cd (p<0.05)	2.3347***					

[#]Mean ± S.E

Mean in a column followed by a same letter (s) are not significantly (P > 0.05) different according to Duncan's Multiple Range Test

,*Significant at P < 0.01, P < 0.001 respectively; ns- non significant.

4.4.5 Total antioxidant capacity

The total antioxidant activity is based on the reduction of Phosphomolybdate ion in the presence of an antioxidant resulting in the formation of a green phosphate complex which is measured spectrophotometrically.

In the present study, the percentage of total antioxidant activity was performed with five solvents of leaf and stem bark extract of *C. mala-elengi* and ascorbic acid as standard. The activity was increased with the increase in the concentration of different solvents of leaf and stem bark extracts. The percentage of inhibition was increased from chloroform extract (63.02±0.19%) to ethanol (79.40±0.19%) for leaf extract at 50 µg/ml, 55.18±0.17% at 50 µg/ml for chloroform stem bark extract to 74.27±0.39% at 50 µg/ml for ethyl acetate stem bark extract. The results were represented in Table 30 and 32. The antioxidant activity of ascorbic acid, a known antioxidant used as the positive control, was comparatively effective than that of *C. mala-elengi* compounds.

The leaf extracts of *C. mala-elengi* revealed the IC_{50} value of petroleum ether, chloroform, ethyl acetate, ethanol and water leaf extract was found to be 31±0.58 µg/ml, 32.33±0.88 µg/ml, 20±1.15 µg/ml, 18.67±0.67 µg/ml and 30.33±2.03 µg/ml. Based on the

IC_{50} value, the leaf extracts were effective in the order ethanol > ethyl acetate > water > petroleum ether > chloroform. The results are represented in Table 31.

In stem bark extract of *C. mala-elengi*, IC_{50} value of petroleum ether , chloroform, ethyl acetate, ethanol and water stem bark extract found to be 42±1.73 µg/ml, 41±0.58 µg/ml, 27.33±0.88 µg/ml, 23.67±1.20 µg/ml and 24.67±0.67 µg/ml. Based on the IC_{50} value, the stem bark extracts were effective in the order ethanol > water > ethyl acetate > chloroform > petroleum ether stem bark, while in IC_{50} value of ascorbic acid 8.67±0.67 µg/ml and they are significantly different at $p<0.05$ level. The results are represented in Table 33.

Table 30

Total antioxidant capacity of *C. mala-elengi* leaf

Concentration (µg/ml)	Percentage of inhibition (%)					
	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Water	Ascorbic acid
10	31.61±0.16^{e}	38.72±0.19^{e}	47.29±0.18^{e}	47.32±0.19^{e}	41.88±0.16^{e}	67.37±0.11^{e}
20	38.62±0.18^{d}	42.92±0.41^{d}	50.83±0.33^{d}	59.38±.0.30^{d}	46.69±0.11^{d}	69.14±0.14^{d}
30	47.19±0.25^{c}	47.58±0.09^{c}	58.46±0.14^{c}	64.01±0.11^{c}	49.58±0.14^{c}	77.14±0.14^{c}
40	55.89±0.18^{b}	55.96±0.17^{b}	63.72±0.19^{b}	70.02±0.14^{b}	53.59±0.16^{b}	85.08±0.34^{b}
50	73.07±0.16^{a}	63.02±0.19^{a}	69.32±0.21^{a}	79.40±0.19^{a}	66.72±0.14^{a}	88.75±0.16^{a}
SED	0.00146					
Cd ($p<0.05$)	0.00292***					

[#]Mean ± S.E

Mean in a column followed by a same letter (s) are not significantly (P > 0.05) different according to Duncan's Multiple Range Test

,*Significant at P < 0.01, P < 0.001 respectively; ns- non significant.

Table 31

IC_{50} values of Total antioxidant capacity of *C. mala-elengi* leaf

IC_{50} (µg/ml)						
S.NO	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Water	Ascorbic acid
1	31±0.58[a]	32.33±0.88[a]	20±1.15[b]	18.67±0.67[b]	30.33±2.03[b]	8.67±0.67[d]
SED	1.4657					
Cd (p<0.05)	3.1934***					

[#]Mean ± S.E

Mean in a column followed by a same letter (s) are not significantly (P > 0.05) different according to Duncan's Multiple Range Test

,*Significant at P < 0.01, P < 0.001 respectively; ns- non significant.

Table 32

Total antioxidant capacity of *C. mala-elengi* stem bark

Concentration (µg/ml)	Percentage of inhibition (%)					
	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Water	Ascorbic acid
10	22.27±0.09[e]	31.09±0.18[e]	46.82±0.35[e]	38.98±0.25[e]	38.98±0.11[e]	67.37±0.11[e]
20	30.94±0.12[d]	38.72±0.11[d]	54.61±0.09[d]	47.32±0.19[d]	46.38±0.20[d]	69.14±0.14[d]
30	39.01±0.35[c]	42.76±0.28[c]	59.71±0.16[c]	57.5±0.16[c]	53.62±0.14[c]	77.14±0.14[c]
40	47.32±0.19[b]	47.34±0.12[b]	60.75±0.20[b]	59.38±0.40[b]	59.84±0.12[b]	85.08±0.34[b]
50	57.71±0.38[a]	55.18±0.17[a]	63.85±0.39[a]	74.27±0.39[a]	66.04±0.18[a]	88.75±0.16[a]
SED	0.00162					
Cd (p<0.05)	0.00324***					

[#]Mean ± S.E

Mean in a column followed by a same letter (s) are not significantly (P > 0.05) different according to Duncan's Multiple Range Test

,*Significant at P < 0.01, P < 0.001 respectively; ns- non significant.

Table 33

IC_{50} value of Total antioxidant capacity of *C. mala-elengi* stem bark

S.NO	IC_{50} ((µg/ml)					
1	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Water	Ascorbic acid
	42±1.73[a]	41±0.58[a]	27.33±0.88[b]	23.67±1.20[c]	24.67±0.67[b]	8.67±0.67[d]
SED Cd (p<0.05)	1.4657 3.1934***					

[#]Mean ± S.E

Mean in a column followed by a same letter (s) are not significantly (P > 0.05) different according to Duncan's Multiple Range Test

,*Significant at P < 0.01, P < 0.001 respectively; ns- non significant.

4.4.2.1 Antimicrobial activity

Plant parts have been used as herbal medicine for their healing properties since ancient times. Some bioactive compounds within these plants are responsible for their medicinal value. The most prominent of these bioactive compounds are alkaloid, tannin, flavonoid and phenolic compound. Their concentrations may vary in different plants which results in unique medicinal properties for a specific plant. During the last decades, the global interest in the study of various medicinal plants has increased rapidly due to their antibacterial activities, low toxicity and the potential to be a cheaper alternative to costly synthetic drugs. The determination of antibacterial activities of different medicinal plants is of special interest these days due to the current global issue of increasing antibiotic resistance of microorganisms. It is assumed that the drug resistance in pathogenic microorganisms is developing due to indiscriminate use of commercial antimicrobial drugs. Antimicrobial resistance threatens the prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi. Therefore, it is highly imperative to determine compounds which can be used to develop novel medicines with higher antimicrobial properties.

Therefore, the antimicrobial activity of extracts of leaf and stem bark of *C. mala-elengi* was tested against belong three gram-positive bacteria such as *Bacillus cereus*, *Bacillus subtilis* and *Staphylococcus aureus*, two gram negative bacteria such as *Citrobacter freundii* and *Klebsiella pneumoniae*. Antifungal activity was taken up against three fungal pathogens *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans*. The extracts such as Petroleum ether, Chloroform, Ethyl acetate, Ethanol and Water were used in both the plant parts and tested for antimicrobial activity.

Ethanol leaf extract of *C. mala-elengi* showed highest zone of inhibition against all the tested microorganism with *Bacillus cereus* (18.0±1.53 mm), *Staphylococcus aureus* (17.33±0.98 mm), *Citrobacter freundii* (17.0±1.15 mm), *Klebsiella pneumoniae* (16.33±1.86 mm), *Aspergillus niger* (30.0±1.15 mm), *Aspergillus flavus* (14.33±0.33 mm), *Candida albicans* (15±1.73 mm) except *Bacillus subtilis* which showed highest zone of inhibition in water extract (20.33±1.20 mm).

Ethanolic stem bark extract of *C. mala-elengi* showed maximum inhibition with *Bacillus cereus* (16.33±0.88 mm), *Klebsiella Pneumoniae* (15.0±0.58 mm), *Aspergillus flavus* (14.33±0.88 mm), *Candida albicans* (12.0±0.58 mm), water extract showed maximum inhibition with *Bacillus subtilis* (20±1.15 mm), *Aspergillus niger* (27±0.88 mm). Ethyl acetate extract showed maximum inhibion *Citrobacter freundii* (16.33±0.88 mm), *Staphylococcus aureus* (18.33±0.33 mm) and they are significantly different at $p<0.05$ level. The results are represented in Table 34, 35 & (Plate-7, 8).

Table 34

Antimicrobial activity of leaf extracts of *C. mala-elengi* against different bacterial and fungal species

(Zone of inhibition in mm)

S. No	Type of the Pathogen	Name of the Pathogen	Negative control	Positive control	Leaf				
					Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Water
1	Gram Positive bacteria	*Bacillus subtilis*	NIL	36.33±2.19[a]	15.33±2.40[b]	18.33±0.88[a]	9.33±0.33[e]	10.33±0.88h	**20.33±1.20[b]**
		Bacillus cereus	NIL	25.33±1.86[c]	12± 0.58[d]	11.33±0.33[b]	12.67±0.67[d]	**18.0±1.53[b]**	11±0.58[g]
		Staphylococcus aureus	NIL	17.67±0.88[d]	NIL	NIL	12.33±0.67[d]	**17.33±0.98[c]**	16±1.15[d]
2	Gram negative bacteria	*Citrobacter freundii*	NIL	26.33±1.45[b]	13.33±3.38[c]	12±1.15[d]	15.33±3.18[b]	**17.0±1.15[d]**	16.33±3.48[c]
		Klebsiella pneumoniae	NIL	23.33±0.67	12±1.15[d]	10.33±0.67[c]	13.67±0.33[c]	**16.33±1.86[e]**	15.33±2.10[e]
3	Fungi	*Aspergillus niger*	NIL	19±1[f]	16±0.58[a]	NIL	21.33±2.03[a]	**30.0±1.15[a]**	21.67±1.86[a]
		Aspergillus flavus	NIL	17±0.58[d]	7±0.58[e]	Nil	13±1.15[c]	**14.33±0.33[g]**	11±3.67[h]
		Candida albicans	NIL	14±1.15[e]	NIL	NIL	NIL	**15.0±1.73[f]**	13.67±0.88[f]
		SED Cd (p<0.05)	0.80123 1.59044***						

[#]Mean ± S.E

Mean in a column followed by a same letter (s) are not significantly (P > 0.05) different according to Duncan's Multiple Range Test

,*Significant at P < 0.01, P < 0.001 respectively; ns- non significant.

Table 35

Antimicrobial activity of stem bark extract of *C. mala-elengi* against different bacterial and fungal species

(Zone of inhibition in mm)

S. No	Type of the Pathogen	Name of the Pathogen	Negative control	Positive control	Stem bark				
					Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Water
1	Gram Positive bacteria	*Bacillus subtilis*	NIL	36.33±2.19^{a}	Nil	8.33±1.45^{f}	10.67±0.88^{f}	10.33±0.33^{g}	**20±1.15^{b}**
		Bacillus cereus	NIL	25.33±1.86^{c}	13.67± 1.33^{a}	12.67±0.88^{c}	15±1.53^{d}	**16.33±0.88^{b}**	12.33±0.88^{e}
		Staphylococcus aureus	NIL	17.67±0.88^{f}	NIL	14±0.58^{b}	**18.33±0.33^{b}**	17.33±0.88^{a}	15±0.58^{c}
2	Gram negative bacteria	*Citrobacter freundii*	NIL	26.33±1.45^{b}	12.67±1.20^{c}	12.33±0.88^{d}	**16.33±0.88^{c}**	15±1.15^{d}	13±0.58^{d}
		Klebsiella pneumoniae	NIL	23.33±0.67^{d}	12.33±1.76^{d}	11.33±0.33^{e}	14±1.15^{e}	**15±0.58^{d}**	11±1.15^{f}
3	Fungi	*Aspergillus niger*	NIL	19±1^{e}	13.33±0.67^{b}	21±1.52^{a}	26.67±1.86^{a}	16±0.58^{c}	**27±0.88^{a}**
		Aspergillus flavus	NIL	17±0.58^{g}	12.33±0.88^{d}	NIL	8±0.58^{h}	**14.33±0.88^{e}**	10.33±0.88^{g}
		Candida albicans	NIL	14±1.15^{h}	5±0.58^{e}	NIL	9±1.15^{g}	**12±0.58^{f}**	11±0.58^{f}
		SED Cd (p<0.05)		0.58267 1.15659**					

[#]Mean ± S.E

Mean in a column followed by a same letter (s) are not significantly (P > 0.05) different according to Duncan's Multiple Range Test

,*Significant at P < 0.01, P < 0.001 respectively; ns- non significant.

Experiment No - 5

Based on the results of the qualitative and quantitative phytochemical analysis, the antioxidant and antimicrobial activities of leaf and stem bark of *C. mala-elengi* were selected as suitable for further study. The ethanol extract was tested for their FTIR, GC-MS and *In vitro* antidiabetic and cytotoxic activities.

4.5.1 FT-IR analysis

FT-IR (Fourier Transform Infrared spectroscopy) is a most powerful analytical tool to provide a snapshot of the metabolic composition of a tissue at a given time. It can be employed to determine the type of chemical bonds present in compounds.

To detect the chemical constituents and elucidate the structure of the compounds of leaf and stem bark of *C. mala-elengi*, this analysis was carried out. Experimental details are given in Materials and Methods (3.5.1).

The FTIR analysis of ethanol leaf extract of *C. mala-elengi* revealed the presence of H bonded alcohol, alkanes, phenols, alkene, alkyl halide, nitro compound, alcohol, carboxylic acids, ethers, esters, amines compounds which revealed major peaks at (3849.92, 3294.42, 2962.66, 2895.15, 2540.25, 2343.51, 1672.28, 1408.04, 1311.59, 1056.99, 871.82, 704.02, 684.73). The most probable absorption bands are recorded in Table 36 & Figure 7.

The FTIR analysis of ethanol stem bark extract of *C.mala-elengi* showed the presence of H bonded alcohol, alkanes, H bonded carboxylic acid, phenol, transition metal carbonyl, alkene, alkyl halide, nitro compound, alcohol, carboxylic acid, ethers, ester compounds which showed major peaks at 3847.99, 3311.78, 3288.63, 2964.69, 2891.30, 2534.46, 2347.37, 1917.24, 1668.43, 1409.96, 1309.67, 1056.99, 854.47, 707.88. The results of FT-IR peak values and functional groups are represented in Table 37 & Figure 8.

Table 36

FT-IR peak values and its functional groups ethanol leaf extract of *C. mala-elengi*

S.NO	Standard (mm)	Bond	Wave number (mm)	Functional group
1	3500-4000	O-H stretching of α cellulose	3849.92	Alcohol, Phenols
2	3200-3600	O-H Stretch	3294.42	H bonded alcohol
3	2850-2970	C-H Stretch	2962.66	Alkanes
4	2970-2850	C-H Stretch	2895.15	Alkanes
5	2400-3400	O-H Stretch	2540.25	Alkanes
6	2350-2300	N-H Stretching of hemicelluloses	2343.51	Phenols
7	1600-1680	C=C stretch	1672.28	Alkenes
8	1400-1000	C-F stretch	1408.04	Alkyl halide
9	1300-1370	N-O asymmetric stretch	1311.59	Nitro compound
10	1000-1320	C-O Stretch	1056.99	Alcohols, Carboxylic acids, ethers, esters.
11	910-665	N-H Stretch	871.82, 704.02, 684.73	Amines

Figure-7

FTIR spectrum ethanolic extract of *C. mala-elengi* leaf

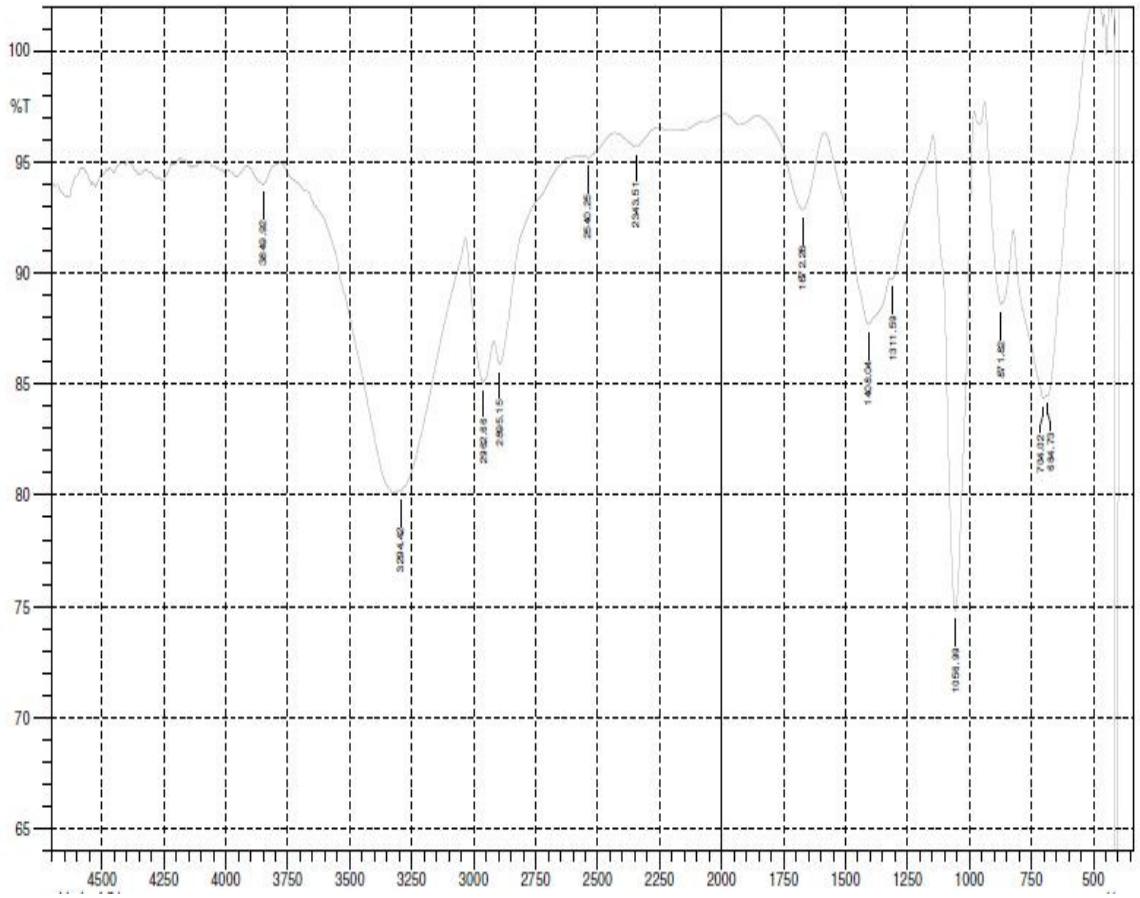


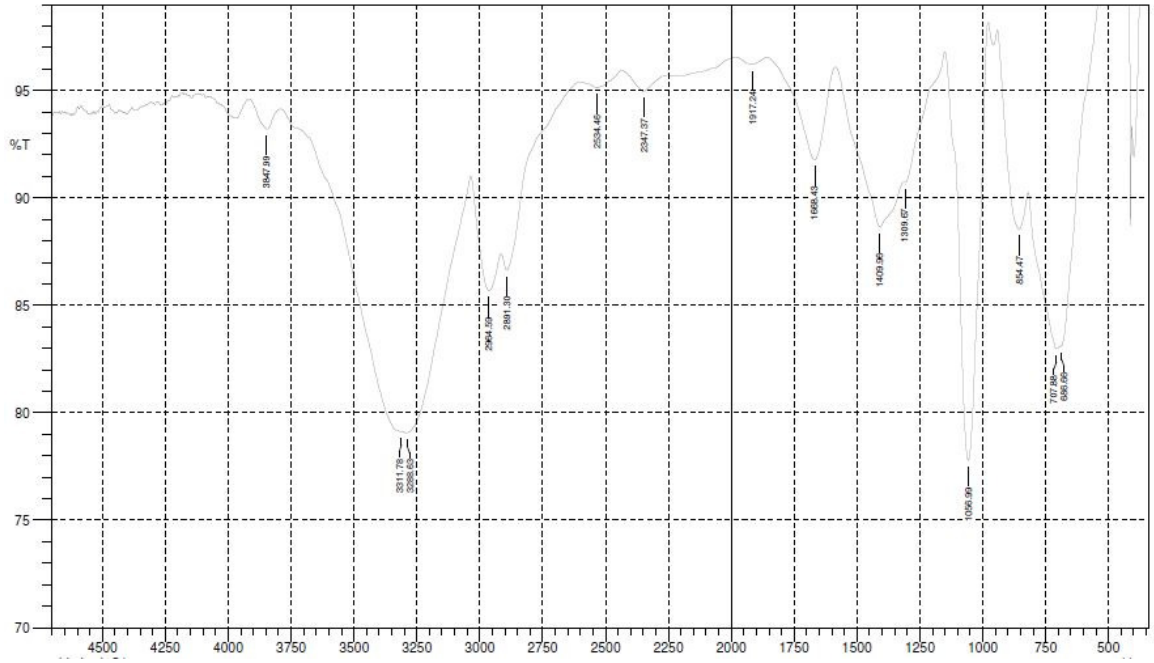
Table 37

FT-IR peak values and its functional groups of ethanolic stem bark extract of *C.mala-elengi*

S.NO	Standard (mm)	Bond	Wave number (mm)	Functional group
1	3500-4000	O-H stretching of α cellulose	3847.99	Alcohols, Phenols
2	3200-3600	O-H stretch	3311.78	H bonded alcohol
3	3200-3600	O-H stretch	3288.63	H bonded alcohol
4	2850-2970	C-H stretch	2964.69	Alkanes
5	2850-2970	C-H stretch	2891.30	Alkanes
6	2400-3400	O-H stretch	2534.46	H bonded carboxylic acid
7	2300-2350	N-H stretching hemicelluloses	2347.37	Phenols
8	2100-1800	Carbonyl compound frequency	1917.24	Transition metal carbonyls
9	1600-1680	C=C stretch	1668.43	Alkenes
10	1400-1000	C-F Stretch	1409.96	Alkyl halides
11	1300-1370	N-O asymmetric stretch	1309.67	Nitro compound
12	1000-1320	C-O stretch	1056.99	Alcohols, carboxylic acids ethers, esters.
13	650-1000	C-H bend	854.47	alkenes
14	650-1000	C-H bend	707.88	Alkenes

Figure-8

FTIR spectrum ethanolic extract of *C. mala-elengi* stem bark



4.5.2 GC-MS analysis of ethanol leaf and stem bark of *C. mala-elengi*

Gas chromatography Mass Spectrometry (GC-MS) is a method that combines the features of gas liquid chromatography and mass spectrometry to identify different substances with in a test sample. GC-MS method proved to be very effective and sensible for the separation and detection of complex mixture of phytochemicals. GC MS can provide meaningful information for components present in ethanol leaf and stem bark extracts of *C. mala-elengi.* Details of methodology are given in Materials and Methods (3.5.2).

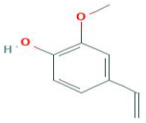
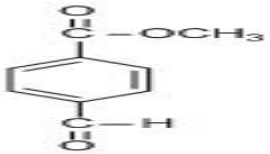
The GC-MS analysis of ethanol leaf and stem bark extract of *C. mala-elengi* showed the presence of phytochemical constituents that could be responsible for the various curative property of the plant. The identification of the bioactive constituents was confirmed based on the peak area, retention time, molecular formula of the active principles with their retention time, name of the compound, molecular weight and peak area in percentage and structure of the compound.

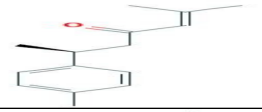
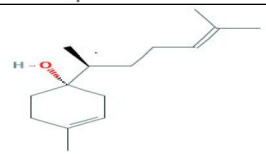
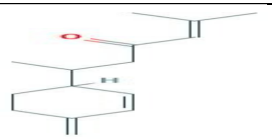
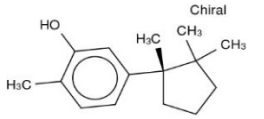
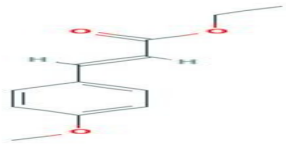
The ethanol leaf extract of *C. mala-elengi* showed the presence of 17 different bioactive compounds. The first compound detected with less retention time of 10.291 min and peak area of 6.90% was p-vinylguaiacol compound and phytol compound was detected with the retension time of 22.309 min and peak area of 8.50%. The GC MS peak values of ethanol leaf extract of *C. mala-elengi* is shown in Table 38 & 39 (Figure - 9 & 10).

The ethanol stem bark extract of *C. mala-elengi* showed the presence of 16 different compounds, the first compound detected with less retension time of 11.336 min and peak area of 1.59% was 2-(4'-methoxy phenyl)-2-(2'-methoxyphenyl) propane. The last compound detected with the retension time of 22.623 min and peak area of 2.71% was phenol, 4-(3,7 – dimethyl-3-ethenylocta-1,6-dienyl)-. The GC MS peak values of ethanol stem bark extract of *C. mala-elengi* is shown in Table 40, 41, (Figure – 11, 12).

Table 38

GC-MS analysis of ethanolic leaf extract of *C. mala-elengi*

S.No	Retention time (minutes)	Name of the compound	Molecular formula	Molecular weight (g/mol)	Peak area %	Compound Structure
1	10.291	p-vinylguaiacol	$C_9H_{10}O_2$	150.17	6.90	
2	11.133	Methylterephthalaldehydate	$C_9H_8O_3$	164.16	1.76	
3	11.327	Phosphoric acid,bis(tri methylsilyl)monomethyl ester	$C_7H_{21}O_4PSi_2$	256.38	6.38	
4	12.333	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	1.54	
5	13.845	Beta-Elemenone	$C_{15}H_{12}O$	218.33	4.23	
6	13.917	B-Asarone	$C_{12}H_{16}O_3$	208.25	1.98	

S.No	Retention time (minutes)	Name of the compound	Molecular formula	Molecular weight (g/mol)	Peak area %	Compound Structure
7	14.577	Ar-tumerone	$C_{15}H_{20}O$	216.32	5.43	
8	14.650	Beta-bisabolol	$C_{15}H_{26}O$	222.37	2.84	
9	15.023	Curlone	$C_{15}H_{22}O$	218.33	1.91	
10	15.589	Cuparophenol	$C_{15}H_{22}O$	202.34	19.26	
11	15.768	Ethyl p-methoxycinnamate	$C_{12}H_{14}O_3$	206.24	13.92	
12	15.970	2H-Benzocyclohepten-2-one,3,4,4A,5,6,7,8,9-octahydro-	$C_{12}H_{18}O$	178.27	7.45	

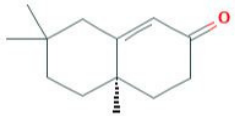
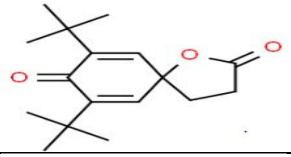
S.No	Retention time (minutes)	Name of the compound	Molecular formula	Molecular weight (g/mol)	Peak area %	Compound Structure
13	16.087	2(3H)-Naphthalenone,4,4A,5,6,7,8-Hexahydro-4A-Methyl-	$C_{11}H_{16}O$	164.244	7.05	
14	16.208	2H-Benzocyclohepten-2-one,3,4,4A,5,6,7,8,9-octahydro-	$C_{12}H_{18}O$	178.27	1.62	
15	17.856	7,9-Di-tert-butyl-1-Oxaspiro(4,5)deca-6,9- diene-2,8-dione	$C_{17}H_{24}O_3$	276	1.54	
16	19.066	Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	7.70	
17	22.309	Phytol	$C_{20}H_{40}O$	296.5	8.50	

Table 39

Bioactivity of the compounds identified in the ethanolic leaf extract of *C. mala-elengi* by GC-MS

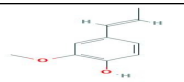
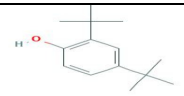
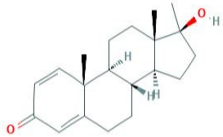
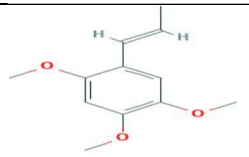
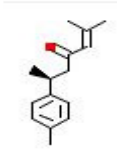
S. No	Retention time (minutes)	Name of the compound	Nature of compound	Activity
1.	10.291	p-vinylguaiacol	Phenolic compound	Antioxidant, antimicrobial and anti inflammatory
2.	11.133	Methylterephthalaldehydate	Unknown	Unknown
3.	11.327	Phosphoric acid, bis(tri methylsilyl)mono methyl ester	Unknown	Unknown
4.	12.333	Methyl tetradecanoate	Fatty acid methyl ester	Unknown
5.	13.845	Beta-Elemenone	Sesquiterpenoids	Unknown
6.	13.917	B-Asarone	Phenyl propanoid	Antidepressant, anti anxiety, anti-alzheimer's, anti parkinsons, antiepileptic, anticancer activity and anti hyperlepidemic, antithrombotic, anti cholestatic and radioprotective activity
7.	14.577	Ar-tumerone	Sesquiterpenoids	Antiangiogenic effect, antiplatelet activity, antileishmanial activity, treating neurogenerative disease, stroke, anti tumor, apoptosis, inhibition of

S. No	Retention time (minutes)	Name of the compound	Nature of compound	Activity
				tumor cell invasion, anti-inflammatory, parkinsion, antidermatophytic activity, alzheimer's disease. anti-angiogenic effects, antiplatelet activity, Cytotoxic activity to HL-60, K-562, L-1210, Hela, U-937 and RBC-2H_3 cells.
8	14.650	Beta-bisabolol	monocyclic sesquiterpene alcohol	Unknown
9	15.023	Curlone	Sesquiterpenoid	Unknown
10	15.589	Cuparophenol	Sesquiterpene	Antimicrobial activity
11	15.768	Ethyl p-methoxycinnamate	Aromatic ester	Anti inflammatory, analgesic activity and treating angiogenesis related disease
12	15.970	2H-Benzocyclohepten-2-one,3,4,4A,5,6,7,8,9-octahydro-	Unknown	Unknown
13	16.087	2(3H)-Naphthalenone,4,4A,5,6,7,8-Hexahydro-4A-Methyl-	Unknown	Anti inflammatory activity
14	16.208	2H-Benzocyclohepten-2-one,3,4,4A,5,6,7,8,9-octahydro-	Unknown	Unknown

S. No	Retention time (minutes)	Name of the compound	Nature of compound	Activity
15	17.856	7,9-Di-tert-butyl-1-Oxaspiro(4,5)deca-6,9- diene-2,8-dione	Oxaspiro compound	Antioxidant
16	19.066	Hexadecanoic acid	Palmitic acid ester	Antioxidant, antiandrogenic and hypocholesterolemic
17	22.309	Phytol	Acyclic Diterpene alcohol	Anxiolytic effect, a precursor of synthetic vitamin A, E and K. It was proven to be cytotoxic against breast cancer cell lines (MCF7), antimicrobial, anti-inflammatory, anticancer, diuretic, resistant gonorrhoea, joint dislocation, headache, hernia, antimalarial, anticonvulsant, antispasmodic, antinociceptive and antioxidant

Table 40

GC-MS analysis of ethanolic stem bark extract of *C. mala-elengi*

S.No	Retention time (minutes)	Name of the compound	Molecular formula	Molecular weight (g/mol)	Peak area%	Compound structure
1	11.336	2-4'-methoxyphenyl)-2-(2'-methoxyphenyl)propane	Unknown	Unkown	1.59	Unknown
2	12.127	(Z)-Isoeugenol	$C_{10}H_{12}O_2$	164.204	5.01	
3	12.617	1-Phenylethylene glycol	$C_8H_{10}O_2$	138.16	1.91	
4	12.681	2,4-Ditert-butylphenol	$C_{10}H_{12}O_2$	206.32	2.62	
5	13.855	1,2-Dehydro-17-methyl testosterone	$C_{20}H_{28}O_2$	300.4	3.55	
6	13.928	Alpha-asarone	$C_{10}H_{12}O_2$	208.25	5.05	
7	14.587	Ar-tumerone	$C_{15}H_{20}O$	216.32	2.40	

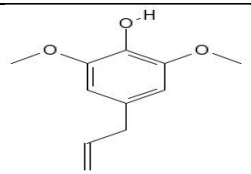
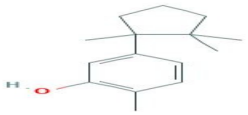
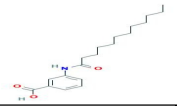
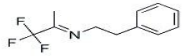
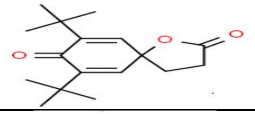
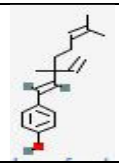
8.	15.108	Methoxyeugenol	$C_{11}H_{14}O_3$	194.18	3.64	
9.	15.596	Phenol,2-methyl-5-(1,2,2-trimethylcyclopentyl)-, (S)-	$C_{15}H_{22}O$	218	12.26	
10	15.896	3-(Dodecanoylamino)benzoic acid	$C_{19}H_{29}NO_3$	319	24.87	
11	16.00	2(3H)-Naphthalenone, 4,4A,5,6,7,8,-Hexahydro-4A,7,7-trimethyl-, (R)-	$C_{13}H_2O$	192.3	14.27	
12	16.100	1H-Inden-1-one,3A,4,5,6,7,7A-hexahydro-5,5-dimethyl-, cis	Unknown	Unknown	14.73	Unknown
13	16.275	Benzeneethanamine,N-(2,2,2-Trifluoro-1-methylethylidene)-	$C_{11}H_{12}F_3N$	215.21	2.71	
14	17.857	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276	0.88	
15	22.311	Phytol	$C_{20}H_{40}O$	296.5	1.81	
16	22.623	Phenol,4-(3,7-dimethyl-3-ethenylocta-1,6-dienyl)-	$C_{18}H_{24}$	256.383	2.71	

Table 41

Bioactivity of the compounds identified in the ethanolic stem bark extract of *C. mala-elengi* by GC-MS

S. No	Retention time(minutes)	Name of the compound	Nature of compound	Activity
1.	11.336	2-4'-methoxyphenyl)-2-(2'-methoxyphenyl)propane	Alcohol	Unknown
2.	12.127	(Z)-Isoeugenol	Phenylpropene	Antiseptic, analgesic, act as flavouring agent, antioxidant and manufacturing perfumeries, essential oil and vanillin
3.	12.617	1-Phenylethylene glycol	Unknown	Unknown
4.	12.681	2,4-Ditert-Butylphenol	Phenolic compound	Antioxidant, antifungal activity, manufacturing pharmaceuticals and fragrances, antimalarial, cytotoxicity against cancer mammalian cell lines such as H_9C_2, Hela and MCF-7.
5.	13.855	1,2-Dehydro-17-methyl testosterone	Unknown	Jaundice, androgenic effect and progestational activity
6	13.928	Alpha-asarone	Phenyl propanoid	Anticonvulsant activity in rodent, antiepileptic activity, antioxidant, anti inflammatory, anti depressant, antianxiety, anti-alzheimer's, anti parkinson's, anticancer

S. No	Retention time(minutes)	Name of the compound	Nature of compound	Activity
				activity, anti hyperlipidemic, antithrombotic activity, anticholestatic and radioprotective activity
7	14.587	Ar-tumerone	Sesquiterpenoid	Treating neurogenerative disease, stroke, anti tumor, apoptosis, inhibition of tumor cell invasion, anti-inflammatory, antiparkinson, antialzheimer's disease, anti-angiogenic effects, antiplatelet activity, antileishmanial activity, antidermatophytic activity and cytotoxic activity to HL-60, K-562, L-1210, U-937 and RBC-2H3 cells.
8	15.108	Methoxyeugenol	Phenol	Unknown
9	15.596	Phenol,2-methyl-5-(1,2,2-trimethylcyclopentyl)-, (S)-	Unknown	Unknown
10	15.896	3-(Dodecanoylamino)benzoic acid	Unknown	Unknown
11	16.00	2(3H)-Naphthalenone, 4,4A,5,6,7,8,-Hexahydro-4A,7,7-trimethyl-, (R)-	Ketone	Anti inflammatory activity.

S. No	Retention time(minutes)	Name of the compound	Nature of compound	Activity
12	16.100	1H-Inden-1-one,3A,4,5,6,7,7A-hexahydro-5,5-dimethyl-, CIS-	Unknown	Unknown
13	16.275	Benzeneethanamine,N-(2,2,2-Trifluoro-1-methylethylidene)-	Unknown	Unknown
14	17.857	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	Oxaspiro compound	Antioxidant
15	22.311	Phytol	Diterpene alcohol	Antioxidant, anxiolytic effect, a precursor of synthetic Vitamin A, E, K. It was proven to be cytotoxic against breast cancer cell lines (MCF7), antimicrobial, anti-inflammatory, anticancer, diuretic, resistant gonorrhoea, joint dislocation, headache, hernia, antimalarial, anticonvulsant, antispasmodic and antinociceptive
16	22.623	Phenol,4-(3,7-dimethyl-3-ethenylocta-1,6-dienyl)-	Unknown	Unknown

Figure-9

GC-MS spectra of ethanolic extract of *C. mala-elengi* leaf

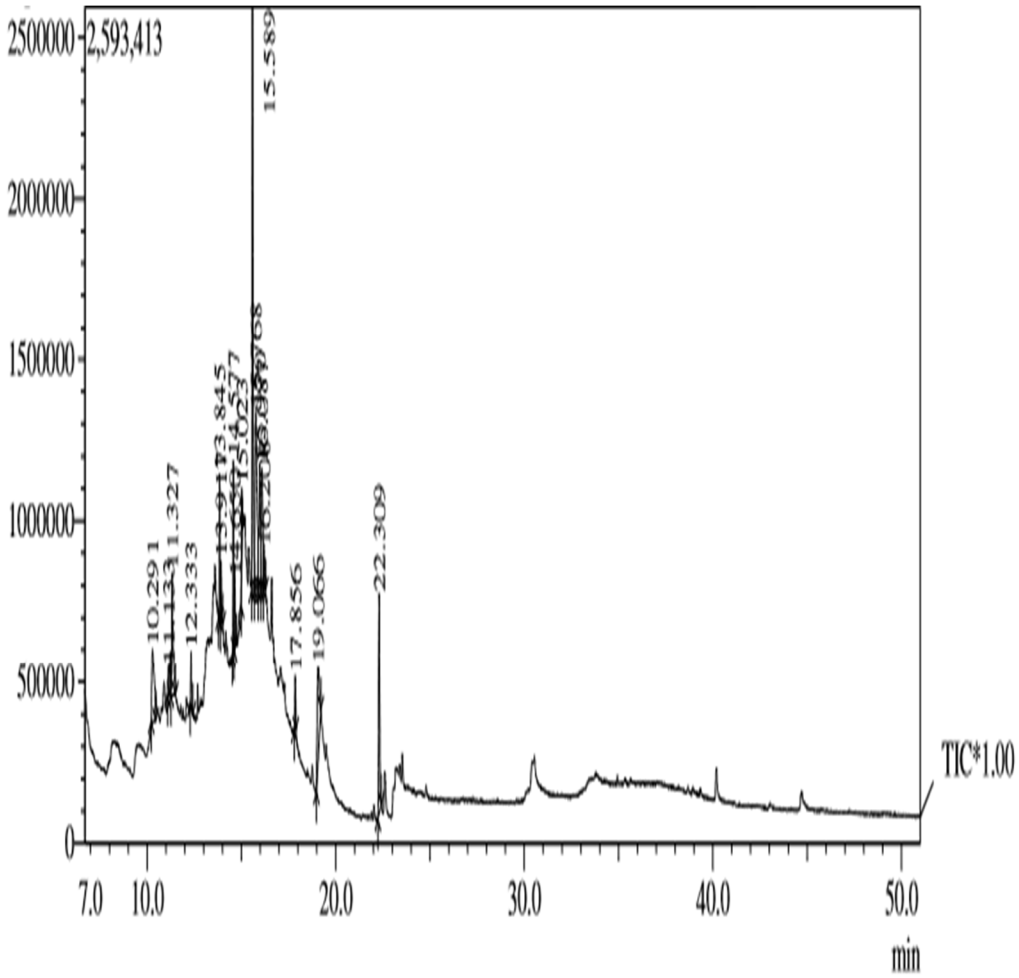
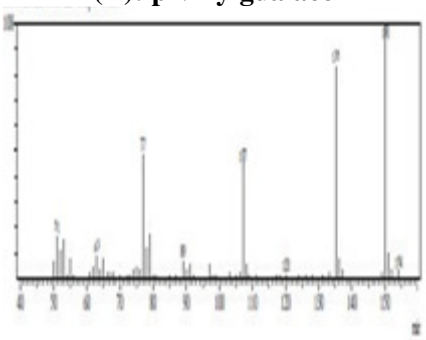


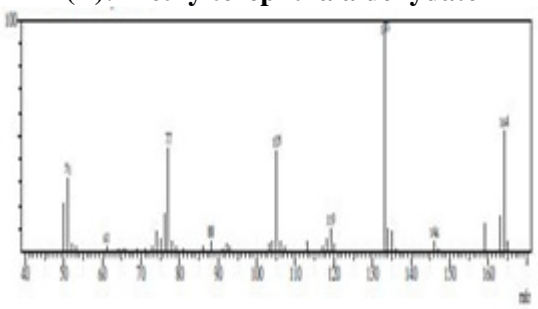
Figure-10

Mass spectra of bioactive compounds present in ethanolic extract of *C. mala-elengi* leaf

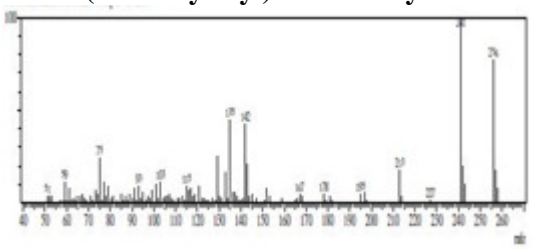
(A): p-vinylguaiacol



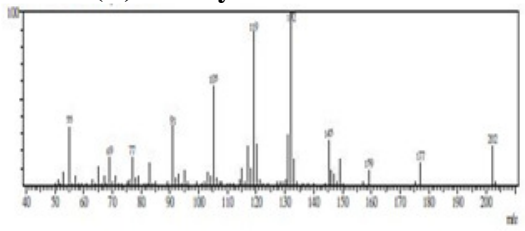
(B): Methylterephthalaldehydate



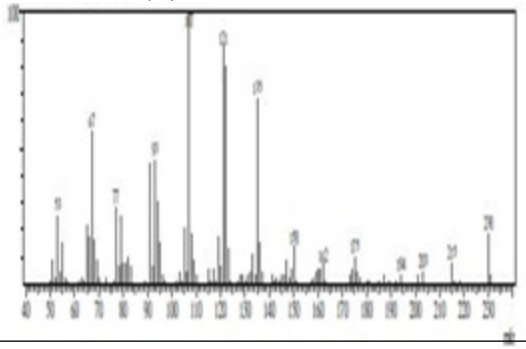
(C): Phosphoric acid, bis(trimethylsilyl)monomethyl ester



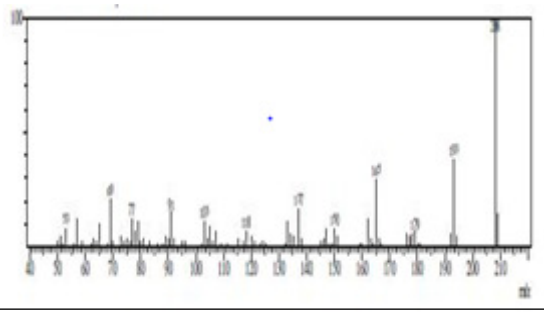
(D): Methyl tetradecanoate



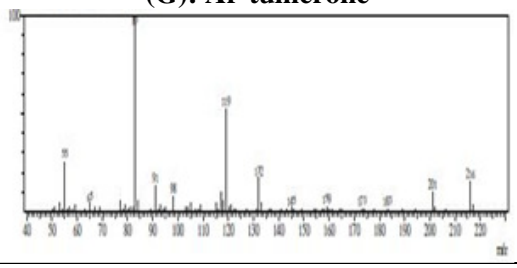
(E): Beta elemenone



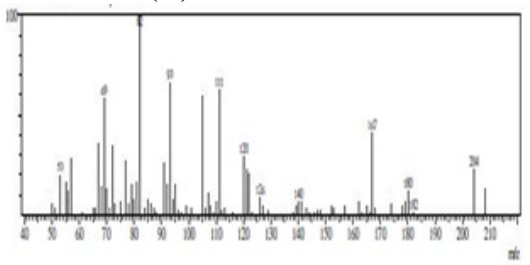
(F): B-Asarone



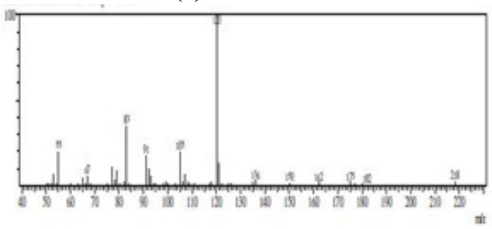
(G): Ar-tumerone



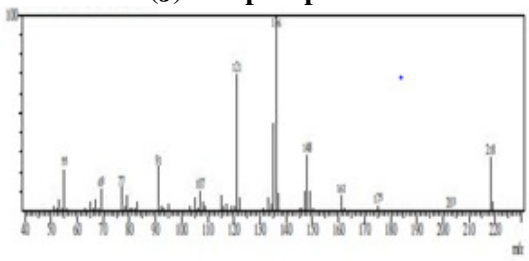
(H):Beta-bisabolol



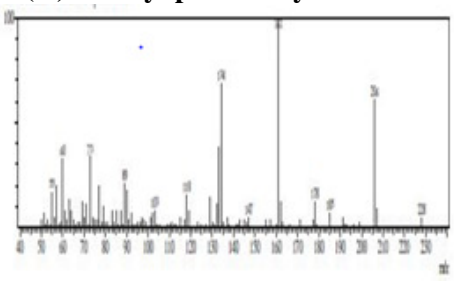
(I): Curlone



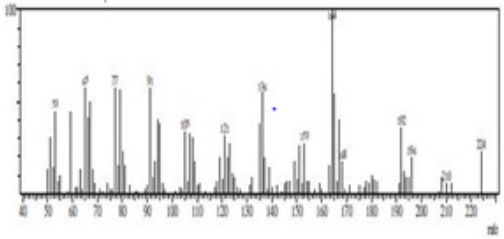
(J): Cuparophenol



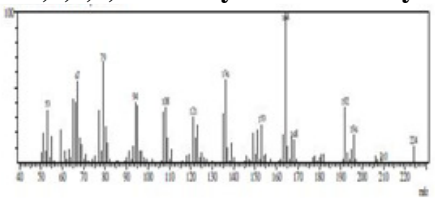
(K): Ethyl p-methoxycinnamate



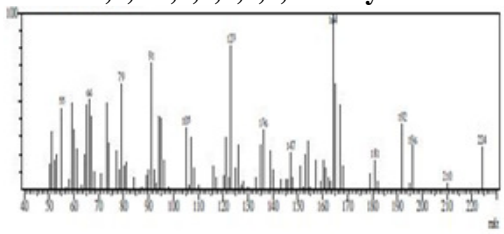
(L): 2H-Benzocyclohepten-2-One,3.4,4A,5,6,7,8,9- octahydro



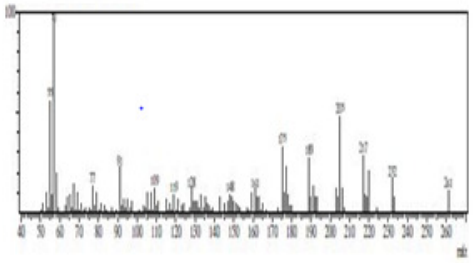
(M): 2(3H)Naphthalenone ,4, 4A,5,6,7,8-Hexahydro-4A-Methyl



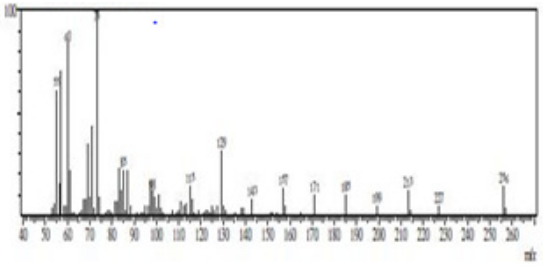
(N):2H-Benzocyclohepten-2-one 3,4,4A,5,6,7,8,9,-octahydro



(O): 7,9-Di tert–butyl-1-Oxaspiro(4,5) deca-6,9-diene-2,8-dione



(P): Hexadecanoic acid



(Q): Phytol

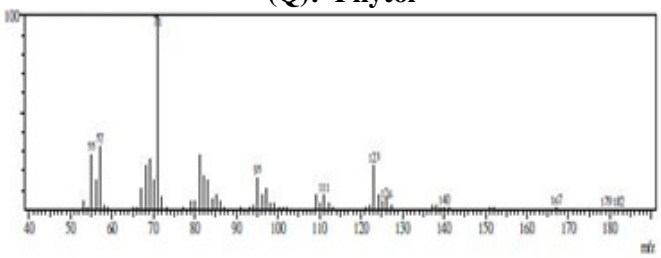


Figure 11

GC-MS spectra of ethanolic extract of *C. mala-elengi* stem bark

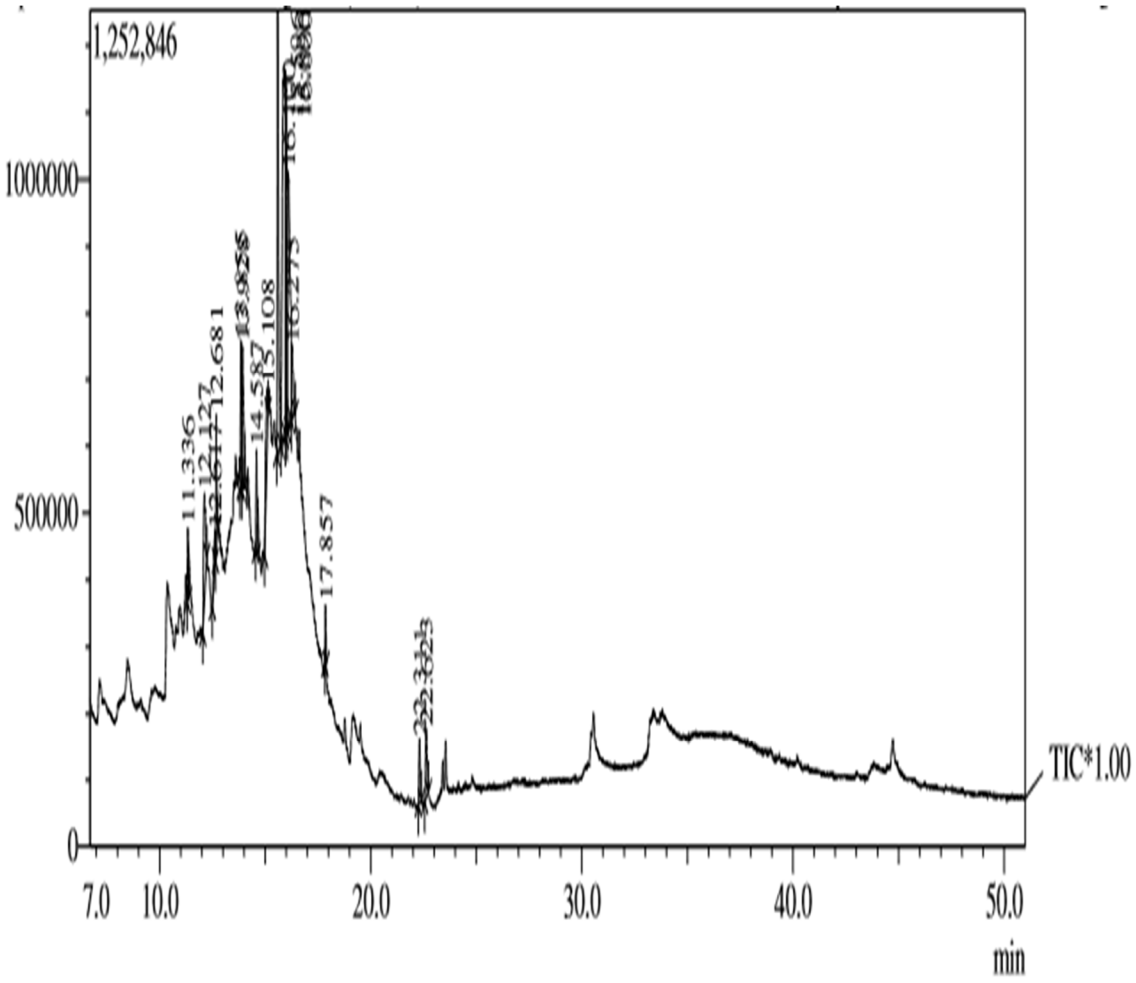
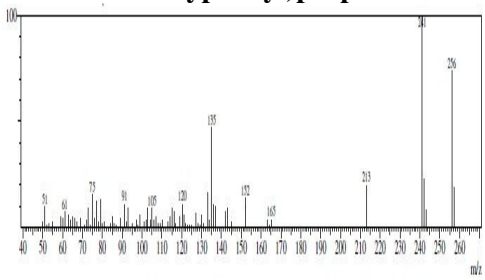


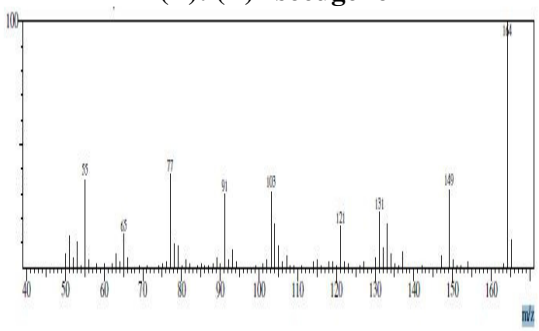
Figure 12

Mass spectra of bioactive compounds present in ethanolic extract of *C. mala-elengi* stem bark

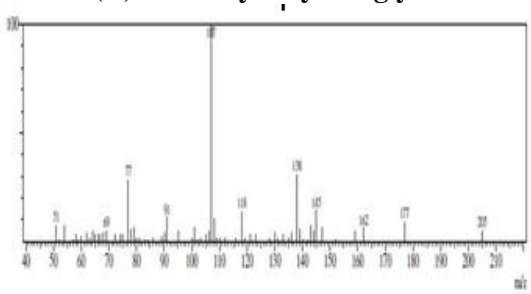
(A): 2-(4'-Methoxyphenyl)-2-(2'-methoxyphenyl)propane



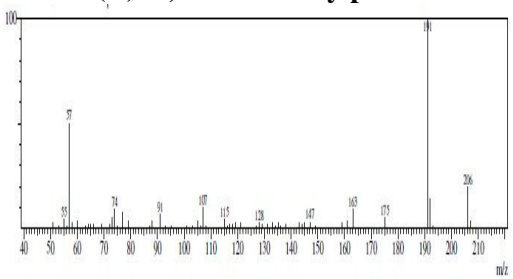
(B): (Z)-Isoeugenol



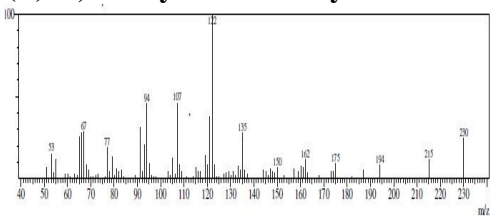
(C): 1-Phenylethylene glycol



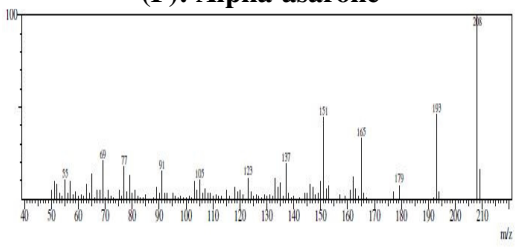
(D): 2,4-Ditert-butylphenol



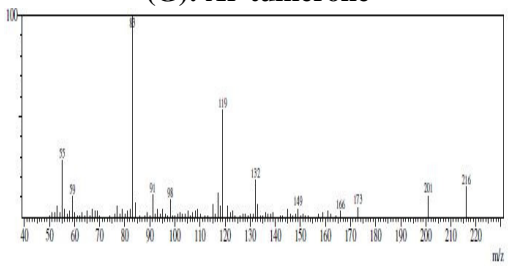
(E): 1,2-Dehydro-17-methyltestosterone



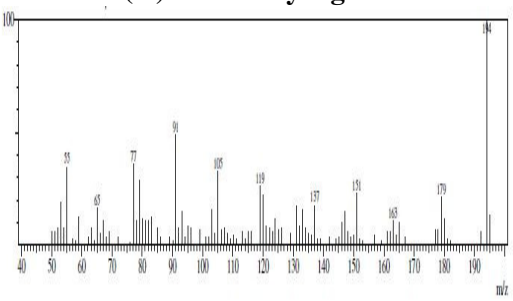
(F): Alpha-asarone



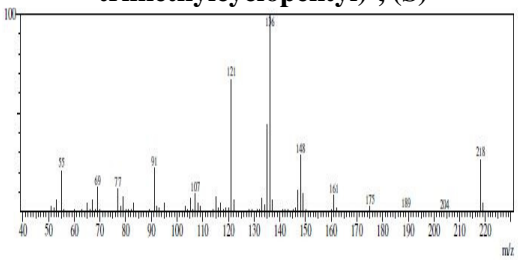
(G): Ar-tumerone



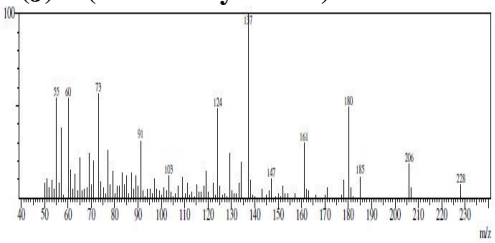
(H): Methoxyeugenol



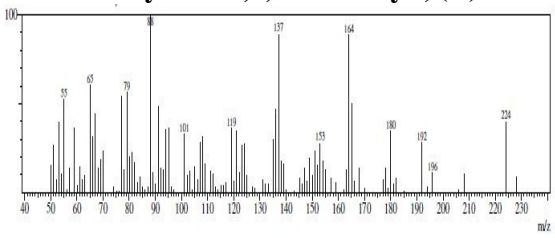
(I): Phenol, 2-methyl-5-(1,2,2-trimethylcyclopentyl)-, (S)-



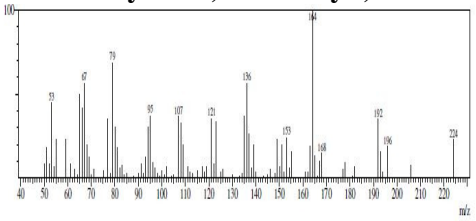
(J) :3(Dodecanoylamino)benzoic acid



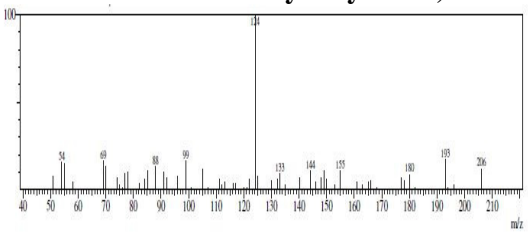
(K): 2(3H)-Naphthalenone, 4,4a,5,6,7,8-hexahydro-4a,7,7-trimethyl-, (R)-



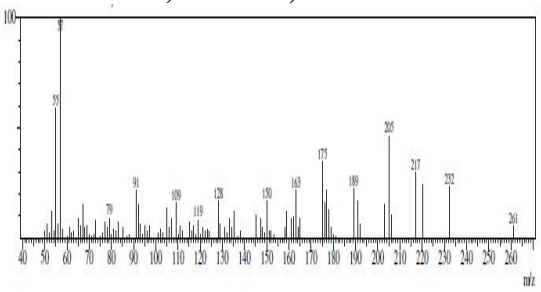
(L): 1H-Inden-1-one, 3a,4,5,6,7,7A-hexahydro-5,5-dimethyl-, CIS-



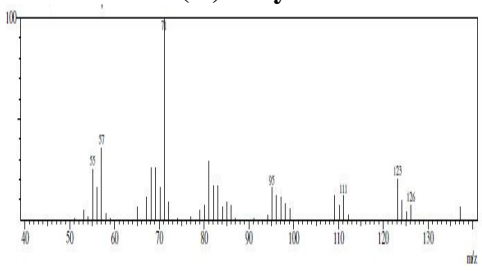
(M): Benzeneethanamine, n-(2,2,2-trifluoro-1-methylethylidene)-



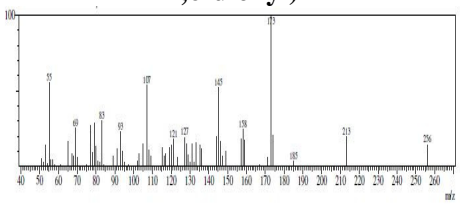
(N): 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8- dione



(O): Phytol



(P): Phenol, 4-(3,7-dimethyl-3-ethenylocta-1,6-dienyl)-



Experiment No - 6

Pharmacology study

Based on the results of antioxidant and antimicrobial analysis, ethanol leaf extract of *C. mala-elengi* was selected as suitable for the further studies.

From the previous experiments, it was understood that the plant *C. mala-elengi* is a rich source of phytochemical which could be helpful to cure various diseases. To confirm whether this plant could be used to treat human ailments. Several tests have been conducted using animal models as described in Materials and Methods (3.8).

4.6. *In vitro* antidiabetic activity

Diabetes is one of the most common causes of premature death worldwide. Lack of insulin secretion affects the metabolism of carbohydrates, proteins, fat and it affects the significant disturbance of water and electrolyte homeostasis. Nowadays, advances in research helps understand the activity of intestinal enzymes α-amylase and α-glucosidase play a vital role in carbohydrate digestion and glucose absorption leads to increase in novel pharmacological drugs. Suppression of the activity of such digestive enzyme (α-amylase and α-glucosidase) wound delay the degradation of starch and oligosaccharides which would in turn causes a decrease in the absorption of glucose. Therefore, the reduction of postprandial blood glucose depends on the α-amylase and α-glucosidase inhibitors, which decreases the absorbtion of carbohydrate. Hence, it is one of the medicinal approaches for reducing Postprandial (PP) blood glucose levels in patients with Diabetes mellitus is to prevent absorbtion of carbohydrate after food intake. Synthetic inhibitor causes various side effects such as abdominal pain, diarrhoea and soft feces in the colon.

Plants are traditionally used worldwide for treating diabetes. They synthesize a wide range of biologically active compounds containing antidiabetic properties. The ethanol leaf extract of *C. mala-elengi* exposed a significant inhibitory action on α-amylase and α-glucosidase enzyme. There was a dose dependent increase in percentage inhibitory activity against α-amylase and α-glucosidase enzyme. The details of methodology are given in materials and methods (3.6)

The inhibitory effects of the extract against α-amylase and α-glucosidase enzyme were compared with those of acarbose. The results are represented in Table 42, 44 and Figure-13, 14. The results showed that the α-amylase and α-glucosidase enzyme percentage of inhibition was increased with the concentration enhancement (25 µg/ml to 200 µg/ml) of ethanol leaf extract and acarbose. At the highest concentration the leaf extract displayed appreciable effect on α-amylase by 67.22±1.40% at 200 µg/ml and α-glucosidase by 69.28±0.98% at 200 µg/ml. However, acarbose as positive control was far more effective on α-amylase 97.3±0.55% at 200 µg/ml than the extract, exhibiting percentage inhibitory activities at 85.27±0.56% at 200 µg/ml against α - glucosidase.

The IC_{50} values of the ethanol leaf extract of α-amylase (134±0.58 µg/ml) and α-glucosidase (129.33±0.33 µg/ml) higher than those of acarbose of α-amylase (120.67±1.20 µg/ml) and α-glucosidase (110.67±0.67 µg/ml). In this study, results revealed that *C. mala-elengi* efficiently inhibit α-amylase and α-glucosidase enzyme. *In vitro* antidiabetic action of *C. mala-elengi* leaf can also be contributed to the intestinal α-amylase and α-glucosidase inhibitory activity. The results are significantly different at $p<0.05$ level. The results are represented in Table 43, 45.

Table 42

α-amylase inhibitory activity of ethanol leaf extract of *C. mala-elengi*

Concentration (µg/ml)	% of inhibition	
	Leaf	**Acarbose**
25	14.50± 0.69[e]	12.38±0.26[e]
50	31.44± 0.61[d]	25.26±0.23[d]
100	42.35± 0.90[c]	53.35±1.18[c]
150	54.28 ± 0.81[b]	73.42±0.42[b]
200	**67.22 ±1.40[a]**	**97.3±0.55[a]**
SED Cd (p<0.05)	1.3097 2.9181***	0.9009 2.0073***

[#]Mean ± S.E

Mean in a column followed by a same letter (s) are not significantly (P > 0.05) different according to Duncan's Multiple Range Test

,*Significant at P < 0.01, P < 0.001 respectively; ns- non significant.

Table 43

IC_{50} values of α-amylase inhibitory activity of ethanol leaf extract of *C. mala-elengi*

IC_{50} values (µg/ml)		T test
Sample	**Acarbose**	
134±0.58	120.67±1.20	16.20**

** - Significant at 1% level (p<0.01)

Figure 13

α-amylase inhibitory activity

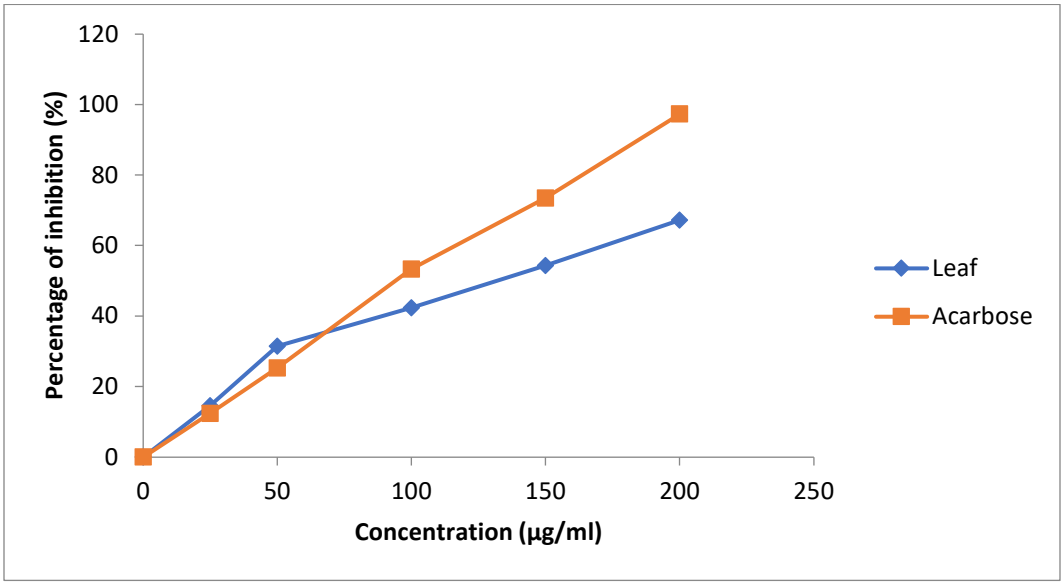


Table 44

α-glucosidase inhibitory activity of ethanol leaf extract of *C. mala-elengi*

Concentration (µg/ml)	% of inhibition	
	Leaf	Acarbose
25	17.32± 0.56^{e}	11.17±0.08^{e}
50	34.48± 0.62^{d}	18.35±0.29^{d}
100	43.29± 0.98^{c}	46.24±0.57^{c}
150	56.28 ± 0.51^{b}	65.80±1.07^{b}
200	**69.28± 0.98^{a}**	**85.27±0.56^{a}**
SED Cd (p<0.05)	1.0714 2.3872***	0.8666 1.9308***

#Mean ± S.E

Mean in a column followed by a same letter (s) are not significantly (P > 0.05) different according to Duncan's Multiple Range Test

,*Significant at P < 0.01, P < 0.001 respectively; ns- non significant.

Table 45

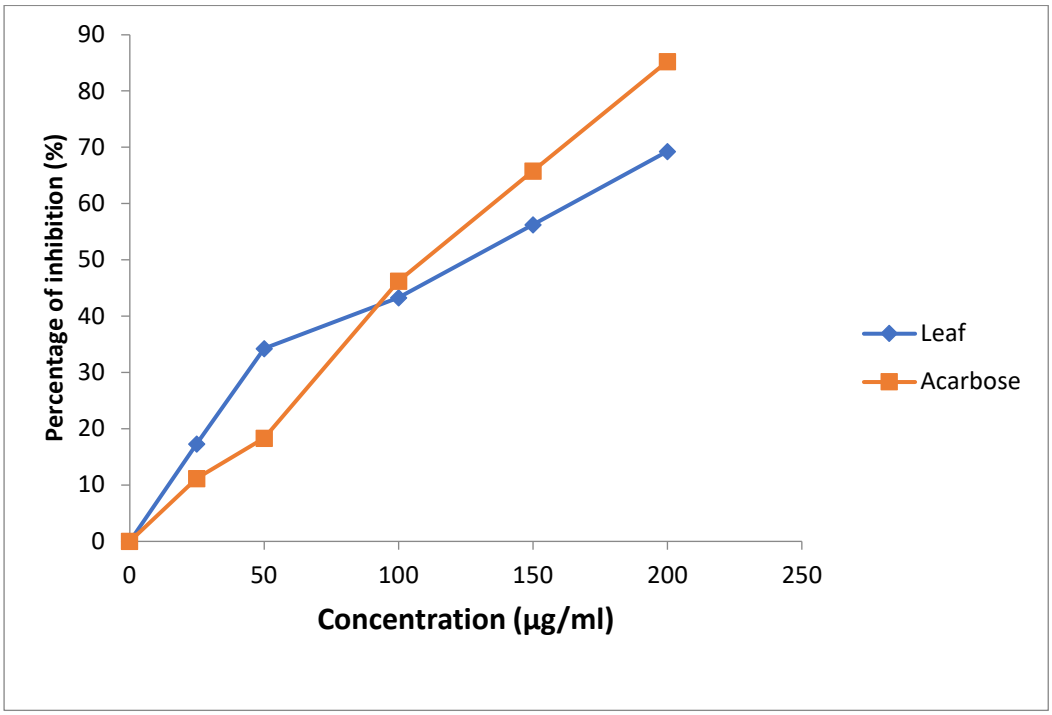
IC_{50} value of α-glucosidase inhibitory activity of ethanol leaf extract of *C. mala-elengi*

IC_{50} values (µg/ml)		T value
Leaf	**Acarbose**	
129.33±0.33	110.67±0.67	21.166**

** - Significant at 1% level (p<0.01)

Figure 14

α - glucosidase inhibitory activity



Experiment No-7

From the previous experimental results, it was understood that ethanol leaf extract of *C. mala-elengi* showed high *in vitro* antidiabetic activity. Thus, further studies were enhanced for detecting anticancer activity of ethanolic leaf extract of *C. mala-elengi.* The details are given in Materials and Methods (3.7).

4.7 *In vitro* cytotoxicity assessment (Anticancer activity)

Cancer is one of the life threatening diseases which occurs with no particular symptoms and age. These is a sustained demand for novel therapies to cure and prevent this devating ailment. Recent treatments such as chemotheraphy, radiotherapy and chemically derived medicines are available to cure cancer. Chemotherapy puts patients under a lot of side effects and strains. However there is a focus on using other therapy and treatment against cancer, specifically from herbal plant.

MTT assay is a commonly used sensitive, quantitative and reliable colorimetric method which tests the viability, proliferation and activation of cells. The technique is based on the ability of mitochondrial dehydrogenase enzymes in living cells turned into the yellow water soluble substrate 3-(4,5-dimethylthiozol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) into a dark blue formazon product which is insoluble in water. This reaction is intermediated by dehydrogenases enzymes related with the endoplasmic reticulum and the mitochondria. MTT assay is mostly used for evaluating anticancer properties of medicinal plants. Evaluation of the cytotoxic activity of the leaf ethanol extract of *C. mala-elengi* human Hepato carcinoma (HepG2) cell lines with different doses (10 to 50 µg/ml) of the extract. From the results, it is confirmed that ethanol leaf extract of *C. mala-elengi* showed significant activity at the concentration of 10 µg/ml showed 94±0.29% and at the concentration of 50 µg/ml it exhibited 28±0.46%. The IC_{50} concentration (The concentration of the extract have the capacity to kill 50% of viable cells) against the HepG2 cells at 22±1.0. The IC_{50} value of standard reference Doxorubicin was found to be 18±1.0 and they are significantly different at $p<0.05$ level. Our results strongly

proposed that the test extract may have potential therapeutic activity against Hepato carcinoma cells depending on the dosage of drug. The results are represented in Table 46, 47, Figure-15, (Plate-9).

Table 46

Cytotoxicity assessment of ethanol leaf extract of *C. mala-elengi* against HepG2 cell line by MTT assay

Concentration (µg/ml)	% of Cell viability	
	Leaf	Doxorubicin
10	**94±0.29[a]**	**94±0.25[a]**
20	64±0.46[b]	52.96±0.10[b]
30	45±0.98[c]	32.18±0.52[c]
40	34±0.17[d]	18.85±0.54[d]
50	28±0.46[e]	12.64±0.57[e]
SED Cd (p<0.05)	0.7755 1.7278***	0.6192 1.3796***

[#]Mean ± S.E

Mean in a column followed by a same letter (s) are not significantly (P > 0.05) different according to Duncan's Multiple Range Test

,*Significant at P < 0.01, P < 0.001 respectively; ns - non significant.

Table 47

IC_{50} value of Cytotoxicity assessment

IC_{50} (µg/ml)		T value
Leaf	**Doxorubicin**	
22±1.0	18±1.0	4.11*

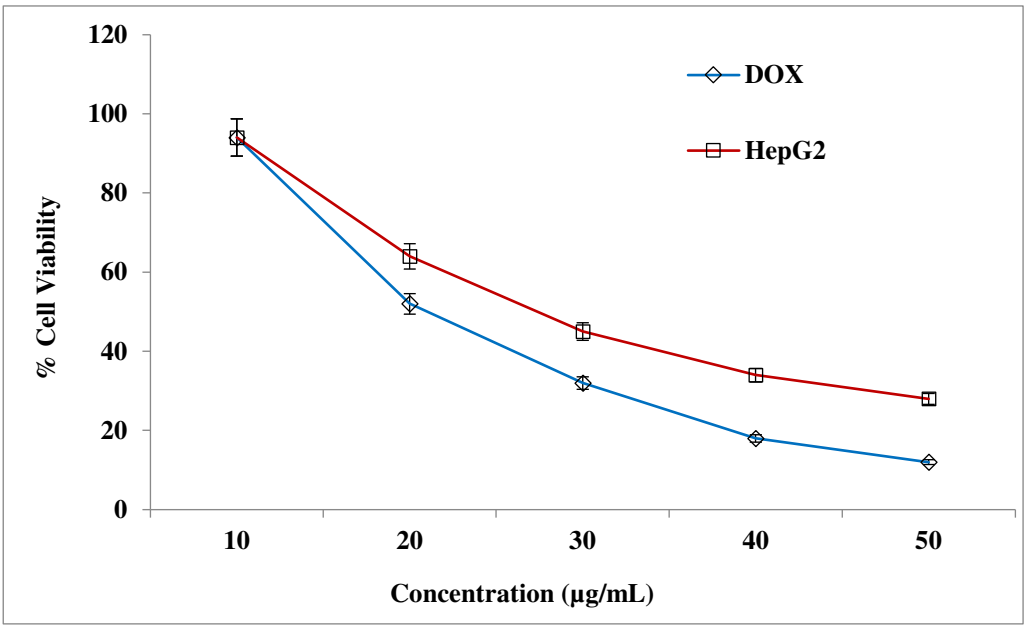
#Mean ± S.E

Mean in a column followed by a same letter (s) are not significantly (P > 0.05) different according to Duncan's Multiple Range Test

,*Significant at P < 0.01, P < 0.001 respectively; ns- non significant.

Figure 15

Cytotoxicity assessment (MTT assay)



DOX-Doxorubicin, HepG2-Hepatoma G2

Experiment No - 8

4.8 *In vivo* pharmacological study

4.8.1 Acute toxicity study

Acute toxicity studies are designed to determine the short term adverse effect of a substance (test drug) when administered in a single or multiple doses within a period of one day (24 hours) in a mammalian species like mice. It is helpful in evaluating the dosage that will cause mortality or severe toxicological effects when given once or over a few administration. The study also provides information regarding doses that should be used in subsequent experimental studies.

The ethanol leaf extract of *C. mala-elengi* did not affect the mortality up to 2000 mg/kg and was assigned as safe (OECD-423 guidelines). The observation of acute toxicity study of ethanol leaf extract of *C. mala-elengi* are shown in Table 48 & 49 and mortality did not occur after the oral administration of tested extract up to 2000 mg/kg given within 24 to 72 hrs. No death or sign of toxic reactions were observed with the tested extract during and after the toxicity study period.

Table 48

Acute toxicity of ethanol leaf extract of *C. mala-elengi*

Group	Drug treatment	No.of animals	Death	Dose difference	Mean death	Dose different × Death
I	Control	3	0	0	-	-
II	5 mg/kg	3	0	5	NM	NM
III	30 mg/kg	3	0	30	NM	NM
IV	100 mg/kg	3	0	100	NM	NM
V	300 mg/kg	3	0	300	NM	NM
VI	1000 mg/kg	3	0	1000	NM	NM
VII	2000 mg/kg	3	0	2000	NM	NM

All treatments were administrated as single dose orally with the help of intragastric catheter tube (IGC) NM-No mortality.

Table 49

Oral administration of ethanol leaf extract of *C. mala-elengi* sign and symptom of acute toxicity in mice

S.NO	General behaviour	*C. mala-elengi*
1	Sedation	-
2	Hypnosis	-
3	Convulsion	-
4	Ptosis	-
5	Analgesia	-
6	Stupar reaction	-
7	Motor activity	-
8	Muscle relaxant	-
9	CNS stimulant	-
10	CNS depressant	-
11	Pilo erection	-
12	Skin colour	-
13	Lacrimation	-
14	Stool consistency	-

(-) : absent

4.8.2 Wound healing activity

Wound healing is the biological process of repairing the injury to the skin and other soft tissue. It is a vital and complex sequence of events in restoring cellular structures and tissue layers in injured tissue as closely as possible to its common and original state. The wound breaking strength is evaluated by the rate of collagen synthesis and attains maximum by the maturation stage where there is covalent binding of collagen fibrils through inter and intra molecular cross linking. The healing process based the large extent of accumulation of collagen and their successive maturation.

Herbal plants play a vital role in treating skin disorders such as cuts and burns. Traditional herbal plants are used for various skin associated ailments. As reported in various literature, 70% of the wound healing drugs are associated with plant origin. The results showed the topical application of ethanol leaf extract of *C. mala-elengi* extract in different concentration (10% and 20%) has revealed good reduction in wound area. The 20% ointment of ethanol leaf extract of *C. mala-elengi* treated animals revealed earlier epithelialisation of wound than the animals treated with 10% extract ointment. The contraction of excission wound was promoted from 1^{st} day of treatment till 12^{th} day. The epithelialization period of 10% ointment is 19.1±0.4 while in 20% ointment is 18.2±0.2 and they are significantly different at $p<0.01$ level. The results are represented in Table 50, (Plate 10, 11, 12).

Table 50

Wound healing activity of ethanol leaf extract of *C. mala-elengi*

Group	Wound contraction (%)				Epithelialization period (day)
	1 day	4 day	8 day	12 day	
Group I (Negative control)	4.32±0.2	18.8±2.6	39.2±1.8	57.3±1.6	23.7±0.8
Group II (Positive control)	5.76±0.3^{ns}	32.6±1.6**	61.7±3.2**	78.2±3.2**	17.4±0.6**
Group III (10% leaf extract of *C. mala elengi*)	5.69±0.4^{ns}	26.9±2.6**	53.7±1.8**	69.3±1.8**	19.1±0.4**
Group IV (20% leaf extract of *C. mala-elengi*)	**5.82±0.4^{ns}**	**30.7±2.2****	**58.1±1.6****	**72.5±1.2****	**18.2±0.2****

Values were mean ± SD, n=6, ^{ns}P > 0.05, *P<0.05, **P<0.01 Vs control (one way ANOVA followed by Dunnett's test).

4.8.3 Hepatoprotective activity

Liver disease is still a worldwide health problem. A large number of drugs of plant origin are endowed with hepatoprotective claims either directly or indirectly. In recent years, the usage of herbal drugs for the treatment of liver diseases has increased all over the world. Herbal drugs are believed to be harmless, free nature and easily available.

Several hundred plants have been examined for use in treating variety of liver disorders. Just a handful has been fairly well researched. There are about 600 commercial herbal formulations, which are claimed to have hepatoprotective activity and many of them are being sold in market all over the world. In India, about 40 patented polyherbal formulations representing a variety of combinations of 93 herbs and 44 families are

formulations representing a variety of combinations of 93 herbs and 44 families are available. Researchers examined and reported that 160 phytocompounds from 101 plants contain hepatoprotective activity. Hepatoprotective plant drug contains various bioactive constituents like phenol, coumarin, lignans, essential oil, monoterpenes, carotenoids, glycosides, flavonoids, organic acids, lipids, alkaloids and xanthones derivatives. Therefore, the present study has been designed to evaluate the hepatoprotective activity of ethanol leaf extract of *C. mala-elengi* in experimental animal models of paracetamol induced hepatic damage in rats.

The effects of plant extracts on the elevated level of Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), Alkaline Phosphatase (ALP), Bilirubin, Urea and Creatinine due to paracetamol intoxication reduced significantly ($P<0.01$) in rats after treated with ethanol leaf extract (Group IV-V) and silymarin at the dose of 50 mg/kg (Group III) the level of the enzymes were found recovering towards normal state. A comparison of all the treated groups, the 250 mg/kg and 500 mg/kg of leaf extract exhibited excellent significant effect which is equivalently potent to silymarin (standard drug). The results are represented in Table 51.

Serum Glutamic Oxaloacetic Transaminase (SGOT)

The ethanol leaf extract of *C. mala-elengi* orally treated animals at the dose of 250 mg/kg and 500 mg/kg showed significant ($p<0.01$) reduction in SGOT liver marker enzyme compared to the paracetamol treated animal group.

Serum Glutamic Pyruvic Transaminase (SGPT)

The ethanol leaf extract of *C. mala-elengi* orally treated at the dose of 250 mg/kg and 500 mg/kg showed significant ($p<0.01$) reduction in SGPT liver marker enzyme compared to the paracetamol treated animal group.

Alkaline Phosphatase (ALP)

Among the test groups, ethanol leaf extract of *C. mala-elengi* at the dose of 250 mg/kg and 500 mg/kg treated animals groups showed significant ($p<0.01$) reduction when compared to paracetamol treated group.

Bilirubin

In groups orally administrated at the dose of 250 mg/kg ethanol leaf extract of *C. mala-elengi* showed significant ($p<0.05$) reduction when compared to paracetamol treated group. In case of 500 mg/kg treated animal groups showed significant ($p<0.01$) reduction when compared to paracetamol treated group.

Urea

Among the test groups, orally administrated at the dose of 250 mg/kg ethanol leaf extract of *C. mala-elengi* showed significant ($p<0.05$) reduction when compared to paracetamol treated group. In case of 500 mg/kg treated animals groups showed significant ($p<0.01$) reduction when compared to paracetamol treated group.

Creatinine

Among the test groups, ethanol leaf extract of *C. mala-elengi* at the dose of 250 mg/kg and 500 mg/kg treated animal groups showed significant ($p<0.01$) reduction when compared to paracetamol treated group

Table 51

Hepatoprotective activity of ethanol leaf extract of *C. mala-elengi*

Group	SGOT Units/ml	SGPT Units/ml	ALP Units/ml	Bilirubin	Urea (mg/dl)	Creatinine (mg/dl)
I (Control)	56.2±1.2	44.9±2.6	167.2±5.2	86±0.02	16.86±1.2	0.78±0.04
II Negative control	141.9±4.8[a]	117.4±5.2[a]	290.7±5.6[a]	1.92±0.06[a]	34.18±2.2[a]	1.46±0.06 [a]
III Positive control	72.6±3.4[c]	61.6±3.8[c]	197.8±6.4[c]	1.06±0.04[c]	19.87±2.6[c]	0.92±0.08 [c]
IV 250 mg/kg *C. mala-elengi*	102.7±5.2[c]	94.9±4.2[c]	242.1±5.8[c]	1.59±0.08[b]	29.02±1.4[b]	1.18±0.04 [c]
V 500 mg/kg *C. mala-elengi*	**86.2±2.8[c]**	**74.6±5.6[c]**	**218.0±6.2[c]**	**1.20±0.04[c]**	**23.14±2.8[c]**	**1.02±0.06 [c]**

Values were mean ± SD; n=6

[a]P<0.01 when compared to control. [b]P<0.05, [c]P<0.01 when compared to paracetamol control (one way ANOVA followed by Dunnett's test. SGOT= U/L, SGPT= U/L, ALP= U/L, Bilirubin= mg/dl, Urea = mg/dl and Creatinine = mg/dl.

4.8.4 Histopathological studies

Histopathological examination of the liver section of rats treated with paracetamol showed binucleation and interface hepatitis. It indicates the inflammation and damage of liver cells. The rats treated at the dose of 250 mg/kg, 500 mg/kg ethanol leaf extract of *C. mala-elengi* and 50 mg/kg of silymarin (Positive control) showed a good sign of protection against toxicants. As it was evident from more number of regeneration of hepatocytes (it mention by lines) and it showed cytoplasmic vascularization in our present histopathology. The photomicrograph of the hepatoprotective activity of each group is presented in (Plate-13).