Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
www.plantsjournal.com
JMPS 2021; 9(4): 04-13

Received: 07-05-2021
Accepted: 11-06-2021

Saraswathi K
Department of Biotechnology, Karpaga Vinayaga College of Engineering and Technology, Madhuranthagam, Kancheepuram, Tamil Nadu, India

Arumugam P
Phytochemistry and Natural Products Laboratory, ARMATS Biotek Training and Research Institute, Guindy, Chennai, Tamil Nadu, India

Sivaraj C
Phytochemistry and Natural Products Laboratory, ARMATS Biotek Training and Research Institute, Guindy, Chennai, Tamil Nadu, India

Corresponding Author:
Dr. Sivaraj C
Phytochemistry and Natural Products Laboratory, ARMATS Biotek Training and Research Institute, Guindy, Chennai, Tamil Nadu, India

Pharmacological evaluation of dried roots of *Glycyrrhiza glabra* Linn. (Liquorice)

Saraswathi K, Arumugam P and Dr. Sivaraj C

Abstract
Glycyrrhiza glabra is one of the most popular medicinal plants belonging to the Fabaceae family (also known as Leguminosae), and its members are now commonly used as feed and food. This species is a native of Mediterranean areas, but it is now also present in India, Russia, and China. The use of liquorice predates the Greek and Roman empires, having a long history of traditional medicines and folk remedies. *Glycyrrhiza glabra* root is employed to prepare a tea that is an excellent thirst quencher. The dried root has been described as a tooth cleanser. Actually, the most important industrial use of *Glycyrrhiza glabra* is the production of food additives, such as flavours and sweetening agents. In the cosmetic field, *Glycyrrhiza glabra* is described as a skin depigmentation agent and is being incorporated in topical products for that purpose. Nutritionally, liquorice is a source of proteins, amino acids, polysaccharides and simple sugars, mineral salts (such as calcium, phosphorus, sodium, potassium, iron, magnesium, silicon, selenium, manganese, zinc, and copper), pectins, resins, starches, sterols, and gums. The antibacterial activity of methanol root extracts of Liquorice (*Glycyrrhiza glabra*) showed maximum zone of inhibition of 22 mm for *Proteus vulgaris* and for *Escherichia coli* as 16 mm at 500 µg/mL concentration. Various antioxidant activities were performed in which methanol root extracts of Liquorice significantly had the capability to scavenge the free radicals in respective mode of mechanism. The maximum haemolytic inhibition for methanol root extracts of Liquorice (*Glycyrrhiza glabra*) was 68.28±0.22% at 120 µg/mL concentration. The anticancer activity for methanol root extracts of Liquorice (*Glycyrrhiza glabra*) was 72.13±0.14% at 350 µg/mL concentration. Bioactive compounds such as Isopropyl stearate, n-Hexadecanoic acid, Coumarine, 3-(2-benzoxazolyl)-8-methoxy- were detected possessing antioxidant, anticoagulant, anticancer, hypocholesterolmic activities by GC-MS analysis.

Keywords: DPPH$^{\cdot}$ radical, ABTS$^{\bullet+}$ radical cation, nitric oxide radical, heat induced haemolysis, HeLa, GC-MS

Introduction
Glycyrrhiza glabra Linn. Is one of the most extensively used medicinal herbs from the ancient medical history of Ayurveda. It is also used as a flavoring herb. The word *Glycyrrhiza* is derived from the Greek term glykos (meaning sweet) and rhiza (meaning root) [1, 2]. This plant is a tall perennial herb, up to 2 m high found cultivated in Europe, Persia, Afghanistan, and too little extent in some parts of India [3] (Figure 1). *Glycyrrhiza* plays important parts in Hindu medicine and is one of the principle drugs of the "susruta." In ancient Egypt, Greece, and Rome, *Glycyrrhiza* was also frequently used. Liquorice is referred by Theophrastus. It is interesting to find that even to this day liquorice is maintaining its place in medicine and pharmacy [4, 5].
The main constituent of roots is glycyrrhizin, a triterpenoid saponin that is almost 50 times sweeter than sucrose, being the primary active ingredient [6]. Glycyrrhizin represents about 10% of the liquorice root dry weight, being a mixture of potassium, calcium, and magnesium salts of glycyrrhizic acid that varies between 2% and 25%. The yellow colour of liquorice is due to the flavonoid content. The major flavonoids are glycosides of liquiritigenin (4′,7-dihydroxyflavanone) and isoliquiritigenin (2′,4,4′- trihydroxychalcone), such as liquiritin, isoliquiritin, liquiritin apioside, and licuraside [7]. In present work antioxidant, antimicrobial and anticancer activities of methanol root extracts of Liquorice (*Glycyrrhiza glabra*) were evaluated with standardized methods.

Fig 1: Habitat of Liquorice (*Glycyrrhiza glabra*)

Materials and Methods

Chemicals and reagents

The chemicals used in the research studies were of analytical grade and procured from Merck India Private Limited.

Extraction process

The roots of *Glycyrrhiza glabra* (Liquorice) were collected from Indian herbal market, Chennai, Tamil Nadu, India. The roots were cleaned, made into fine pieces and soaked in methanol for 72 hours. The thick brown coloured extract was sterile filtered and condensation was carried out in a rotary evaporator at 50°C, which yields brown extract [8, 9].

Determination of total phenols, flavonoids and steroids

Qualitative analysis for the methanol root extracts of Liquorice (*Glycyrrhiza glabra*) was performed in order to find the derivatives of various phytochemicals [8]. Total phenolic content of the methanol root extracts of Liquorice (*Glycyrrhiza glabra*) was determined following the methodology [10] and was estimated as gallic acid equivalent (GAE/mg of extract). Total flavonoid content of the methanol root extracts of Liquorice (*Glycyrrhiza glabra*) was assessed by aluminium chloride reagent method [11] and was estimated as quercetin equivalent (QE/mg of extract). The total steroids content of methanol root extracts of Liquorice (*Glycyrrhiza glabra*) were determined by potassium hexacyanoferrate (III) method [12] and was estimated as cholesterol equivalent (Cholesterol/mg of extract).

Thin layer chromatography analysis

Thin layer chromatography (TLC) was carried out for the methanol root extracts of Liquorice (*Glycyrrhiza glabra*) in silica gel pre-coated TLC aluminium sheets (Merck/60 F254) [13]. The prepared root extract was spotted at 0.3 mm above from the bottom of the TLC plate (1.5x5 cm). The spotted TLC plate was placed in a 100 mL beaker containing solvent mixture of Toluene (1.5 mL): Chloroform (1 mL): Methanol (0.5 mL) and the chromatogram was developed. The spots were visualized in UV light chamber at 254 nm as well as under iodine crystals and the R_f values of coloured spots were calculated.

Antibacterial activity by Agar well-diffusion method

The antibacterial activity of methanol root extracts of Liquorice (*Glycyrrhiza glabra*) was performed by agar well diffusion method [14]. The solidified nutrient agar in the petriplates was inoculated by dispensing the inoculum (gram positive and gram negative) using sterilized cotton swabs and spread evenly onto the solidified agar medium. Five wells were created in each plate with the help of a sterile well-borer of 8 mm diameter. The methanol root extracts of Liquorice (*Glycyrrhiza glabra*) were loaded into each well to get desirable concentrations. Tetracycline was used as the standard with the concentration of 30 µg. All the plates containing root extract loaded wells were incubated for 24 hours at 37°C. After the incubation period, zone of inhibition in each plate, for each concentration of root extract and standard were measured by calculating the diameter of zone of inhibition.

In vitro antioxidant activities

DPPH˙ radical scavenging activity

The radical scavenging activity of methanol root extracts of Liquorice (*Glycyrrhiza glabra*) (20-120 µg/mL) was carried out by the reduction DPPH˙ free radical method [15]. The decrease in absorbance was measured at 517 nm using UV-Vis spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of inhibition was calculated as:

$$\% \text{ of DPPH}^{\cdot} \text{ radical inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}}\right] \times 100$$

$ABTS^{\bullet+}$ (2,2-azinobis (3-ethylbenzo thiazoline-6-sulfonic acid) radical cation scavenging activity

The methanol root extracts of Liquorice (*Glycyrrhiza glabra*) (5-30 µg/mL) was performed according to the method [16]. The absorbance was measured at 734 nm using UV-Vis spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of inhibition was calculated as:

$$\% \text{ of } ABTS^{\bullet+} \text{ radical cation inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}}\right] \times 100$$

Hydroxyl radical ($OH^{\bullet}$) scavenging activity

Hydroxyl radical ($OH^{\bullet}$) scavenging activity for methanol root extracts of Liquorice (*Glycyrrhiza glabra*) (10-60 µg/mL) was measured by the salicylic acid method with some modifications [17]. The absorbance was measured at 510 nm using UV-Vis Spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of inhibition was calculated as:

$$\% \text{ of Hydroxyl } (OH^{\bullet}) \text{ radical inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}}\right] \times 100$$

Nitric Oxide radical scavenging activity

Nitric oxide radical scavenging activity for methanol root extracts of Liquorice (*Glycyrrhiza glabra*) (10-60 µg/mL) was carried out by the sodium nitroprusside method [18]. The absorbance was measured at 546 nm using UV-Vis spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of inhibition was calculated as:

$$\% \text{ of Nitric oxide radical inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}}\right] \times 100$$

Phosphomolybdenum reduction activity

The antioxidant capacity of methanol root extracts of Liquorice (*Glycyrrhiza glabra*) (10-60 µg/mL) was assessed as described [19]. The absorbance of the coloured complex was measured at 695 nm using UV-Vis spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of reduction was calculated as:

$$\% \text{ of Phosphomolybdenum reduction} = \left[\frac{\text{Sample} - \text{Control}}{\text{Sample}}\right] \times 100$$

Ferric (Fe^{3+}) reducing power activity

The reducing power of methanol root extracts of Liquorice (*Glycyrrhiza glabra*) (10-60 µg/mL) was determined by potassium ferricyanide method [20]. The absorbance was measured at 700 nm using UV-Vis spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of reduction was calculated as:

$$\% \text{ of } Fe^{3+} \text{ reduction} = \left[\frac{\text{Sample} - \text{Control}}{\text{Sample}}\right] \times 100$$

Anti-inflammatory activity by Heat induced haemolysis Membrane stabilization - Preparation of Red Blood cells (RBC's) suspension

The Blood was collected from healthy human volunteer. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The volume of blood was measured and re-constituted as 10% (v/v) suspension with normal saline. Varying concentrations (20-120 µg/mL) of methanol root extracts of Liquorice (*Glycyrrhiza glabra*) was carried out by the haemolytic inhibition method [21]. The absorbance was measured at 560 nm using UV-Vis spectrophotometer. Diclofenac was used as a standard reference. The percentage of inhibition was calculated as:

$$\% \text{ of haemolytic inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}}\right] \times 100$$

Anticancer activity

HeLa cell line was obtained from NCCS (National Centre for Cell Science, Pune) was cultured in Dulbecco Modified Eagle's Medium (DMEM), supplemented with 10% fetal bovine serum, antibiotics. The cell culture was maintained at 37°C in a humidified atmosphere of 5% CO_2. Cells were allowed to grow to confluence over 24 hours before use. With slight modifications, HeLa cells were seeded at a density of 5×10^3 cells/well in 96-well plates for 24 hours in 200 µL of DMEM with 10% FBS. Then culture supernatant was removed and DMEM containing various concentrations of methanol root extracts of Liquorice (*Glycyrrhiza glabra*) (50-350 µg/mL) was added and incubated for 48 hours. After treatment cells were incubated with (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) MTT solution (20 µL, 5 mg/mL in PBS, pH-7.2) at 37°C for 4 hours and then with DMSO at room temperature for 1 hour [22]. The absorbance was read at 570 nm on a scanning multi-well spectrophotometer and the IC_{50} value was calculated.

Gas chromatography–Mass Spectrometry (GC–MS) analysis

For GC-MS analysis, the methanol root extracts of Liquorice (*Glycyrrhiza glabra*) were injected into a HP-5 column (30 m X 0.25 mm i.d with 0.25 µm film thickness), Agilent technologies 6890 N JEOL GC Mate II GC-MS model. Following chromatographic conditions were used: Helium as carrier gas, flow rate of 1 mL/min; and the injector was operated at 200°C and column oven temperature was programmed as 50-250°C at a rate of 10°C/min injection mode. Following MS conditions were used: ionization voltage of 70 eV; ion source temperature of 250°C; interface temperature of 250°C; mass range of 50-600 mass units.

Identification of components

The database of National Institute Standard and Technology (NIST) having more than 62,000 patterns was used for the interpretation on mass spectrum of GC-MS. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library [23].

Results and Discussion

Phenolic acids, known for diverse biological applications, are the main polyphenols produced by plants and work as ancestor for bioactive molecules regularly used in therapeutics, cosmetics, and food industries [24, 25]. The key advantage of using phenolic acids is their metabolizing ability by natural microbes; therefore provide an essential alternate to man-made chemicals which are harmful to environment also (Table 1). Phenolic compounds are known as direct antioxidants; however they also showed indirect antioxidant activity by inducing endogenous protective enzymes and positive regulatory effects on signalling pathways [26].

Table 1: Phytochemical analysis of methanol root extracts of Liquorice (*Glycyrrhiza glabra*)

S. No	Phytochemicals	Tests	Results
1	Alkaloids	(a) Mayer's test	Positive
		(b) Hager's test	Positive
2	Phenols	Ferric chloride test	Positive
3	Tannins	Lead acetate test	Positive
4	Flavonoids	Sodium hydroxide test	Positive
5	Glycosides	Legal's test	Negative
6	Steroids	Libermann-Burchard test	Positive
7	Terpenoids	Salkowski test	Positive
8	Saponins	Foam test	Positive
Quantitative Estimations			
1	Phenols	376.53±0.38 GAE/mg	
2	Flavonoids	108.27±0.20 QE/mg	
3	Steroids	94.16±0.17 Cholesterol/mg	

Plant phenolics can offer an opportunity in this regard and more than half of all anticancer prescription drugs approved internationally between the 1940s and 2006 were natural products or their derivatives and lots of clinical trials are continuing. Plant steroids possess many interesting medicinal, pharmaceutical and agrochemical activities like anti-tumor, immunosuppressive, hepatoprotective, antibacterial, plant growth hormone regulator, antihelminthic, cytotoxic and cardiotonic activity [27].

Thin layer chromatography analysis

The active spots by TLC analysis and retention factor for the compounds were calculated based on the solvent front (Figure 2). Iodine vapour reversible reactions occur with a wide range of organic lipophilic molecules, e.g. hydrocarbons, fats, waxes, some fatty acids and esters, steroids, antioxidants, detergents, emulsifiers, antibiotics and many miscellaneous pharmaceuticals [28].

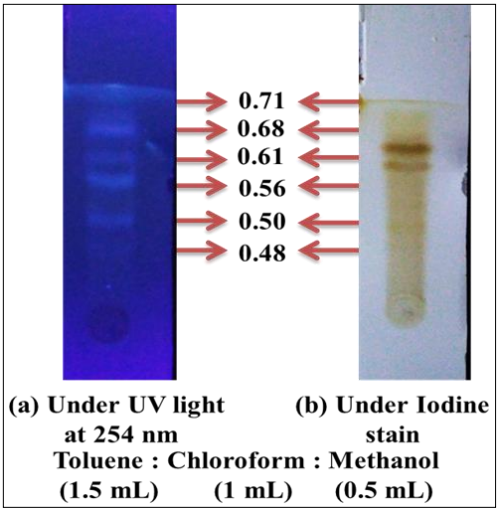


Fig 2: Separation of distinct compounds by Thin Layer Chromatography

Antibacterial activity by Agar well-diffusion method

The cell inhibition is expressed by perturbation of the membrane with phenolic compounds, interacting genetic material with coumarins, chelating metals with flavonoids and flavonols. The antimicrobial effect of a certain antimicrobial agent depends on three main factors: the psycho-chemical properties of the antimicrobial agent (dissociation constant of the acids, solubility, organoleptic properties and hydrophobicity/lipophilicity ratio), the environmental factors (pH, water activity, temperature and structure of food), and the microbiological factors (initial and/or competitive microflora and type, genus, species and strain of the target microorganism) [29-32].

Table 2: Antibacterial activity of methanol root extracts of Liquorice (*Glycyrrhiza glabra*)

Microorganisms	Zone of inhibition (mm)			
	Standard-Tetracycline (30 µg/mL)	250 µg/mL	375 µg/mL	500 µg/mL
B.subtilis	12	13	14	14
M.luteus	16	14	14	15
S.aureus	14	12	14	15
E.coli	18	14	15	16
S.flexneri	14	12	13	13
P.vulgaris	19	20	21	22

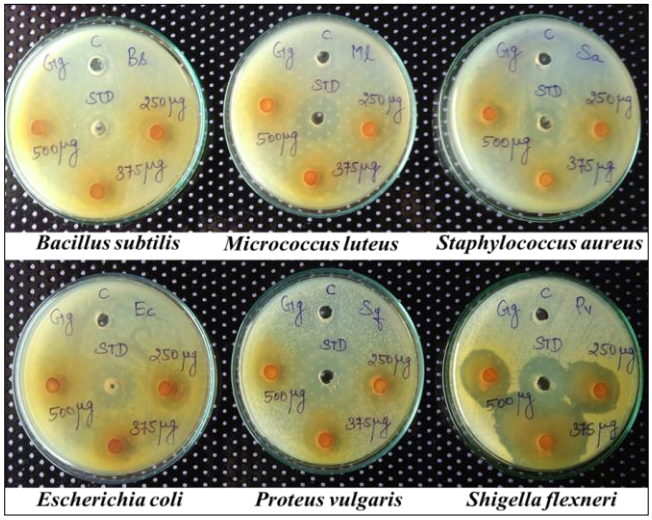


Fig 3: Antibacterial activity of methanol root extracts of Liquorice (*Glycyrrhiza glabra*)

Even if the plant based antimicrobials are obtained from different sources by different methods, the antimicrobial agent has three ways to inhibit a bacterial cell. These targets are destruction of the cell wall, inactivation of essential enzymes and damage of genetic material [33]. The antibacterial activity of methanol root extracts of Liquorice was higher against gram negative pathogens (Table 2 and Figure 3). Plant based antimicrobials that are effective on microbial cells with different mechanisms are generally more effective on Gram-positive than Gram-negative bacteria. Gram-negative bacteria possess an outer membrane that surrounds the cell wall and protects diffusion of hydrophobic substances through its lipopolysaccharide covering [34-36].

In vitro antioxidant activities

Antioxidant capacity calculates might be generally confidential as single electron transfer (SET) and hydrogen atom transfer (HAT) based assays. Preponderances of HAT assays like DPPH and ABTS are kinetics based and include an economical reaction system in which antioxidant and substrate contend for free radicals thermally generated through the disintegration of azo compounds. SET assays measure the capacity of an antioxidant in the reduction of an oxidant which changes colour when reduced. SET assays are easier than HAT assays. SET assays like Phosphomolybdenum (PM) were certain to analyse the reduced capacity. The antioxidant activity of phenolic is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers [37].

DPPH˙ radical scavenging activity

The free radical scavenging activity of the root extract was measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH. The absorbance disappears due to the reduction of 2, 2'-diphenyl-1-picrylhydrazyl radical (purple color solution) to 2, 2'-diphenyl-1- picrylhydrazine (yellow color solution)[38,39]. DPPH radical scavenging activity increased with increasing phenolic components such as flavonoids, phenolic acids and phenolic diterpenes. These phenolic constituents have several hydroxyl groups, containing an o-dihydroxy group which have very strong radical scavenging effect and antioxidant power [40]. The IC_{50} value for methanol root extracts of Liquorice (*Glycyrrhiza glabra*) was 67.90 µg/mL concentration (Table 3 and Figure 4) and was compared with standard ascorbic acid (IC_{50} = 14. 18 µg/mL concentration).

Table 3: DPPH˙ radical scavenging activity of methanol root extracts of Liquorice (*Glycyrrhiza glabra*)

S. No.	Concentration (µg/mL)	% of inhibition*
1	20	14.6±0.28
2	40	22.77±0.13
3	60	36.14±0.16
4	80	58.91±0.40
5	100	74.01±0.38
6	120	77.72±0.20

(*Average of duplicates)

$ABTS^{\bullet+}$ (2,2-azinobis (3-ethylbenzo thiazoline-6-sulfonic acid) radical cation scavenging activity

ABTS radical scavenging assay comprises a process that generates a blue/green/blues green ABTS+ chromophore through the reaction of ABTS and potassium persulfate. The ABTS radical cation is produced by the oxidation of ABTS (Figure 2) with potassium persulfate, reduction capability of

ABTS radical is determined by the decrease in its absorbance since hydrogen donating at 734 nm, induced by antioxidants. It is visible as a change in color from dark bluish green to colorless [41]. The IC_{50} value for methanol root extracts of Liquorice (*Glycyrrhiza glabra*) was 15.99 µg/mL concentration (Table 4 and Figure 4) and was compared with standard ascorbic acid (IC_{50} = 9.73 µg/mL concentration).

Table 4: $ABTS^{\bullet+}$ radical cation scavenging activity of methanol root extracts of Liquorice (*Glycyrrhiza glabra*)

S. No	Concentration (µg/mL)	% of inhibition*
1	5	28.13±0.39
2	10	40.63±0.30
3	15	46.88±0.17
4	20	53.13±0.26
5	25	62.5±0.34
6	30	91.74±0.25

(*Average of duplicates)

Hydroxyl ($OH^{\bullet}$) radical and Nitric oxide radical scavenging activities

The hydroxyl radical is the most reactive of the ROS which induces severe damage in adjacent biomolecules [42]. Iron can stimulate lipid peroxidation by the Fenton reaction and accelerate peroxidation by decomposing lipid peroxide into peroxyl and alcoxyl radical that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation [43]. Hydroxyl radicals (OH) generated in the human body may play an important role in tissue injury at sites of inflammation in oxidative stress-originated diseases. Hydroxyl radicals were formed in free solution and were detected by their ability to degrade 2-deoxy-2- ribose into fragments formed a pink chromogen upon heating with TBA at low pH. Ferric – EDTA was incubated with H_2O_2 and ascorbic acid at pH 7.4 [44]. The IC_{50} value for methanol root extracts of Liquorice (*Glycyrrhiza glabra*) was 51.21 µg/mL concentration (Table 5 and Figure 4) and was compared with standard ascorbic acid (IC_{50} = 14. 18 µg/mL concentration).

Nitric oxide is an unstable free radical involved in many biological processes which are associated with several diseases. It reacts with oxygen to produce stable product nitrate and nitrite through intermediates and high concentration of nitric oxide can be toxic and inhibition of over production is an important goal [45]. The IC_{50} value for methanol root extracts of Liquorice (*Glycyrrhiza glabra*) was 57.53 µg/mL concentration (Table 5 and Figure 4) and was compared with standard ascorbic acid (IC_{50} = 20.69 µg/mL concentration).

Table 5: Hydroxyl ($OH^{\bullet}$) radical and Nitric oxide radical scavenging activities of methanol root extracts of Liquorice (*Glycyrrhiza glabra*)

S. No	Concentration (µg/mL)	% of inhibition*	
		Hydroxyl ($OH^{\bullet}$) radical	Nitric oxide radical
1	10	22.62±0.43	8.97±0.10
2	20	28.57±0.38	11.03±0.27
3	30	32.14±0.24	26.21±0.19
4	40	36.9±0.18	33.79±0.46
5	50	48.81±0.36	43.45±0.17
6	60	63.1±0.11	53.79±0.33

(*Average of duplicates)

Phosphomolybdenum reduction and Ferric (Fe^{3+}) reducing power activities

In TAC by phosphomolybdenum method there is a affinity of extract and fractions to gain color light yellow to dark greenish blue due to reduced molybdate ions in phosphomolybdenum complex [46]. PM assay gives an instant result of a great range of separate antioxidants in dose-response way. Higher degree of color creation designates the more reducing power of analyte [19]. It includes in thermally producing auto-oxidation during lengthy incubation period at higher temperature. It gives a direct estimation of reducing the capacity of antioxidant [47]. The RC_{50} value for methanol root extracts of Liquorice (*Glycyrrhiza glabra*) was 30.59 µg/mL concentration (Table 6 and Figure 4) and was compared with standard ascorbic acid (RC_{50} = 12.90 µg/mL concentration).

The reducing power assay was carried out by the reduction of Fe^{3+} to Fe^{2+} by the methanol root extracts of Liquorice and the subsequent formation of ferro-ferric complex. The reduction capacity increases with increase in concentration of the extract. The RC_{50} value for methanol root extracts of Liquorice (*Glycyrrhiza glabra*) was 34.04 µg/mL concentration (Table 6 and Figure 4) and was compared with standard ascorbic acid (RC_{50} = 10.16 µg/mL concentration). The reducing capacity of the extract was performed using Fe^{3+} to Fe^{2+} reduction process as the yellow colour changes to green or blue colour depending on the concentration of antioxidants [48]. The antioxidants such as phenolic acids and flavonoids were present, considerable amount in methanol root extracts of Liquorice and showed the reducing capacity in a concentration dependent manner.

Table 6: Mo^{6+} reduction and Fe^{3+} reduction activities of methanol root extracts of Liquorice (*Glycyrrhiza glabra*)

S. No	Concentration (µg/mL)	% of reduction*	
		Mo^{6+} reduction	Fe^{3+} reduction
1	10	33.28±0.19	24.18±0.21
2	20	42.19±0.35	37.3±0.39
3	30	49.03±0.47	44.06±0.15
4	40	54.47±0.12	52.29±0.41
5	50	59.04±0.28	65.25±0.30
6	60	73.35±0.34	71.33±0.16

(*Average of duplicates)

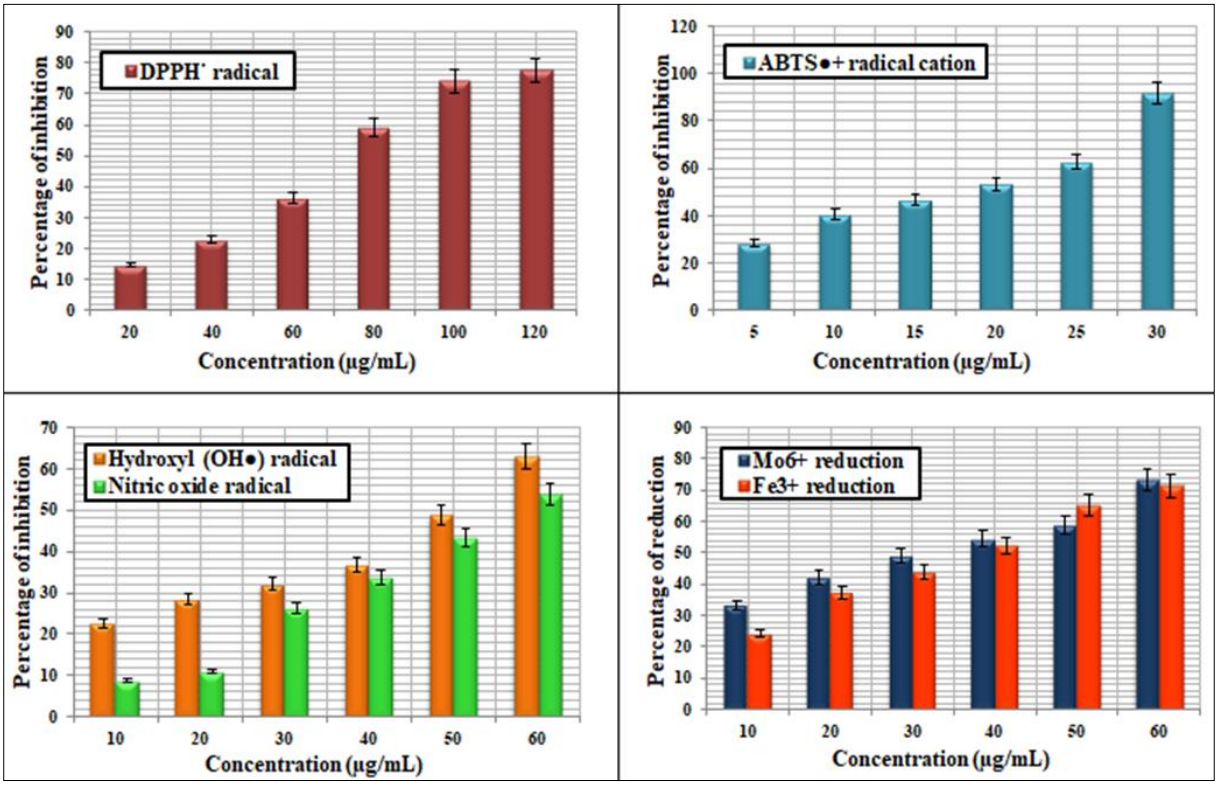


Fig 4: *In vitro* antioxidant activities of methanol root extracts of Liquorice (*Glycyrrhiza glabra*)

Anti-inflammatory activity by Heat induced haemolysis

Haemolysis is due to red blood cells destruction which resulted from lysis of membrane lipid bilayer. Erythrocytes are considered as major target for the free radicals owing to the presence of both high membrane concentration of poly unsaturated fatty acids (PUFA) and the oxygen transport associated with redox active haemoglobin molecules, which potent promoters of activated oxygen species. The erythrocyte model has been widely used as the direct indication of toxicity of injectable formulations as well as general indication of membrane toxicity [49].

The IC_{50} value for methanol root extracts of Liquorice (*Glycyrrhiza glabra*) was 83.03 µg/mL concentration (Table 7 and Figure 5) and was compared with standard Diclofenac (IC_{50} = 18.56 µg/mL concentration).

Table 7: Heat induced haemolysis of methanol root extracts of Liquorice (*Glycyrrhiza glabra*)

S. No	Concentration (µg/mL)	% of inhibition*
1	20	16.35±0.48
2	40	22.43±0.29
3	60	33.42±0.17
4	80	48.17±0.13
5	100	54.04±0.30
6	120	68.28±0.22

(*Average of duplicates)

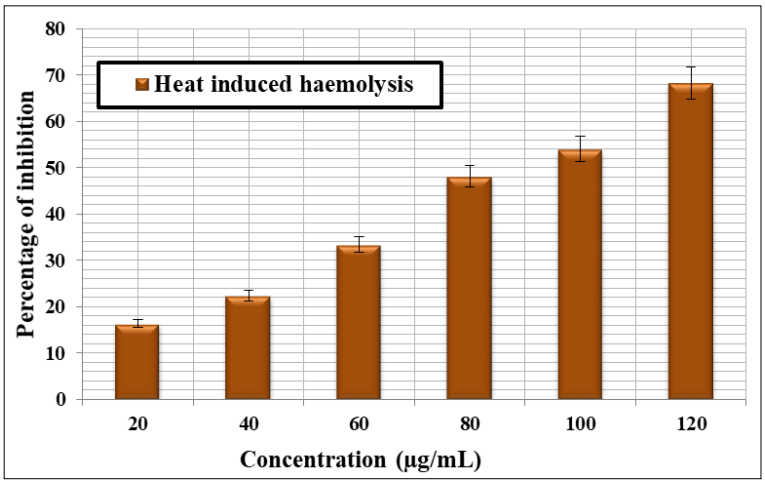


Fig 5: Heat induced haemolysis of methanol root extracts of Liquorice (*Glycyrrhiza glabra*)

Anticancer activity

Licochalcone (LA) is a novel estrogenic flavonoid isolated from *Glycyrrhiza glabra* showed significant antitumor activity in various malignant human cell lines. Evaluation of its anti-cancer activities were carried out in LA-elicited growth control and induction of apoptosis using androgen-independent p53-null PC-3 prostate cancer cells. Licochalcone induced modest level of apoptosis but had more pronounced effect on cell cycle progression arresting cells in G2/M, accompanied by suppression of cyclin B1 and cdc2. It also inhibited phosphorylation of Rb, decreased expression of transcription factor E2F concurrent with reduction of cyclin

D1, down-regulation of CDKs 4 and 6, but increased cyclin E expression [50].

MTT assay in the present research study, revealed that the Liquorice (*Glycyrrhiza glabra*) root extracts can exhibit effective cytotoxic activity in a dose dependent manner. The maximum effect of cytotoxicity of Liquorice (*Glycyrrhiza glabra*) root extracts on HeLa cells was found to be 72.13±0.14% at 350 µg/mL concentration (Table 8 and Figure 6). The IC_{50} value for the root extracts was 185.63 µg/mL concentration (Figure 7). Further, the isolated pure compound from *Glycyrrhiza glabra* shall be subjected to cytotoxic evaluation to understand the exact mode of root extracts against HeLa cell lines.

Table 8: Cytotoxicity effect of Liquorice (*Glycyrrhiza glabra*) root extracts on HeLa cells

S. No	Concentration (µg/mL)	% of cytotoxicity* HeLa cells
1	50	23.45±0.26
2	100	25.01±0.17
3	150	36.6±0.32
4	200	53.87±0.11
5	250	58.16±0.43
6	300	69.36±0.29
7	350	72.13±0.14

(*Average of duplicates)

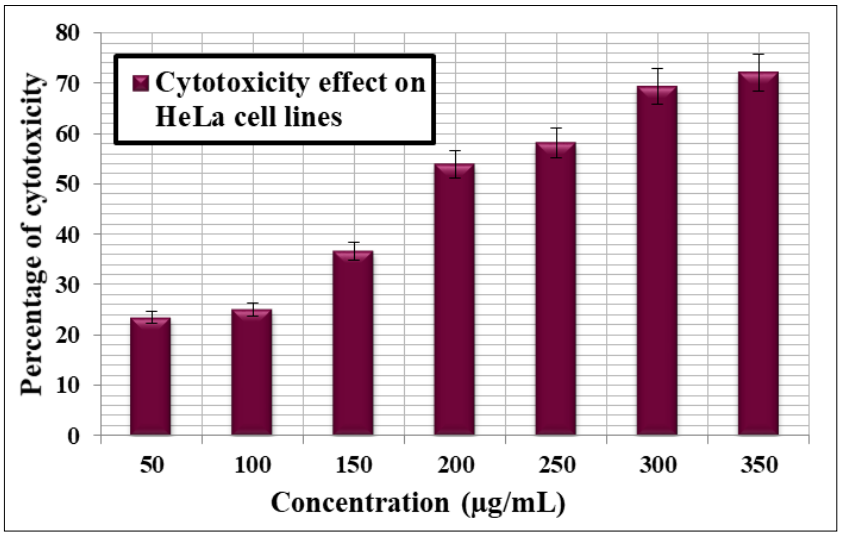


Fig 6: Cytotoxicity effect of Liquorice (*Glycyrrhiza glabra*) root extracts on HeLa cells

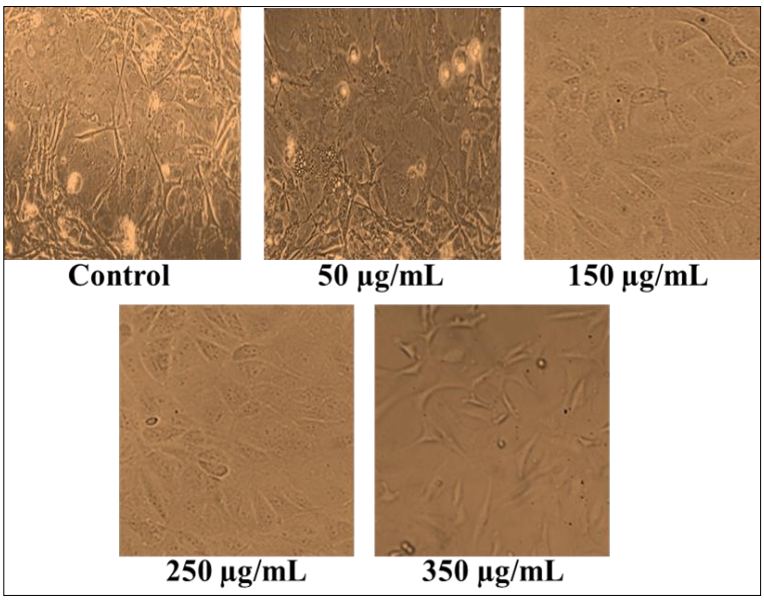
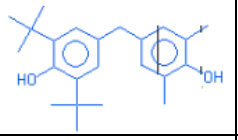
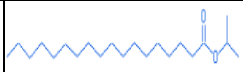
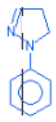
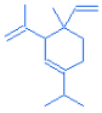
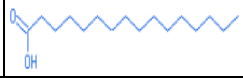
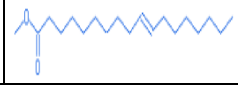
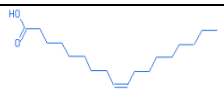
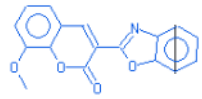
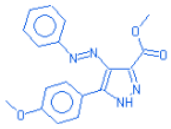


Fig 7: Microscopic view of cytotoxicity effect of Liquorice (*Glycyrrhiza glabra*) root extracts on HeLa cells

Gas chromatography–Mass Spectrometry (GC–MS) analysis

GC-MS is an analytical technique used for many applications which has very high sensitivity and specificity. In recent years GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of nonpolar components and volatile essential oil, fatty acids, lipids and alkaloids. It also plays a fundamental role as an analytical technique for quality control and standardization of phytochemical molecules [51-53]. The GC-MS analysis of methanol root extracts of Liquorice (*Glycyrrhiza glabra*) (Table 9) revealed the presence of different bioactive compounds (phytochemical constituents) that could contribute the antioxidant, antimicrobial and anticancer activities (Table 10). The identification of the phytochemical compounds was confirmed based on the retention time, molecular formula and molecular weight (Figure 8).

Table 9: GC-MS analysis of methanol root extracts of Liquorice (*Glycyrrhiza glabra*)

S. No	RT	Compound Name	Compound Structure	Molecular Weight (g/moL)	Molecular Formula
1	25.85	Phenol, 2,6-bis(1,1-dimethylethyl)-4-[(4-hydroxy-3,5-dimethylphenyl)methyl]-		339.98	$C_{23}H_{32}O_2$
2	21.55	Isopropyl stearate		326.01	$C_{21}H_{42}O_2$
3	11.05	1H-Pyrazole, 4,5-dihydro-1-phenyl-		146.65	$C_9H_{10}N_2$
4	16.05	Alpha Elemene		204	$C_{15}H_{24}$
5	16.55	n-Hexadecanoic acid		256	$C_{16}H_{32}O_2$
6	17.57	10-Octadecenoic acid, methyl ester		296	$C_{19}H_{36}O_2$
7	18.12	Oleic acid		282	$C_{18}H_{34}O_2$
8	18.97	Coumarine, 3-(2-benzoxazolyl)-8-methoxy-		293	$C_{17}H_{11}NO_4$
9	23.38	Pyrazole-3-carboxylic acid, 5-(4-methoxyphenyl)-4-phenylazo-, methyl ester		336	$C_7H_{10}N_2O_2$

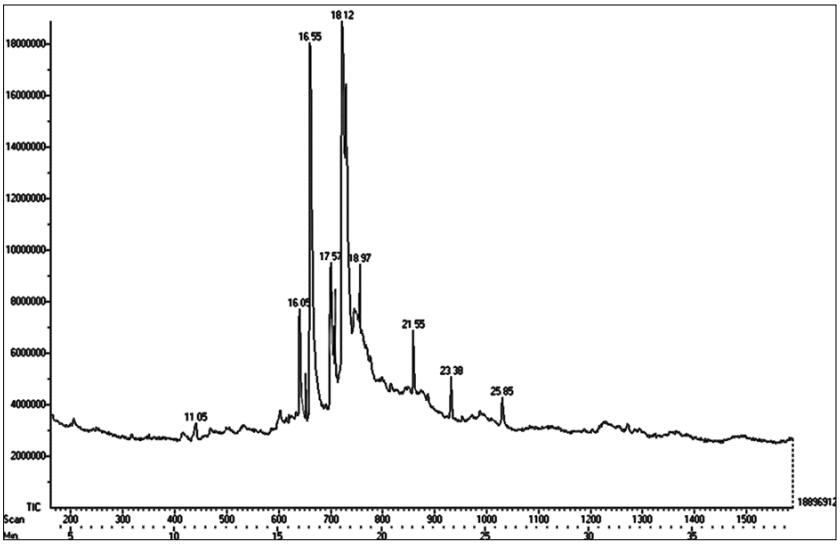


Fig 8: GC-MS Chromatogram of methanol root extracts of Liquorice (*Glycyrrhiza glabra*)

Table 10: Pharmacological activity of methanol root extracts of Liquorice (*Glycyrrhiza glabra*)

S. No	Compound Name	Pharmacological activity [54-58]
1	Phenol, 2,6-bis(1,1-dimethylethyl)-4-[(4-hydroxy-3,5-dimethylphenyl)methyl]-	Antimicrobial activity, Antioxidant activity, Antimalarial activity, Immuno-modulatory effect
2	Isopropyl stearate	An emollient, skin conditioner Binder and humectant activities
3	n-Hexadecanoic acid	Antioxidant activity, Hypocholesterolmic, Nematicide, Pesticide,

		Lubricant, Antiandrogenic activity, Flavor, Haemolytic and 5- Alpha reductase inhibitor activities
4	10-Octadecenoic acid, methyl ester	Antioxidant and antimicrobial activities
5	Oleic acid	5 alpha reductase inhibitor, Hypocholestrolemic activity, Perfumery and flavour, Cancer preventing agent, Anti-inflammatory activity, Antibacterial activity
6	Coumarine, 3-(2-benzoxazolyl)-8-methoxy-	High Protein edema, Chronic Infections, Cancer treatment, Blood Coagulation and Anticoagulant, Inflammation

Conclusion

Historically, pharmacological screening of compounds of natural or synthetic origin has been the source of innumerable therapeutic agents. Researchers are increasingly turning their attention to natural products looking for leads to develop better drugs against many microbial infections. The present study showed that the methanol root extracts of Liquorice (*Glycyrrhiza glabra*) has excellent antioxidant, antimicrobial and potent anticancer activities. Consumers are increasingly interested in complementary and alternative medicines, including herbal medicine, as they perceive these forms of healing as being both safe and effective. This trend in use of alternative and complementary healthcare has prompted scientists to investigate the various biological activities of medicinal plants.

Acknowledgement

The authors are thankful to SAIF, IIT, Madras and Armats Biotek Training and Research Institute for providing facilities to carry out research work.

References

1. Velvizhi S, Annapurani S. Estimation of total flavonoid, phenolic content, and free radical scavenging potential of *Glycyrrhiza glabra* root extract. Asian Journal of Pharmaceutical and Clinical Research 2018;11(4):231-235.
2. Chopra PK, Saraf BD, Inam F. Antimicrobial and antioxidant activities of methanol extract roots of *Glycyrrhiza glabra* and HPLC analysis. International Journal of Pharmacy and Pharmaceutical Sciences 2013;5:157-60.
3. Visavadia NP, Soni B, Dalwadi N. Evaluation of anti-oxidant and anti atherogenic properties of *Glycyrrhiza glabra* roots using *in vitro* models International Journal of Food Sciences and Nutrition 2009;60:135-49.
4. Singh G, Gupta A, Jjalhan S, Jindal A. Pharmacological activities on *Glycyrrhiza glabra*-a review. Asian Journal of Pharmaceutical and Clinical Research 2013;11:5-7.
5. Pastorino G, Cornara L, Soares S, Rodrigues F, Oliveira MBPP. Liquorice (*Glycyrrhiza glabra*): A phytochemical and pharmacological review. Phytotherapy Research 2018;32:2323-2339.
6. Yu JY, Ha JY, Kim KM, Jung YS, Jung JC, Oh S. Anti-inflammatory activities of licorice extract and its active compounds, glycyrrhizic acid, liquiritin and liquiritigenin, in BV2 cells and mice liver. Molecules 2015;20(7):13041-13054.
7. Rizzato G, Scalabrin E, Radaelli M, Capodaglio G, Piccolo O. A new exploration of licorice metabolome. Food Chemistry 2017;221:959-968.
8. Harborne JB. Phytochemical Methods, A guide to Modern Techniques of Plant analysis, second ed. Chapman and Hall, London 1998, 54-84.
9. Raaman N. Phytochemical techniques. New India Publishing Agency, New Delhi 2006, 306.
10. Liu X, Dong M, Chen X, Jiang M, Lv X, Yan G. Antioxidant activity and phenolics of endophytic *Xylaria* sp. from *Ginkgo biloba*. Food Chemistry 2007;105:548-554.
11. Spanos GA, Wrosltad RE. Influence of processing and storage on the phenolic composition of Thompson seedless grape juice. Journal of Agricultural & Food Chemistry 1990;38:1565-1571.
12. Madhu M, Sailaja V, Satyadev TNVSS, Satyanarayana MV. Quantitative phytochemical analysis of selected medicinal plant species by using various organic solvents. Journal of Pharmacognosy and Phytochemistry 2016;5(2):25-29.
13. Stahl E. Thin Layer Chromatography, 2nd ed., Springer Pvt. Ltd., New Delhi, 2005, 85.
14. Eloff JN. Which extractant should be used for the screening and isolation of antimicrobial components from plants. Journal of ethnopharmacology 1998;60:1-8.
15. Khalaf NA, Shakya AK, Al-Othman A, El-Agbar Z, Farah H. Antioxidant activity of some common plants. Turkish Journal of Biology 2008;32:51-55.
16. Arnao MB, Cano A, Acosta M. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chemistry 2001;73:239-44.
17. Smirnoff N, Cumbes QJ. Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry 1989;28:1057-1060.
18. Garrat DC. (The quantitative analysis of drugs), Champman and Hall, Japan 1964;3:456-458.
19. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Analytical Biochemistry 1999;269:337-341.
20. Oyaizu M. Studies on products of browning reaction: antioxidative activities of products of browning reaction prepared from glucosamine. Japanese Journal of Nutrition and Dietetics 1986;44:307-315.
21. James O, Nnacheta OP, Wara HS, Aliyu UR. *In vitro* and *in vivo* studies on the anti-oxidative activities, membrane stabilization and cytotoxicity of water spinach from ipogi ponds (Nigeria). International Journal of Pharm Tech Research 2009;1(3):474-482.
22. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. Journal of Immunological Methods 1983;65:55-63.
23. Brintha S, Sivaraj C, Saraswathi K, Arumugam P,

Jeyanthi Rebecca L. Antioxidant, Antibacterial activities and GC-MS analysis of Methanol extract of buds of *Hypericum hookerianum* Wight and Arnott. Research Journal of Pharmacy and Technology 2020;13(8):0974-3618.
24. Naresh Kumar, Nidhi Goel. Phenolic acids: Natural versatile molecules with promising therapeutic applications 2019;24:e00370.
25. Croft KD. The chemistry and biological effects of flavonoids and phenolic acids, Ann. N.Y. Acad. Sci 1998;854:435-442.
26. Stevenson DE, Hurst RD. Polyphenolic phytochemicals-just antioxidant or much more? Cellular and Molecular Life Sciences 2007;64:2900-2916.
27. Petersen M, Simmonds MSJ. Rosmarinic acid. Phytochemistry 2003;62:121-125.
28. Wall PE, Merck Ltd, Poole, Dorset UK. Chromatography: Thin-layer (PLANAR)/Spray Reagents 2000.
29. Burt S. Essential oils: their antibacterial properties and potential applications in foods: a review. International Journal of Food Microbiology 2004;94:223-253.
30. Juneja VK, Dwivedi HP, Yan X. Novel Natural Food Antimicrobials, Annual review of food science and technology 2012;3:381-403.
31. Lucera A, Costa C, Conte A, Del Nobile MA. Food applications of natural antimicrobial compounds. Frontiers in microbiology 2012;3:1-13.
32. Negi PS. Plant extracts for the control of bacterial growth: efficacy, stability and safety issues for food application. International journal of food microbiology 2012;156(1):7-17.
33. Raybaudi-Massilia RM, Mosqueda-Melgar J, Soliva-Fortuny R, Martin-Belloso O. Control of pathogenic microorganisms in fresh-cut fruits and fruit juices by traditional and alternative natural antimicrobials. Comprehensive reviews in food science and food safety 2009;8:157-178.
34. Rasooli I. Food preservation: a biopreservative approach, Food 2007;1(2):111-136.
35. Tajkarimi MM, Ibrahim SA, Cliver DO. Antimicrobial herb and spice compounds in food. Food control 2010;21(9):1199-1218.
36. Marija R, Pavle S, Dean J, Ljupco A, Sandra M, Mirko P. Antimicrobial activity of plant extracts on some food borne pathogenic and saprophytic bacteria, Days of Veterinary Medicine, 3rd International Scientific Meeting, Macedonia 2012, 176-178.
37. Rice Evans CA, Miller NJ, Bowell PG, Bramley PM, Pridham JB. The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Radical Research 1995;22(4):375-383.
38. Aadesariya MK, Ram VR, Dave PN. Evaluation of antioxidant activities by use of various extracts from *Abutilon pannosum* and *Grewia tenax* in the kachchh region. MOJ Food Processing & Technology 2017;5(1):216-230.
39. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature 1958;181:1199-1200.
40. Matkowski A, Tasarz P, Szypuła E. Antioxidant activity of herb extracts from five medicinal plants from Lamiaceae, subfamily Lamioideae. Journal of medicinal plant research 2008;2(1):321-330.
41. Sahreen S, Khan MR, Khan RA. Evaluation of antioxidant activities of various solvent extracts of *Carissa opaca* fruits. Food Chemistry 2010;122(4):1205-1211.
42. Roghini R, Vijayalakshmi K. Free Radical Scavenging Activity of Ethanolic Extract of *Citrus paradisi* and Naringin -An *In vitro* Study. International Journal of Pharmacognosy and Phytochemical Research 2018;10(1):11-16.
43. Gutteridge JMC. Reactivity of hydroxyl and hydroxyl like radicals discriminated by release of thiobarbituric acid-reactive material from deoxy sugars, nucleosides and benzoate. Biochemical Journal 1984;224:761-767.
44. Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine. 2, illustr ed. Oxford, England, UK: Clarendon Press 1989, 543.
45. Menaga D, Ralakumar S, Ayyasamy PM. Free radical scavenging activity of methanolic extract of *Pleurotus florida* mushroom. International journal of pharmaceutical sciences research 2013;5(4):601-606.
46. Mashwani Z, Khan MA, Irum S, Ahmad M. Antioxidant potential of root bark of *Berberis lyceum* Royle from Galliyat, western Himalaya, Pakistan journal of botany 2013;45:231-234.
47. Marinova EM, Yanishlieva N. Antioxidative activity of extracts from selected species of the family Lamiaceae in sunflower oil. Food Chemistry 1997;58(3):245-248.
48. Stadtman ER. Metal ion-catalyzed oxidation of proteins: Biochemical mechanism and biological consequences. Free Radical Biology and Medicine 1990;9:315-325.
49. Mohammedi Zohra, Atik Fawzia. Hemolytic activity of different herbal extracts used in Algeria. International Journal of Pharma Sciences and Research 2014;5(08):0975-9492.
50. Yue Fu, Tze-chen H, Junqiao G, Jan K, Marietta Y Lee, Zbigniew D *et al*. Licochalcone- A: A novel flavonoid isolated from licorice root (*Glycyrrhiza glabra*), causes G2 and late-G1 arrests in androgen independent PC-3 prostate cancer cells. Biochemical and biophysical research communications 2004;322:263-70.
51. Jie MSF, Choi CYC. J Int. Fed. Clin. Chem 1991;3:122.
52. Betz JM, Gay ML, Mossoba MM, Adams S, Portz BS. J AOAC Int 1997;80:303.
53. Andrew Marston. Role of advances in chromatographic techniques in phytochemistry. Phytochemistry 2007;68:2785-2797.
54. Sivaraj C, Aswitha V, Srinidhi M, Saraswathi K, Arumugam P. Antibacterial, antioxidant activities and GC-MS analysis of leaves extract of *Millingtonia hortensis* L. The Pharma Innovation Journal 2019;8(1):513-521.
55. Boominathan P, Chittibabu CV, Sivaraj C, Saraswathi K, Arumugam P. Antioxidant, antibacterial and GC-MS analysis of ethanol root extract of *Hemidesmus indicus* (L.) R.Br. The Pharma Innovation Journal 2019;8(2):307-315.
56. Elaiyaraja A, Chandramohan G. Comparative Phytochemical Profile of *Crinum Defixum* Ker-Gawler Leaves Using GC-MS. Journal of Drug Delivery and Therapeutics 2018;8(4):365-380.
57. Saraswathi K, Sivaraj C, Arumugam P. Antioxidant Activities, Thin Layer Chromatographic Analysis and GCMS Analysis of *Capsicum annuum* L.: A Comparison of Green and Red Chilli. Journal of Biological and Chemical Research 2019;36(1):184-197.
58. Jain PK, Himanshu Joshi. Coumarin: Chemical and Pharmacological Profile. Journal of Applied Pharmaceutical Science 2012;02(06):236-240.