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Research Article

Evaluation of Phytochemical Constituents and Pharmacological Activities of Ethanolic Extract of *Piper nigrum* Leaves Against DLA in Swiss Albino Mice

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ABSTRACT

The ethanolic extract of *Piper nigrum* leaves against Dalton lymphoma ascites in Swiss albino mice scheduled on phytochemical analysis and various pharmacological activitieswere investigated. Phytochemicals analysis, Antimicrobial and antioxidant activitieswere evaluatedby the standard methods.Anticancer activity was evaluated by liver histopathology, serum biochemical parameters.The phytochemicals analysis showed the presence of alkaloids, saponins, flavonoids and phenols. Total phenolic content was determined using Folin-Ciocalteau reagent found to be 4.78 mg/g of phenolic content and flavonoid content was about 4.88mg/g. It showed a broad-spectrum of antimicrobial and antioxidant activities. Anticancer activity was evaluated by liver histopathology, serum biochemical parameters. The specific 25 compounds were identified by Gas Chromatography–Mass Spectrometryanalysis. Biochemical and histopathological observations indicated that it exerted remarkable hepatoprotective efficacy to its antioxidant mechanisms. The present study suggested that anti-cancer activity of ethanolic extract might be mediated through scavenging of free radicals and it has a significant potential to use as a natural antioxidant agent due to its significantly higher amount of phenolic content.

Key words:*Piper nigrum*, anticancer, antioxidant, Swiss albino mice

INTRODUCTION

Globally a positive trend has blossomed in support of traditional and integrative health science both in research and practices. Natural products are a source of synthetic and traditional herbal medicine (Johnson et al., 2012). Herbal medicine mainly in the developing countries is to promote primary health care with better cultural acceptability and human compatibility (Prashant et al., 2011). Medicinal plants are expensive gift from nature to human (Janakiraman et al., 2012). Recently, there has been a worldwide trend towards the use and ingestion of natural antioxidants present in different parts of plants due to their phytochemical constituents.

Reactive oxygen species (ROS) are various forms of activated oxygen that include Oxygen-derived free radicals (Selvi et al., 2012). Antioxidants may guard against ROS toxicities by the prevention of ROS construction, by disruption of ROS attack, by scavenging reactive metabolites and converting them to less reactive molecules or by enhancing the resistance of sensitive biological target to ROS attack (Siddique et al., 2010). Natural antioxidants are known to exhibit a wide range of biological effects including antimicrobial, anti-inflammatory and antiallergic activities (Ankita et al., 2012). Numerous Natural plant products constituents have proven to show free radical scavenging or antioxidant activity (Valentin et al., 2012).

Cancer is the second leading disease leads to cause of death worldwide next to cardiovascular diseases (Zalkeet al., 2010) and increasing demand to utilize alternative approaches in cancer chemo-prevention. The plant *P.nigrum L.* (Family-Piperaceae), "King of Spices" is highly valuable spicethat widely used in traditionalIndian system of medicines(Ramesh kumar et al., 2011) for various ailments due to its important healthcare system, well-researched phytochemical and anticancer drug which may be synthetic chemicals, microbial products, plant or animal extracts (Saeidnia et al., 2011).

The aim of my present study has been carried out to evaluate the antitumor activity of the ethanolic extract of pepper leaves. There is an ongoing need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and reemerging infectious diseases.Therefore; it was exploration for their potential antioxidant and antimicrobial activity. Further, specific compounds were identified by GC-MS analysis and carried out the hepatic and renal architecture by histopathological studies. Many plants with strong anticancer effect lie unexplored or remain under explored. So there is a need for systematic studies to identify and scientifically validate compounds.

MATERIALS AND METHODS

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Table 1: Quantitative analysis of ethanolic extract of *Piper nigrum* leaves

	Various Extract of Pepper Leaves (100µg/ml)	
Quantitative analysis	Ethanol	Standard
Total phenolic content, GAE (mg/g)	4.78	3.98
Total flavonoid content, GAE (mg/g)	4.88	4.03
DPPH radical scavenging assay	77	88.3
ABTS radical scavenging assay	86.12	82.5
Total antioxidant capacity	1.01	1.132
Reducing power	1.312	1.563
Hydrogen peroxide scavenging activity	20.7	46.9
H_2O_2induced damage activity untreated	36	34.3
treated	28.6	33.2
Nitric oxide generation method	51.3	56.6
superoxide generation method	49.6	66.4

All values are expressed as mean ± SD for three determinations
GAE- Gallic acid equivalent

Plant material*:* The plant *P.nigrum* in the family Piperaceae was collected from the surrounding area of North Parur, Kerala. The leaves were collected, shade dried and coarsely powdered by standard method (Chitravadivu et al., 2009) and stored in an airtight container for further work.

Preparation of extract*:* The fresh leaves parts of *P.nigrum* were dried under shade & powder in a mixture grinder. 5 g powder of plant material used for the extraction. Powdered material extract with 150ml of ethanol in Soxhlet apparatus separately for 3 h. Then ethanol filtrate was concentrated separately on water bath to a thick paste and dried under rotary evaporator (Chitravadivu et al., 2009).The residue was weighed to give the yield of 0.8 g and stored at 4°C in cold room.

Chemicals*:* All chemicals and solvents used in this study were of analytical grade obtained commercially. All chemicals were purchased from Sigma Chemicals Co.,Sd Fine Chemicals Pvt. Ltd, Merck Limited, Mumbai and Hi media, Mumbai.

Qualitative analysis of phytochemicals: Preliminary phytochemical analysis was carried out using the standard analytical procedure(Apurba et al., 2012)to investigate the presence or absence of secondary metabolites.

Quantitative determination of phytochemicals-

Determination of total phenolics content*:* The method with little modification by using Folin-Ciocalteu reagent (Shanmugapriya et al., 2011) was used to determine total phenolics content. 0.5 mL of the extract (1 mg/mL) was mixed with 0.5 mL of 10% Folin-Ciocalteu and was left to stand at room temperature for 1 min. 2.5 mL of sodium carbonate solution was added sequentially in each tube. The resulting mixture was vortexed for 15 sec and incubated at room temperature in the dark at 40°C for 30 min for color development. Reagent blank using distilled water was prepared. The absorbance of total phenolics was measured at 725 nm against the reagent blank using a spectrophotometer. Total phenolics content was expressed as mg/g Gallic acid equivalent (GA).The experiment was conducted in triplicate and the results were expressed as mean ± SD values.

Determination of total flavonoid content (TFC*):* The total flavonoid content was determined using a colorimetric method (Saidu Garda et al., 2012). The extracts were prepared as a 1 mg/ml 60% ethanol solution. Extract was mixed with 0.4 ml of a 5% $NaNO_2$ solution. The mixture was allowed to stay at room temperature for 6 min; 0.4 ml of a 10% $AlCl_3.9H_2O$ solution was added for 6 min followed by the addition of 4 ml 4% NaOH solution and kept at room temperature for 15 min. Absorbance was measured immediately against the reagent blank at 510 nm using a spectrophotometer. Gallic acid was used as the standard and total flavonoid content equivalents (w/w) were determined and expressed as Gallic acid equivalent in mg/g of extract.

Determination of total antioxidant potential-

DPPH radical scavenging assay: The antioxidant activity or Free Radical scavenging activity of extracts against DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) was performed according to the DPPH spectrophotometric method (Abiodunet al., 2011). One milliliter of 0.3 mM DPPH methanol solution was added to 2.5 ml solution of the extract or standard (100µg/ml) and allowed to react at room temperature for 30 min. Methanol (1.0 ml) plus extract solution (2.5 ml) was used as blank. 1 ml of 0.3 mM DPPH plus methanol (2.5 ml) was used as a negative control. Solutions of ascorbic acid served as positive controls. This assay was carried out in triplicates for each concentration. An IC_{50} value denotes the concentration of extracts required to scavenge 50% of DPPH free radicals. After 30 min, the discoloration of DPPH with purple color is reduced to yellow colored diphenyl picryl hydrazine when the antioxidants are present in the sample which was measured at 515 nm in a spectrophotometer. The percentage inhibition of DPPH radical by the sample was calculated using the following formula

DPPH $^+$ scavenging activity = {(Abs control – Abs sample)/ (Abs control)} × 100

Where; Abs control is the absorbance of DPPH + methanol; Abs sample is the absorbance of DPPH + plant extract or standard.

ABTS radical scavenging assay: The method of Balaji et al. (2011) was adopted for the determination of ABTS (2, 2'-azinobis (3-ethylbenzthiazoline)-6-sulphonic acid) activity of the plant extract. The working solution was prepared by mixing two stock solutions of 7 mM ABTS

Table 2: Antimicrobial activity of ethanolic extract of *Piper nigrum* leaves

Microorganisms	Zone of Inhibition (mm)				MIC (µg/ml)
	Ethanolic	Gent	Chl	Nys	
Enterobacterfecalis	14	17	18	NT	04
Haemophilussp	26	18	20	NT	04
Yersinia sp	-	06	24	NT	100
Escherichia coli	18	12	32	NT	100
Bacillus subtilis	14	32	20	NT	100
Staphylococcus aureus	-	12	18	NT	100
Cephalosporiumsp	12	NT	NT	13	100
Aspergillusniger	10	NT	NT	08	50
Penicillumnotatum	10	NT	NT	12	200
Candida albicans	12	NT	NT	08	150

Gent-Gentamycin (10µg/disc),
Chl-Chloramphenicol (30µg/disc),
Nystatin (10µg/disc).
'-'– No significant result, NT- not tested

and 2.45 mM potassium per sulphate in equal amounts and allowed to react for 12-16 hours at room temperature in the dark to yield a concentration of 1 mg/ml. The aliquot of 200 µl of methanolic test solution of each sample was added to 2000 µl of ABTS free radical cation solution. 0.5ml of plant extract was added to 0.3ml of ABTS solution and the resulting solution was further diluted with ethanol by mixing 1 ml of freshly prepared ABTS solution to obtain an absorbance spectrophotometrically at 734 nm after 7 min using spectrophotometer. The percentage inhibition of $ABTS^+$ radical by the extract was calculated and compared with that of BHT as positive control using the above said formula.

Determination of total antioxidant capacity: The antioxidant activity of the extract was evaluated by the Phosphomolybdenum method according to the standard procedure (Badarinath et al., 2010). A 0.3 ml (75mg) of extract was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95ºC for 90 min. Then the absorbance of the solution was measured at 695 nm using a UV-visible spectrophotometer against a reagent blank after cooling to room temperature. The experiment was conducted in triplicates. The antioxidant activity is expressed as the number of gram equivalents of ascorbic acid.

Reduction Potential: The reducing power of *P.nigrum* was evaluated according to the method previously described (Shanmugapriya et al., 2011). Extract (0.5 ml) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) and the absorbance was measured spectrophotometrically at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power of the plant extract. Ascorbic acid (0.2 mg/ml) was used as the standard. Phosphate buffer (pH 6.6) was used as blank sample solution. The experiment was conducted in triplicates and values are expressed as equivalents of ascorbic acid in µg / mg of extract.

Determination of free radical scavenging activity

Hydrogen Peroxide Scavenging Activity: The ability of the ethanolic leaf extract of *P.nigrum* to scavenge hydrogen peroxide was determined (Rameshkumar et al., 2011). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH7.4). Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230 nm in a spectrophotometer. Plant extracts (4 ml) at concentration of 10 mg/10 µl in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40 mM) and incubated for 10 min. Absorbance of hydrogen peroxide at 230 nm was determined after ten minute against a blank solution containing in phosphate buffer buffer (0.1 M pH 7.4) without hydrogen peroxide. The percentages of scavenging of hydrogen peroxide of ethanolic leaf extract of *P.nigrum* and compared with that of BHT as positive control using equation that presented earlier.

Hydroxyl Radical Scavenging Assay: Hydroxyl radical scavenging activity was assayed as described (Thambiraj et al., 2012) with a slight modification. 20 µl of the plant extracts were added and made up final volume of 1.0 ml. The reaction mixture contained, in a final volume of 1 ml, 2-deoxy-2-ribose (2.8 mM); KH_2PO_4-KOH buffer (20 mM, pH 7.4); $FeCl_3$ (100 µM); EDTA (100 µM); H_2O_2 (1.0 mM); ascorbic acid (100 µM) and various concentrations (0–200 µg/ml) of the test sample or reference compound. After incubation for 1 h at 37°C, 0.5 ml of the reaction mixture was added to 1 ml 2.8% TCA, then 1 ml 1% aqueous TBA was added and the mixture was incubated at 90°C for 15 min to develop the pink colour. After cooling, the absorbance was measured at 532 nm against an appropriate blank solution. All tests were performed three times.

Nitric oxide radical scavenging activity: The nitric oxide radical scavenging activity of ethanolic leaf extract of *P.nigrum* was assayed (Sudhanandh et al., 2012). 3.0 ml of 10 mM sodium nitroprusside in phosphate buffer is added

Table 3a: Anticancer activity by assessment of enzymatic antioxidants

Group	Catalase (CAT) (U/mgprotein)		Superoxide dismutase (SOD) (U/mg protein)		Glutathione peroxidase (GPx) (U/mg protein)		Glutathione reductase (U/mg protein)	
Day	7thday	14thday	7thday	14th day	7thday	14thday	7thday	14thday
Normal Control	5.9 ±0.04	6.19 ±0.02	1.92 ±0.02	2.12 ±0.01	0.084 ±0.01	0.091 ±0.02	0.447 ±0.02	0.456 ±0.02
DMSO*	6.1 ±0.01	6.15 ±0.03	2.23 ±0.03	2.46 ±0.03	0.065 ±0.02	0.083 ±0.01	0.414 ±0.02	0.419 ±0.04
Pepper*	6.01 ±0.03	6.21 ±0.01	2.56 ±0.01	2.96 ±0.02	0.103 ±0.02	0.113 ±0.03	0.465 ±0.03	0.469 ±0.03
DLA Control**	3.55 ±0.01	3.57 ±0.04	2.42 ±0.02	2.8 ±0.02	0.006 ±0.03	0.015 ±0.02	0.303 ±0.01	0.311 ±0.02
DLA+ extract***	4.37 ±0.02	4.51 ±0.03	1.03 ±0.03	1.41 ±0.01	0.043 ±0.04	0.48 ±0.01	0.356 ±0.04	0.365 ±0.01
Silymarin+ DLA***	5.1 ±0.01	5.93 ±0.03	0.85 ±0.01	1.06 ±0.02	0.103 ±0.01	0.123 ±0.04	0.375 ±0.02	0.396 ±0.02

The values are the mean ± standard deviation of six animals.

*Where, *- No significance **- p<0.01 significance ***- p<0.05 significance*

to 2.0 ml of extract and reference compound in different concentrations (20-100µg/ml). The resulting solutions are then incubated at 25°C for 60 min. To 5.0 ml of the incubated sample, 5.0 ml of Gries's reagent is added and absorbance of the chromophore formed is measured at 540 nm. After incubation, 0.5ml of the reaction mixture containing nitrite was removed, 1 ml of sulphanilic acid reagent was mixed well and allowed to stand for 5 minutes for completingdiazotization, and then 1ml of 1-Naphthylamine (5%) was added, mixed and allowed to stand for 30 minutes. A pink colored chromophore is formed in diffused light. Ascorbic acid is used as a positive control.

Superoxide anion radical scavenging assay*:* Measurement of superoxide anion scavenging activity was performed (Rameshkumar et al., 2011). The assay mixture contained sample with 0.1ml of Nitro blue tetrazolium (1.5 mM NBT) solution, 0.2 ml of EDTA (0.1M), 0.05 ml riboflavin (0.12 mM) and 2.55 ml of phosphate buffer (0.067 M phosphate buffer). The reaction mixture was illuminated for 30 min and the absorbance at 560 nm was measured against the control samples. Ascorbic acid was used as the reference compound. All the tests were performed in triplicates. The difference in optical density before and after illumination is the generation of superoxide by the test sample and calculated by comparing with the OD of the control.

Antimicrobial activity

Antimicrobial screening*:* The bacterial and fungal test organisms were obtained from School of Biotechnology, Dr.G.R.Damodaran College of science, Coimbatore. The bacterial strains included three gram-negative (*Escherichia coli, Bacillus subtitis* and *Staphylococcus aureus*) and three gram positive bacteria (*Enterobacter* sp., *Haemophilus* sp. and *Yersinia* sp.). Four fungal strains included *Cephalosporin sp, Aspergillus niger, Penicillum notatum* and *Candida albicans*. The organisms were maintained on nutrient agar and Sabouraud dextrose broth at 4 ºC and subcultured before use.

Antimicrobial susceptibility test: Antimicrobial activity of the ethanolic extract were performed against five six bacterial strains and four fungal strains using disc diffusion method (Shanmugapriya et al., 2011). Under aseptic condition, the liquid nutrient agar medium for bacteria was poured in sterilized Petriplates and spread evenly to a depth of 4mm. Sterile filter paper disc's (Whatman No.1, 6mm) was impregnated with 0.5 ml and 1ml of the plant extract to give a final concentration ranges from 50 to 200 µg/ml and left to dry under laminar flow cabinet for few minutes. Standard antibiotic concentration of Chloramphenicol (30 µg/disc) and Gentamycin (10 µg/disc) was used to confirm that all the microorganisms tested were inhibited by the antibiotics. The fungal isolates were treated in a slightly different way. A sample concentration range from 50 to 200µg/ml was compared with standard Nystatin (10µg/disc). The bacterial plates were incubated at 37ºC for 24 h, while the fungal plates were incubated at 25ºC (room temp.) for 72 h. The zones of inhibition were measured in mm diameter and recorded.

Determination of Minimum Inhibitory Concentration (MIC): The Minimum Inhibitory Concentration of the crude extracts was carried out using the method of agar disc diffusion (Shanmugapriya et al., 2011). One hundred microliter of the standardized cell suspensions of bacteria and fungi were spread on a molten Nutrient agar and Potato dextrose agar aseptically, mixed gently in sterile Petri-dishes, and then allowed to set respectively. Filter discs were prepared from whatman No.1 and sterilized. The discs were placed on agar plates and impregnated with different dilution of extracts. The plates were later incubated in bacteria at 37 °C for 24 h and in fungi at 25 °C for 72 h respectively after about 30 min of inoculation. The lowest concentration preventing visible growth of the test microorganism in each determination was regarded as the minimum inhibitory concentration of the extracts. The mean of three replicate determinations was obtained.

Evaluation of anticancer studies against DLA in Swiss albino mice: The anticancer activity of ethanolic extract of *P.nigrum* leaf extract against Ehrlich ascites carcinoma (EAC) in Swiss albino mice was evaluated (Venu Gopal et al., 2012). Dalton's Lymphoma ascites cell line inoculated mice was supplied byC. Abdul Hakeem College,

Melvisharam, Tamil Nadu, India with the prior permission of the Institutional Animal Ethics Committee (No.1011/c/06/CPCSEA, dated 19.12.2006) for my present Investigation. It was selected for *In vivo* studies by the intraperitoneal administration in ethanolic extract of Pepper leavesto examine antioxidant activity and histophathological examination of liver tissues.

Animals: The experiment was carried out on Swiss albino mice with weighing between 20-25gm. The animals were housed in micro nylon boxes under control temperature of 25±2ºC for 12hrs dark/ light cycle with standard laboratory diet and water *ad libitum*. The study was conducted after obtaining permission from institutional animal ethical committee regulations. All the groups were injected with DLA cells expect normal group as day zero. All experiments were performed during morning according to CPCSEA guidelines for care of laboratory animals and the ethical guideline for investigations of experimental pain in conscious animals. Number of mice in each group was five and divided into six groups. It includes Group I: Normal control; Group II: DMSO control; Group III: Ethanolic extract of Pepper leaves; Group IV: DLA control; Group V: Pepper leaves extract and DLA; Group VI: Silymarin control.

Experimental Design: DLA cells were obtained through the courtesy of Amala Cancer Research Center, Thrissur and maintained by weekly intraperitoneal inoculation (Sasmalet al., 2012). All the groups were injected with EAC cells except the group I. This was taken as day zero. From day, normal control was given in Group I, 0.1ml of Dimethyl sulfoxide every day, intraperitoneally and served as a vehicle control for the experimental groups 2 to 4; the ethanolic extract of pepper leaves (100 mg/kg) intraperitoneally were given to Group III; Nearly $1x10^6$ DLA tumor cells, intraperitoneally and was served as DLA control to this group IV; *P.nigrum* leaf extract as plant sample were given. DLA tumor cells and plant extract were administered on the next day and administration of extract continued for 15 days for group V, Group VI served as positive control and fed with 0.3ml of standard antioxidant silymarin in DLA tumor cells. On 15^{th} day, all mice from each group were sacrificed, 24h after last dose and an overnight fasting. The parameters were used for determination of antioxidants and Histopathological study.

In vivo Assessment of Enzymatic antioxidants: Enzymatic antioxidants such as Catalase, Superoxide dismutase, Glutathione peroxidase and Glutathione Reductase were assessed in the liver of Swiss Albino mice by the standard procedure (Rameshkumaret al., 2011; Periyanayagamet al., 2012). All the methods were carried in triplicates.

In vivo Assessment of non-enzymatic antioxidants: The levels of the non-enzymatic antioxidants such as Vitamin A, E and C were also assessed in the liver by standard method (Periyanayagam et al., 2012). All the methods were carried in triplicates.

In vivo Assessment of liver marker enzymes: Liver marker enzymes include reduced glutathione, Lipid Peroxides (TBARS), Acid Phosphatase (ALP), Alkaline Phosphatase (ACP), Serum Glutamate Oxaloacetate Transaminase (SGOT) and Serum Glutamate Pyruvate Transaminase (SGPT) Venu (Gopal et al., 2012; Periyanayagamet al., 2012) were assessed in the liver of Swiss albino mice by standard method. Blood is collected by cardiac puncture and the serum is separated by centrifugation. The preparation of serum sample was stored at 2-8°C. All the methods were carried in triplicates.

Histopathological examination of tissues: The mice were sacrificed and the desired liver of each group were collected at the end of the experiment to evaluate for histopathological study in ethanolic extract of pepper leaves (Venu Gopal et al., 2012; Periyanayagamet al., 2012).

Gas Chromatography-Mass Spectroscopy analysis (GC-MS***):*** The compounds present in ethanolic extract of pepper leaves was analyzed by GC-MS. The column used was TR5-MS Capillary standard Non-Polar Column. The GC column dimension was 30X0.25mmX0.25µm. The GC condition maintained were; the injector temperature was about 250 ºC, column temperature isothermal at 100 ºC, then programmed to rise upto 250 ºC at 6 ºC/min and be held at this temperature for 10 min. The ion source temperature was 200ºC and the interface temperature is 259 ºC. Helium gas was engaged as a carrier gas at the rate of 1 ml/min. The spectra were obtained in the EI mode with 700 x ionization energy. The compounds were identified based on the comparison of retention indices (RI), retention time (RT) and mass spectra by comparing with standard of the mass spectra was matched with inbuilt library (Sundarammal et al., 2012).

RESULTS

Qualitative analysis of phytochemicals: Plants constitute an important source of many secondary metabolites. All the pepper leaves extracts were tested preliminary for the presence of constituents such as alkaloids, flavonoids, saponins, tannins and phenols. Among all standard tests, ethanolic extracts showed the presence of alkaloids, saponins, flavonoids and phenols whereas tannin was absent. Based on this qualitative analysis, pepper leaves showed good source of phytochemicals.

Quantitative analysis of phytochemicals: The total phenolic and total flavonoid content were found to be 4.78µg/ml and 4.88µg/ml respectively (Table1).

Antioxidant activity: All the extract (100µg/ml) showed good scavenging activity of DPPH radical compared with standard antioxidant, Butyl Hydroxyl Toluene at 20µg/ml. Among them, ethanolic extract of pepper leaves revealed good antioxidant activity. ABTS radical cation decolorization assay is also another total antioxidant activity screening method. Averagely the extracts of pepper leaves showed the inhibition of ABTS radical. Ethanolic extract showed the maximum percentage of scavenging ABTS radical. The results obtained on the detection of total antioxidant activity of various extract pepper leaves at 695 nm. At 700 nm absorbance the ethanolic extract of pepper leaves indicated high reducing power compared to reducing ability of BHT standard. From the result, it appeared that the hydrogen peroxide scavenging activity of the extract of pepper leaves is negligible when compared to the standard BHT. The

Table 3b: Anticancer activity by assessment of Non-enzymatic antioxidants

Group	Vitamin A(mg/kg tissue)		Vitamin E(mg/kg tissue)		Vitamin C(mg/kg tissue)	
Day	7thday	14th day	7th day	14th day	7th day	14th day
Normal Control	0.786±0.01	0.793±0.03	1.234±0.01	1.346±0.04	2.069±0.04	2.248±0.04
DMSO*	0.732±0.02	0.748±0.02	1.459±0.02	1.528±0.01	2.483±0.01	2.698±0.02
Pepper*	0.724±0.01	0.835±0.04	2.489±0.04	2.53±0.04	3.025±0.03	3.369±0.01
DLA Control**	0.622±0.04	0.635±0.02	2.513±0.01	2.582±0.01	3.019±0.01	3.439±0.03
DLA+ extract***	0.675±0.03	0.698±0.04	1.031±0.05	1.124±0.02	1.516±0.01	1.878±0.01
Silymarin + DLA***	0.697±0.01	0.7±0.01	0.98±0.02	1.08±0.04	1.128±0.02	1.419±0.02

The values are the mean ± standard deviation of six animals.
Where,- No significance **- p<0.01 significance ***- p<0.05 significance*

ethanolic extract showed 20.7 % of scavenging of hydrogen peroxide. The effect of selected medicinal plant leaf extract on H_2O_2 induced damaged to deoxyribose was quantified as the amount of TBARS formed. Among all, ethanolic extract exhibited potential inhibiting activity against NO generation. 51.3 % of ethanolic extract is compared with 56.6 % of standard BHT in inhibition of nitric oxide (Table2). Ethanolic extract of pepper leaves have maximum ability to inhibit this NBT formation. The decrease in absorbance at 560nm with antioxidant indicates the consumption of superoxide anion in the reaction mixture. All the results obtained were summarized in Table 1 and suggested that the phenolic compounds may be responsible for the free radical scavenging activity as well as inhibitory action against the radical generation.

Antimicrobial activity: The antibacterial activity of ethanolic leaves extract of pepper was evaluated and screened for activity against *S. aureus*, *E. coli*, *Haemophilus* sp., *Yersinia* sp.,*B. subtilis* and *Enterobacter fecalis* by agar well diffusion method. Results were compared with the standard drugs such as gentamycin and chloramphenicol for bacterial cultures. The extract at the concentration of 50 mg/ml showed appreciable zone of activity against bacterial pathogens tested.The highest zone was against the *Haemophilus* sp. and the moderate zone was seen against *E. coli*, *B. subtilis*, *Enterobacter fecalis* and *S. aureus.* There was no zone in *Yersinia* sp. The potency of the crude extracts was comparable to antibiotics which are pure substances. Gentamycin (10 µg/disc) and Chloramphenicol (10 µg/disc) have a universal activity against the entire test organism, with zones of inhibition ranging from 6 to 32 mm and 18 to 32 mm respectively (Table2).

The antifungal activity of ethanolic extract of pepper leaves was evaluated using agar well diffusion method. The extracts were tested against *C. albicans*, *A.niger*, *Cephalosporium* sp., and *P. notatum* at 100 mg/ml and 200 mg/ml concentration. It exhibited significant activity against all the tested fungi compared with the standard drug, Nystatin (10µg/disc). The extract showed moderate inhibition against *C. albicans* and *P.notatum.* No appreciable zone of inhibition was observed against *A. niger* and *Cephalosporium* sp. The ethanolic extract showed good activity against the fungal isolates with zones of inhibition ranging from 12 to 14 mm (Table 2). In conclusion, the results showed that the ethanolic leaves extract of *piper nigrum* is a broad spectrum agent which can be used against both gram positive and gram negative bacteria and also fungi.

Anticancer activity of the drug against Ehrlish ascites carcinoma*:* Body weight of the experimental animals were recorded both in the treated and control groups at the beginning of the experiment (day 0) and sequentially on 7th and 14th days. The weight of the normal animals showed no increases in 7th and 14th days. The DMSO controlled and pepper controlled groups indicated that least increases in the body weight. The DLA tumor inoculated control animals recorded significant weight gain by day 0. They gained maximum weight 18.5 % by day 15th. The extract treated with DLA showed reduction in weight gain compared to the control on day 15th. The Standard drug silymarin treated animals also showed least increases in weight.

In vivo Assessment of enzymatic antioxidants: The ethanolic extract of pepper leaves showed significant anti-tumor and antioxidant activity in DLA control bearing mice. Group I served as normal mice and group II and III showed the effect of DMSO and pepper extract in normal mice. Group III detected the DLA bearing mice and group IV and V studied for antitumor activity of ethanolic extract of pepper leaves in DLA cells and the standard Silymarin activity. It was found to be significantly increased in mice administered with ethanolic content of pepper leaves when compared to control mice and decreased in DLA induced mice when compared to control mice. It also increased when administered with Silymarin and showed significant activity indicates the protective effects. The results were tabulated in Table3a.

In vivo Assessment of non-enzymatic antioxidants*:* In non-enzymatic treated mice, the DLA inoculated mice showed significant ($p<0.01$) decreases in vitamin A, E and C when compared with normal mice. After administration with ethanolic extract showed significant increases ($p<0.05$) in vitamins level when compared to control mice and DLA alone induced mice. The vitamins activity was also increased when administered with Silymarin at 20 mg/kg. Significant increase in vitamins content increase in mice treated with DLA and plant extract. The resulted raise

Table 3c: Anticancer activity by assessment of liver marker enzymes

Group	Reduced glutathione(GSH) (mg/kg tissue)		Lipid Peroxidation (mg/kg tissue)		SGOT (mg/kg tissue)		SGPT (mg/kg tissue)		ALP (mg/kg tissue)		ACP (mg/kg tissue)	
Day	7th day	14th day	7th day	14th day	7th day	14th day	7th day	14th day	7th day	14thday	7th day	14th day
Normal Control	9.989 ±0.01	10.035 ±0.02	0.255 ±0.04	0.261 ±0.02	53.21 ±0.02	56.72 ±0.04	64.59 ±0.04	66.72 ±0.04	52.31 ±0.01	124 ±0.02	120.16 ±0.03	53.3 ±0.01
DMSO*	10.486 ±0.01	10.689 ±0.03	0.295 ±0.02	0.301 ±0.04	60.23 ±0.01	62.01 ±0.03	62.61 ±0.02	61.63 ±0.03	53.68 ±0.01	124.3 ±0.01	121.62 ±0.03	52.69 ±0.01
Pepper*	12.265 ±0.04	12.483 ±0.01	0.221 ±0.03	0.229 ±0.01	51.34 ±0.02	54.62 ±0.02	53.22 ±0.03	56.18 ±0.03	48.56 ±0.02	113.16 ±0.01	111 ±0.02	49.32 ±0.04
DLA Control**	12.407 ±0.02	12.819 ±0.02	0.199 ±0.04	0.205 ±0.03	49.34 ±0.01	52.71 ±0.02	51.12 ±0.03	54.92 ±0.01	47.61 ±0.01	105.14 ±0.01	180.12 ±0.02	85.61 ±0.02
DLA+ extract***	10.017 ±0.01	10.224 ±0.03	0.481 ±0.03	0.486 ±0.01	76.45 ±0.02	78.8 ±0.01	53.72 ±0.02	56.03 ±0.01	73.42 ±0.01	155.24 ±0.02	153.21 ±0.02	70.32 ±0.03
Silymarin+ DLA***	9.039 ±0.03	9.478 ±0.04	0.518 ±0.01	0.526 ±0.02	82.5 ±0.01	84.6 ±0.01	63.86 ±0.02	65.38 ±0.02	82.54 ±0.02	182.22 ±0.01	143.12 ±0.01	54.61 ±0.02

The values are the mean ± standard deviation of six animals.
*Where, *- No significance **- p<0.01 significance*
ALP-Acid Phosphatase; ACP-Alkaline Phosphatase;
SGOT -Serum Glutamate Oxaloacetate Transaminase; SGPT- Serum Glutamate Pyruvate Transaminase.

in vitamins treated with ethanolic extract of pepper leaves was illustrated in Table3b.

In vivo Assessment of liver marker enzymes*:* Lipid peroxidation, an autocatalytic free radical chain propagating reaction is known to be associated with pathological conditions of a cell. After administration with ethanolic extract at 100 mg/kg, significantly ($p<0.05$) reduced the MDA levels when compared with DLA control animals. This significant ($p<0.05$) reduction of MDA is also seen in standard Silymarin (20 mg/kg). Results were expressed as nmoles MDA/mg proteins/ml. The levels of reduced and acid GSH were found to be increased on administration with ethanolic extract at 100mg/kg and standard silymarin at 20 mg/kg when compared with DLA control groups. The result for LPO, reduced glutathione, TBARS, reduced GSH, SGOT and SGPT in liver tissue of experimental animals were tabulated in Table 3c. Effect of ethanolic extract of the pepper leaves at 100mg/kg on serum alkaline phosphatase and acid phosphatase of tumor inoculation in normal and tumor bearing mice was detected. All these results clearly indicated that the ethanolic extract of pepper leaves have a remarkable capacity to inhibit the growth of solid tumor and increases the life span by decreasing the activities fluid volume and arresting the tumor induced by DLA in experimental animals.

Histopathological examination of tissues*:* The results obtained with the liver section were as follows: Group I sacrificed on the 7th and 14th day showed structure of liver. The hepatocytes in all the three zones, sinusoids, portal triads, Kupffer cells and central veins appeared normal. Group II on the 7th and 14th day showed large confluent areas of hepatocellular necrosis with peripheral rim of surviving hepatocytes that showed focal steatosis and balloon degeneration. There was no obvious tumor identified. Group III on the 7th and 14th day showed structure of liver. The hepatocytes in all the three zones, sinusoids, portal triads, Kupffer cells and central veins appear normal. There is no evidence of liver cell necrosis-suggestive of reversal of tissue injury. Group IV showed structure of liver. The hepatocytes in all the three zones, sinusoids, portal triads, Kupffer cells and central veins appeared normal. There was no evidence of any pathological changes-DMSO did not cause structural changes to liver morphology. Group V on 7th day showed focal areas of hepatocellular necrosis that found to involve in zone 3 hepatocytes. There were also lymphocytes and histiocytes in this region. Group V on the 14th day showed complete absence of hepatocellular necrosis. The third zone hepatocytes [around the central veins] did not show evidence of necrosis / fatty change or inflammation. There are also no lymphocytes and histiocytes in suggestive regions of complete reversal of lesions. Group VI on the 7th and 14th day showed structure of liver. The hepatocytes in all the three zones, sinusoids, portal triads, Kupffer cells and central veins appeared normal. There was no evidence of any pathological changes of plant extract did not cause structural changes to liver morphology.

GC-MS analysis*:* Results of the GC-MS analysis showed 25 compounds were present in ethanolic extract of pepper leaf based on their molecular weight and area percentage (Figure1) and identified through mass spectrometry attached to GC and the data were tabulated in Table4.

DISCUSSION

Qualitative analysis of phytochemicals: Based on this qualitative analysis, pepper leaves showed good source of phytochemicals. Phytochemical reports of *P.nigrum* showed different class of compounds such as lignans, amides, long-chain esters, terpenoids, steroids, pyrones, chalcones and flavonoids. It also possesses anti-mutagenic, anti-tumor, and antioxidant activities, and the compound also enhances the bioavailability of drugs (Rameshkumar et al., 2011).Saponin is used as mild

Table 4: Compounds identified in ethanolic extract of *Piper nigrum* leaves by GC-MS analysis

S.No	Compounds	M. Formula	M.W	Area %
1	1,2,3-Tri-tert-butyldimethylsilyloxybenzene	$C_{24}H_{48}O_3Si_3$	468	0.31
2	Tsitsixenicin B	$C_{24}H_{34}O_6$	418	0.38
3	(6R)-2,6-Dimethyl-7-[4-methyl-5-trimethylsilyl-2-furanyl]-6-[(trimethylsilyl)oxy]-2,3-heptanediol	$C_{20}H_{40}O_4Si_2$	400	0.54
4	3-Nonyl-N-nitro-5-sydnone-imine	$C_{11}H_{20}N_4O_3$	256	0.39
5	7-(3,5-Dinitrobenzoyloxy)-7-vinylbicyclo[4.2.0]octane	$C_{17}H_{18}N_2O_6$	346	0.47
6	Stigmast-5-en-3-ol, (3á)- (CAS)	$C_{29}H_{50}O$	414	0.40
7	1-Dotriacontanol (CAS)	$C_{32}H_{66}O$	466	0.80
8	1,2-Benzenedicarboxylic acid, butyl octyl ester (CAS)	$C_{20}H_{30}O_4$	334	0.37
9	Ethyl (Z)-2,2-difluoro-3-hydroxyoctadec-13-enoate	$C_{20}H_{36}F_2O_3$	362	0.41
10.	Methyl Ester Of Ricinoleic Acid	$C_{19}H_{36}O_3$	312	0.51
11.	4-{2'-[(t-Butoxycarbonyl)amino]ethyl]-2,2-bis(trifluoromethyl)-1,3-oxathiol an-5-one	$C_{12}H_{15}F_6NO_4S$	383	0.88
12.	betulonal	$C_{30}H_{46}O_2$	438	0.87
13.	15-acetoxy-à-cadinol	$C_{17}H_{28}O_3$	280	0.64
14.	ethyl ester of eicosanoic acid	$C_{22}H_{44}O_2$	340	0.58
15	n-Cyclohexylmethoxymethylketenimine	$C_{10}H_{17}NO$	167	0.95
16	Tsitsixenicin B	$C_{24}H_{34}O_6$	418	0.91
17.	1,3-bis[(t-Butyldimethylsilyl)oxy]-5-[(p-methoxybenzyl)oxy]-2-methylpentane	$C_{26}H_{50}O_4Si$	482	1.94
18.	Isophytol	$C_{20}H_{40}O$	296	296
19.	Permethrin	$C_{21}H_{20}C_{l2}O_3$	390	1.31
20.	3,5,7-trioxononanoic acid	$C_9H_{12}O_5$	200	2.53
21.	Di-isodecyl phthalate	$C_{28}H_{46}O_4$	446	1.31
22.	1-Propyl 3-(phenylamino)-2-(phenylseleno)-3-(phenyl) propanoate	$C_{24}H_{25}NO_2Se$	439	2.24
23.	Alpha-Bisabolol	$C_{15}H_{26}O$	222	1.80
24.	Pentatriacontane (CAS)	$C_{35}H_{72}$	492	5.08
25.	bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	73.3

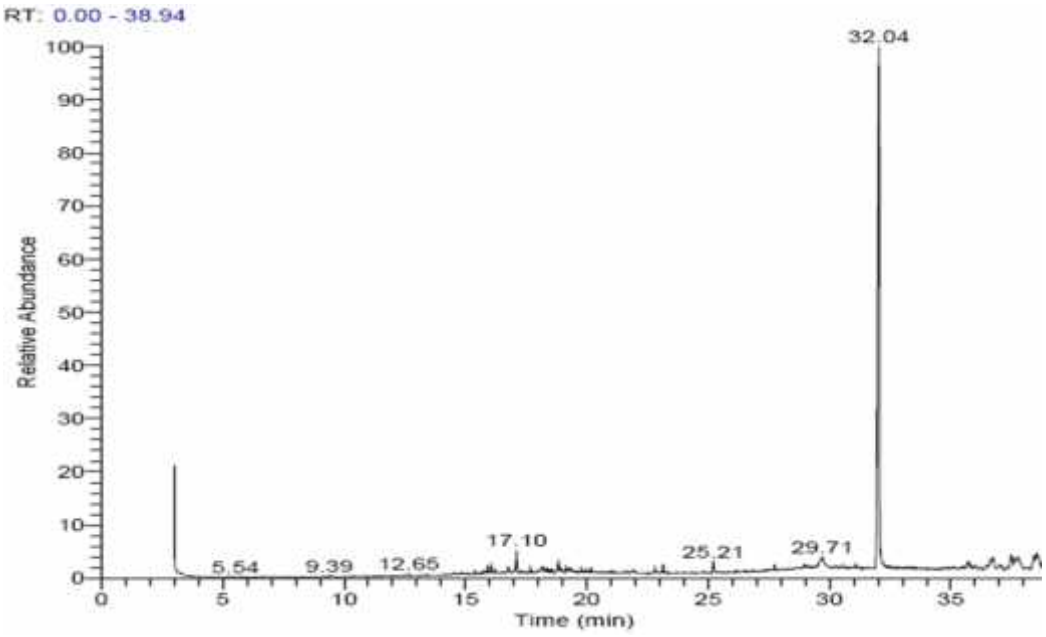

Figure 1: GC-MS analysis of ethanolic extract of Piper nigrum leaves

Scale of Figure 1 : Dimensions-854×591 pixels, width -91% , height-70%, horizontal resolution- 149.98 dpi, vertical resolution -149.98 dpi, bit depth-32, compression- uncompressed , resolution- unit 2, color representation –black and white

detergents and in intracellular histochemical staining. It is also used to allow antibody access in intracellular proteins. In medicine, it is used in hypercholestrolaemia, hyperglycaemia, antioxidant and anticancer (Muraleedharannair et al., 2012). Plant steroids are known to be important for their cardiotonic activities and also possess insecticidal and antimicrobial properties.

The secondary metabolites from plants are good sources for combination therapy. The substances that constitute a total extract may be academically divided into active substances, co-effectors, and matrix formers and the

interaction between them can protect the active substances from decomposition. This may be one of the reasons why Indian traditional Aurvedic medicine, incorporates into many of its specific formulas a combination of black pepper (*Piper longum*) and ginger (Murali and Smitha, 2012).There is growing awareness in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity. Screening active compounds from plants has lead to the invention of new medicinal drugs which have efficient protection and treatment roles against various diseases, including cancer and Alzheimer's disease (Janakiraman et al., 2012).

Quantitative analysis of phytochemicals: The plants known as medicinal are rich in secondary metabolites which include alkaloids, glycosides, flavonoids, insecticides, steroids, related active metabolites. They are of great medicinal value and have been extensively used in the drug and pharmaceutical industry (Johnson et al., 2012). Many tannin-containing drugs are used in medicine as astringent.Tannins have been found to have antiviral, antibacterial, antiparasitic effects, anti-inflammatory, antiulcer and antioxidant property for possible therapeutic applications(Valentine Chi Mbatchou&SulemanDawda,2012).

Saponins are considered as a key ingredient in traditional Chinese medicine and are responsible for most of the observed biological effects. Saponins are known to produce inhibitory effect on inflammation. There is tremendous, commercially driven promotion of saponins as dietary supplements and nutriceuticals. Saponin possesses specific physical, chemical and biological activities that make them useful as drugs. Some of these biological properties include antimicrobial, anti-inflammatory, anti-feedent, and hemolytic effects (Kannika Panyaphu et al., 2012).Alkaloid is one of the largest phytochemical groups in plants has amazing effect on humans and this has led to the development of powerful pain killer medications(Johnson et al., 2012).

Flavonoids, the major group of phenolic compounds are reported for their antimicrobial, antiviral and spasmolytic activity. Flavonoids are able to scavenge hydroxyl radicals, superoxide anion radicals and lipid peroxy radicals, which highlights many of the flavonoid health-promoting functions in organism (Sangameswaran et al., 2012). They are important for prevention of diseases associated with oxidative damage of membrane, proteins and DNA. Flavonoids in human diet may reduce the risk of various cancers, as well as preventing menopausal symptoms. Flavonoids, on the other hand, are potent water-soluble antioxidants and free radical scavengers, which prevent oxidative cell damage and have strong anti-cancer activity(Johnson et al., 2012). Flavonoids are reported to be genotoxic and possess oxidant activity. This pro-oxidant activity has been attributed to the apoptotic inducing property of flavonoids and hence implicated in cancer chemo prevention (Janakiraman et al., 2012). The components of pepper extract are of great interest in medicinal chemistry as these compounds show a broad range of biological activity are exploited in the pharmaceutical industries.

Antioxidant activity*:* DPPH radical scavenging activity was determined by a spectrophotometric method based on the reduction of the stable free radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) (Ramesh kumar et al., 2011). In the present study, ethanolic extract of pepper leavescould readily scavenge ABTS radical cation indicating the presence of phytochemical components such as flavonoids and phenols, which substantiate their antioxidant action.DPPH is commercially available nitrogen centered stable free radical which is destroyed by a free radical scavenger. The method is based on the measurement of the loss of deep purple color of DPPH after reaction with the test compound functioning as a proton radical scavenger or hydrogen donor (Laizuman Nahar et al., 2012). DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Antioxidant on interaction with DPPH, transfer electron or hydrogen atom to DPPH and thus neutralizing its free radical character and convert it to 1-1diphenyl-2-picryl hydrazine and the degree of discoloration indicates the scavenging activity of the drug (Alak et al., 2012). The decrease in absorbance of DPPH radical caused by antioxidants is due to the reaction between antioxidant molecules and radical progress which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a change in color from purple to yellow. Hence, DPPH is usually used as a substance to evaluate the antioxidant activity. In the present study, the scavenging effect of different concentrations of the extracts on the DPPH radical is illustrated (Thambiraj et al., 2012).

Phosphomolybdenum assay is the effective assay for the measurement of total antioxidant activity of the extract. This assay has been successful in the quantification of α-tocopherol antioxidant capacity and being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to plant extracts. The present study investigated the Fe^{3+} to Fe^{2+} transformation in the presence of a various extract of pepper leaves for measurement of reductive ability. Total antioxidant capacity was determined by phosphomolybdenum method which is based on the reduction of Mo (VI) to Mo (V) by the antioxidant and the formation of a green phosphate/ Mo (V) complex with a maximal absorption at 695 nm. As this method is simple, rapid and independent of other antioxidant measurements commonly employed, it was decided to extend its application to the rice extracts (Alaket al., 2012).

Hydrogen peroxide is a week oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydroxyl radical scavenging assay depends on the competition between deoxyribose and the plant extracts for hydroxyl radical generated from Fe3+-EDTA-ascorbate system (Ramesh kumar et al., 2011).The decomposition of H_2O_2 by plant extract may result from its antioxidant and free radical scavenging activity (Alak et al., 2012). Thus, the observations made in the present study showed that the extract of pepper leaves exhibited good scavenging of H_2O_2 in the biological system, thus preventing the stress induced by progressive

increases in malondialdehyde and other free radicals which cause oxidative damages. The extent of damage caused in H_2O_2 untreated groups were fixed at 100 % and the damage caused in other groups were calculated relative to this value. Hydroxyl radical is the most reactive free radical in the biological system and it has been regarded as the highly damaging to almost every molecule found in the biological system. It can conjugate with nucleotides in DNA and cause strand breakage which leads to ultimately mutagenesis, carcinogenesis, and cytotoxicity (Alak et al., 2012). The present study shows the ability of the leaf extract to inhibit hydroxyl radical mediated deoxyribose degradation in a concentration dependent manner (Thambiraj et al., 2012).

Nitric oxide (NO) is a potent pleiotropic mediator of physiological processes (Shanmugapriya et al., 2011). The percentage inhibition of nitric oxide generation by different extract of pepper leaves at 100 µg/ml concentration was compared with standard BHT. Numerous biological reactions generate superoxide anions which are highly toxic species. The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture.

Superoxide anion scavenging assay depends on the light-induced superoxide generation by riboflavin and the corresponding reduction of nitroblue tetrazolium. It is also very harmful to cellular components and most abundantly produced free radical (Ramesh kumar et al., 2011). Pepper extracts from variable polar fractions showed the different antioxidant activities. Antioxidant activity was strongly correlated with the content of bioactive compounds from peppers. A comparative study of the antioxidant activity was also reported as active constituents from *Piper* species, which contribute to pungency and physiological, pharmacological, insecticidal, and medicinal properties (Ramesh kumar et al., 2011).

Antimicrobial activity: The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of phytomedicine to act against microbes (Shanmugapriya et al., 2011).Plants are able to produce compounds that, although having no apparent function in the primary metabolism of the plant, have good activity against bacterial pathogens when they are able to find their way to accumulate in them (Angel et al., 2013). These specific compounds have had an extensive history of use as therapeutic agents (Murali & Smitha, 2012).

Anticancer activity of the drug against Ehrlish ascites carcinoma: Drug discovery from the medicinal plants has played an important role in the treatment of cancer and induced new clinical application of plant secondary metabolites and their derivatives over the last half-century have been made towards combating cancer. The antitumor and antioxidant activity was evaluated by assessing body weight and cytotoxicity parameters in Swiss albino Mice. The improper balance between Reactive Oxygen Metabolites and antioxidant defences result in "Oxidative Stress", which deregulates the cellular functions leading to various pathological conditions including cancer (Ankita et al., 2012). Hence DLA-bearing mice, a regular rapid increase in ascites tumor volume were noted. SOD, CAT and glutathione peroxidase are involved in the clearance of superoxide and hydrogen peroxide. Glutathione, a potent inhibitor of the neoplastic process plays an important role in the endogeneous antioxidant systems (Sasmal et al., 2012). Antitumor activity is either through induction of apoptosis or by inhibition of neovascularization.

Histopathological examination of tissues: Histopathological studies of sections of liver of the control and the experimental mice were carried to test the antitumor effect of ethanolic extract of pepper leaves. There was no evidence of any pathological changes of plant extract did not cause structural changes to liver morphology.

GC-MS analysis: The separation of all phytochemical components in a sample was analysed by GC-MS method. It showed the existence of various compounds with variable chemical structure (Janakiraman et al.,2012).

CONCLUSION

Cancer is the exponential, out-of-control growth of cells. Black pepper is one of the most common condiments worldwide in most curry recipes. The result showed that pepper leaves are good source of phytochemicals. The inhibition produced against particular microorganism depends upon the members of phenolic compounds present in the leaves and identified by GC-MS analysis. Antitumor activity is carried out by inhibition of neovascularization and augmenting endogenous non-enzymatic and enzymatic antioxidant defense systems. Histopathological studies of liver also supported as an active standard antioxidant agent. Many plants with strong anticancer effect lie unexplored or remain under explored. There need to be systematic studies to identify and scientifically validate such compounds, which can results in ways of combating the serious disease of cancer.

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