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Original Article

GC-MS ANALYSIS AND EVALUATION OF BIOACTIVITIES OF *KAEMPFERIA PARISHII*-A NATURAL SOURCE OF TOTAROL

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ABSTRACT

Objective: The present study was aimed for phytoconstituent analysis, *in vitro* antioxidant and antimicrobial activities of leaf and rhizome extracts of an unexplored plant, *Kaempferia parishii* (Zingiberaceae).

Methods: The extracts were analyzed by gas chromatography/mass spectrometry to determine volatile chemical constituents. Antioxidant activity of extracts was determined using DPPH assay whereas the antimicrobial effects were tested by inhibition zone diameter and minimum inhibitory concentration.

Results: GC/MS analysis revealed the presence of 7 and 8 identified components accounting for 92.1% and 82.86% of the leaf and rhizome extract of *Kaempferia parishii* respectively. In leaf extract phytol (72.55±0.5%), hexadecanoic acid methyl ester (4.94±0.2%), hexahydro farnesyl acetone (3.78±0.2%), dibutyl phthalate (3.31±0.2%) were found to be the major constituents and those of rhizome extract were totarol (74.96±0.86%), cembrene (2.83±0.2%), borneol (1.23±0.15). Both the extracts exhibited low to moderate antioxidant activity. They possess very weak activities against some tested microorganisms while the extracts had no activity against some microorganisms.

Conclusion: Totarol, an antimicrobial agent, was found to be the major constituent of *Kaempferia parishii* rhizome extract. Thus, *Kaempferia parishii* can be used as a natural source of totarol. This is the first report on the unexplored plant, *Kaempferia parishii*.

Keywords: *Kaempferia parishii*, GC-MS analysis, Totarol, Phytol, Antioxidant activity, Antimicrobial activity

INTRODUCTION

Medicinal plants are very important for the treatment of many diseases in humans from ancient times and a vital part of the Indian traditional medicinal system, such as the Ayurveda and Siddha [1]. Globally, an extensive activity of research is undergoing on diverse plant group and their remedial values. At present, about 25 percent of the active constituent has been identified from plants that are used as prescribed medicines [2]. About 80,000 plant species are utilized by the different systems of Indian medicine [3]. Scientifically, medicinal flora has proven to be a rich resource of biologically active compounds, many of which have already been formulated into valuable remedial substances or have provided an origin for the development of novel lead molecules for pharmaceuticals. Thus, it is essential to develop phytochemical profiles which represent the bioactive constituents of the herbal drugs.

Modern day synthetic and chemical drugs often exhibit some side effects, but traditional herbals are safer and easy to access. Nowadays, the interests in natural products are looking into sources of a substitute, more natural and environment-friendly anti-microbials and antioxidants agents. A massive pool of bioactive compounds exists in various species of plants, but merely a small proportion of which have been examined and sustained to be and significant source of bioactive agents.

Zingiberaceae is a family of medicinal and economic significance. *Kaempferia parishii* belongs to family Zingiberaceae. The plant is characterized by shiny, ribbed green foliage having purple with pink flowers continuously all the way through summer. There is no published report available for this plant which indicates that the plant is in unexplored condition. Although *Kaempferia* species have been utilized for food flavouring and in traditional medicine, no information is available on chemical constituents and bioactivity of *Kaempferia parishii*. This propels us to study this plant for evaluating its phytochemical contents and its bioactivity potential.

MATERIALS AND METHODS

Plant material

The rhizomes of *Kaempferia parishii* were collected from West Bengal, and the specimen was authenticated by Dr. P. C. Panda, Senior Scientist, Taxonomy and conservation division, Regional Plant Resource Centre, Bhubaneswar. The collected rhizomes were maintained in the greenhouse of Centre of Biotechnology. The leaves and rhizomes of *K. parishii* were taken, washed, chopped into pieces and dried under shade for 10-15 d.

Preparation of extract

The fresh leaves and rhizomes of the plants were taken, washed and chopped into pieces and dried under shade for 15-20 d. The shade dried materials were grounded to a coarse powder. The resulting materials were extracted with methanol for 24 h in soxhlet apparatus. The methanol extracts were filtered and then concentrated and dried by using rotary evaporator. Each methanol extraction was run in triplicate.

Phytochemical screening

The leaf and rhizome methanol extracts of *K. parishii* were separately subjected to preliminary phytochemical screening for the identification of different chemical groups [4, 5].

Determination of total phenolic content

Total Phenolic Contents (TPC) of methanol extracts of leaf and rhizome of *K. parishii* were determined by Folin-Ciocalteu method [6] with little modifications, using Gallic acid as a standard phenolic compound. The extracts were diluted with distilled water to a known concentration in order to obtain the readings within the standard curve range of 0.0 to 600 µg of Gallic acid/ml. 250 µl of diluted extracts or Gallic acid solution was mixed with 1 ml of distilled water in a test tube followed by the addition of 250 µl of Folin-Ciocalteu reagent. The samples were mixed well and then

allowed to stand for 5 min at room temperature in order to allow complete reaction with the Folin-Ciocalteu reagent. Then, 2.5 ml of 7% sodium carbonate aqueous solution was added to the test tube and the final volume was made up to 6 ml with distilled water. After incubating the samples for 90 min, the absorbance of the resulting blue colour solution was measured at 760 nm using a spectrophotometer. The results were expressed as mg of Gallic acid equivalents (GAE)/g of the extract by using an equation that was obtained from standard Gallic acid graph. All the experiment was conducted in three replicates.

Determination of total flavonoid content

Total flavonoid content (TFC) of leaf and rhizome methanol extracts of *K. parishii* was estimated by the method of Ordon *et al.*, (2006) with little modification [7]. This method is based on the formation of a complex flavonoid-aluminium. A volume of 1 ml of 2% $AlCl_3$ ethanol solution was added to 1 ml of extract solution and then it was kept in the dark at room temperature for 1 h. The absorbance was measured at 420 nm using UV-VIS spectrophotometer. TFC was calculated by extrapolating the absorbance of the reaction mixture on the calibration curve of Quercetin. TFC was expressed as mg Quercetin equivalent/g of the extract. All the experiments were conducted in three replicates, and the results were averaged.

GC-MS analysis of plant extracts

GC-MS (gas chromatography coupled with mass spectrometry) analysis was carried out on a 6890 series instrument (Agilent Technologies, Palo Alto, CA, USA) equipped with a mass selective detector with quadrupole analyser, MSD 5973. The electron ionisation energy was 70 ev, ion source temperature 230 °C and the interface temperature 280 °C. A split-split less injection (split ratio 1:100) at 250 °C injector temperature was employed. A fused silica column HP-5 (30 m x 0.25 mm i. d and 0.25 µm film thickness) was used. The oven temperature was programmed as follows: from 50 °C –240 °C at 4 °C/min; from 240 °C to 270 °C at 15 °C/min; held isothermal at 50 °C for 1 min and at 270 °C for 15 min. A sample of 1µl was injected. Data acquisition was performed with the software for the mass ranges 50-600amu with a scan speed of 1 scan/s. carrier gas helium was used at a flow rate of 1.0 ml/min. The identification of compounds was performed by comparing their mass spectra with data from US National Institutes of Standards and Technology (NIST, USA).

Evaluation of antioxidant activity

Radical scavenging activity of the methanolic extracts of *Kaempferia parishii* was determined by a spectrophotometric method based on the reduction of a methanol solution of DPPH (1,1-diphenyl-2-picrylhydrazyl) using the method of Blois (1958) with little modification [8]. 1.5 ml of various concentrations of extracts (1, 5, 10, 20, 50 and 100µg) in methanol was added to 1.5 ml of 0.1 mM methanol solution of DPPH. The mixture was shaken vigorously and left to stand at room temperature for 30 min in the dark.

Then the absorbance was measured at 517 nm on a UV/visible spectrophotometer. Absolute methanol was used to zero the spectrophotometer. The DPPH solution was freshly prepared, stored in a flask covered with aluminium foil and kept in the dark. Tests were carried out in triplicate (sample size is three). Ascorbic Acid, a standard antioxidant was used as positive control. The absorbance of the control, the DPPH radical without a sample, was measured. Special care was taken to minimize the loss of free radical activity of the DPPH stock solution. Radical scavenging activity was expressed as percentage inhibition of DPPH radical and was calculated by the following equation-

$$\% \text{ Inhibition} = [(A_{control} - A_{test})/A_{control}] \times 100$$

Where, Acontrol is the absorbance of the control (solution without extract), and Atest is the absorbance of samples (extract and ascorbic acid). The antioxidant activity of each sample was expressed in terms of IC_{50} (micromolar concentration required to inhibit DPPH radical formation by 50%), calculated from the graph after plotting inhibition percentage against extract concentration.

Evaluation of antimicrobial activity

Microorganism

Total 6 microorganisms namely two gram-positive bacteria: *Enterococcus faecalis* and *Staphylococcus aureus*, two gram-negative bacteria: *Acinetobacter baumannii* and *Escherichia coli* and two fungi: *Candida albicans* and *Aspergillus niger* were taken for the present study. All the microorganisms were maintained at 4 °C.

Initial screening

For initial screening, the disc diffusion method (DDM) as described previously was followed with slight modifications [9]. Briefly, Nutrient Agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed with freshly grown cultures of the test pathogens by the help of a pre-sterilized cotton swab. Sterile Whatmann No. 1 filter paper discs (5 mm diameter) were kept on the above plates at equidistance. Varying volumes (2, 5 and 10 mg) of extracts were loaded over the sterile filter paper discs and allowed to diffuse for half an hour at 4ºC. The plates were incubated at 37 °C for 18-24 h for bacteria, 48 h for fungi. Disc containing 5% DMSO served as negative control and Gentamycin (5 mg) served as positive control. The plates were observed for the presence of inhibition of microbial growth that was indicated by the clear zone around the disc which indicated positive microbicidal activity of the extracts. The size of the zone of inhibition (including disc) was measured in millimetres. The absence of zone inhibition was interpreted as the absence of activity. All the experiments were carried out in triplicate.

Determination of MIC

Minimum inhibitory concentration (MIC) of extracts was determined by the serial dilution method [10]. The samples were diluted with NBT & PDBT (Nutrient and potato dextrose broth supplemented with 0.75% of Tween-20) to give sample concentration of 0.5 mg/ml to 30 mg/ml. The samples were then filtered through a membrane filter. 50 µl of (fresh culture) of the test organisms was inoculated into 1 ml of NBT and PDT containing various concentrations of both the samples. Growth control was prepared by inoculating 50 µl of each culture suspension on 1 ml each of NBT & PDBT medium without any extract or solvent. Solvent control was prepared by pouring 100 µl of DMSO to 1 ml of NBT & PDBT medium followed by the cultures. The tubes were incubated at 37 °C, for 18-24h, (48 h for fungi) and the lowest concentration of antimicrobial agent that inhibited the visible growth (no turbidity) of a microorganism after overnight incubation was noted as MIC.

Test for bactericidal and fungicidal effect

In order to evaluate the effect (microbicidal/microbiostatic) of the extracts, one loop from the MIC tube was subcultured on to the NA & PDA plates which were then incubated at 37 °C overnight to check whether the extracts merely had bactericidal or fungicidal activity i.e. no growth on subculturing. Minimum bactericidal concentration (MBC) was regarded as the lowest concentration of the samples that require to kill the microorganism.

RESULTS

Phytochemical screening

Preliminary phytochemical screening of the leaf and rhizome extract of *Kaempferia parishii* revealed the presence of different phytoconstituents which are represented in the table (1). It was seen that leaf extract contained alkaloids, flavonoids, saponins and glycosides whereas rhizome extract was found to contain alkaloids, steroids, triterpenoids.

Determination of TPC and TFC

Total Phenolic Content of *Kaempferia parishii* extracts was determined and represented in terms of GAE. TPC of the leaf and rhizome extracts of *Kaempferia parishii* was found to be 31.75±0.44 mg GAE/g and 27±0.36 mg GAE/g of the extract. Total Flavonoid Content of *Kaempferia parishii* leaf and rhizome extract was found to be 39.46±0.1 and 27.30±0.43 mg Quercetin equivalent/g of extract.

Identification of chemical constituents by GC-MS analysis

The leaf and rhizome extracts of *Kaempferia parishii* were analyzed by GC-MS for determining their chemical constituents, and it revealed the presence of 7 and 8 identified components accounting for 92.1% and 82.86% of the leaf and rhizome extract respectively (Fig: 1 and 2). In leaf extract phytol (72.55±0.5%), hexadecanoic acid methyl ester (4.94±0.2%), hexahydro farnesyl acetone (3.78±0.2%), dibutyl phthalate (3.31±0.2%) were found to be the major constituents and in case of rhizome extract totarol (74.96±0.86%), cembrene (2.83±0.2%), borneol (1.23±0.15) were the major constituents (table 2 and 3).

Table 1: Preliminary phytochemical screening of leaf and rhizome extract of *Kaempferia parishii*

Phytoconstituents	Test performed	K. P leaf	K. P rhizome
Alkaloids	Dragendroff's test	+ve	-ve
	Mayer's test, Wagner's test, Hager's test	+ve	+ve
Steroids	Libermann Burchard test, Salkowski test	-ve	+ve
Flavonoids	Alkaline reagent test, Shinoda test	+ve	-ve
Triterpenoids	Libermann Burchard test, Salkowski test	-ve	+ve
Carbohydrates	Molisch's test, Fehling's test	-ve	-ve
	Barfoed's test, Benedict's test	-ve	-ve
Tanins	$FeCl_2$ test	-ve	-ve
Saponins	Foam test	+ve	-ve
Aminoacids	Millon's test, Ninhydrin test	-ve	-ve
Glycosides	Killer-Kiliani test	+ve	-ve
	Brontrager's test	+ve	-ve

(+): Indicates the presence of chemical constituents, (-): Indicates the absence of chemical constituents

Table 2: Chemical composition of leaf extract of *Kaempferia parishii*

S. No.	Compound name	Area % (mean±SD)	Retention time
1	β-Farnesene	1.04±0.1	22.748
2	Hexahydro farnesyl acetone	3.78±0.2	37.362
3	Hexadecanoic acid methyl ester	4.94±0.2	40.280
4	Dibutyl phthalate	3.31±0.2	41.202
5	9,12-Octadecenoic acid, methyl ester	3.48±0.3	45.489
6	Methyl linolenate	3.00±0.4	45.663
7	Phytol	72.55±0.5	45.942

Data are given in mean±SD, n=3

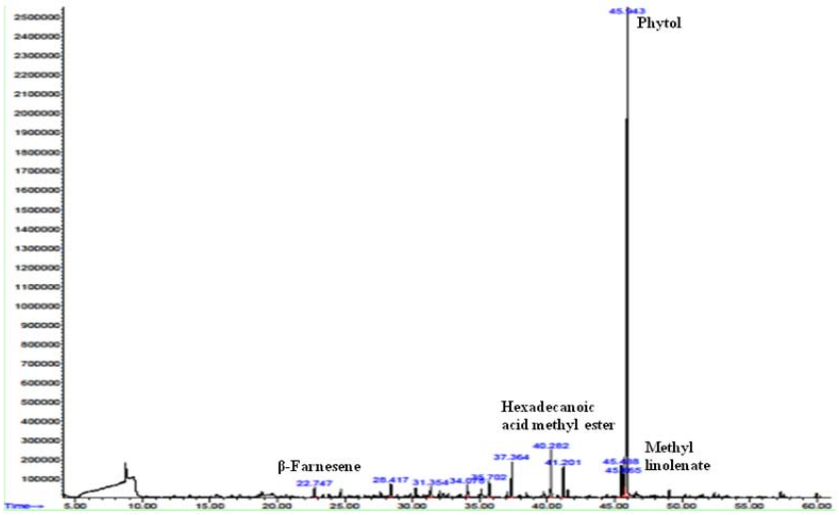

Fig. 1: GC MS Chromatogram of *Kaempferia parishii* leaf extract

Table 3: Chemical composition of rhizome extract of *Kaempferia parishii*

S. No.	Compound name	Area % (mean±SD)	Retention time
1	Borneol	1.23±0.15	9.715
2	L-bornyl acetate	0.60±0.03	14.202
3	Aromadendrene	0.72±0.1	20.862
4	Ledol	0.24±0.08	26.232
5	Dehydrobietan	1.37±0.1	41.197
6	Cembrene	2.83±0.2	41.912
7	Totarol	74.96±0.86	49.498
8	Longipinocarveol, trans-	0.91±0.1	50.407

Data given in mean±SD, n=3

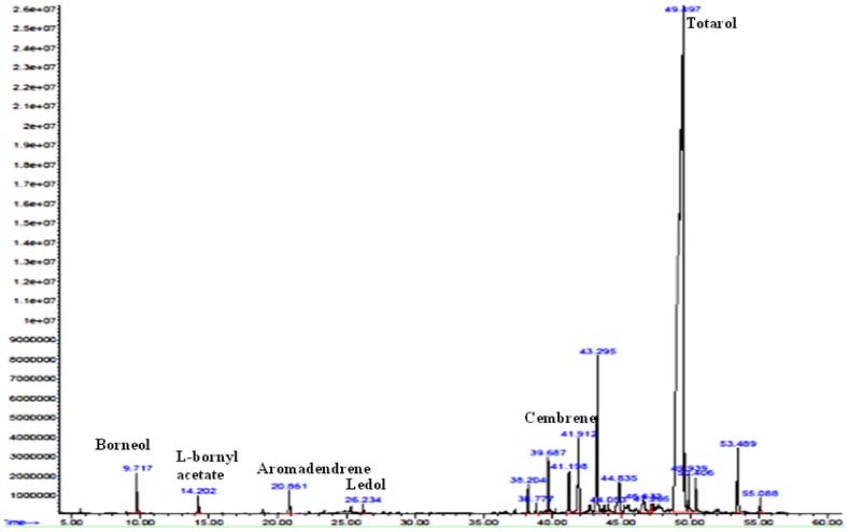

Fig. 2: GC MS Chromatogram of *Kaempferia parishii* rhizome extract

Evaluation of antioxidant activity

The antioxidant activity of the methanolic extracts obtained from *Kaempferia parishii* leaves and rhizomes were evaluated using DPPH free radical scavenging assay. In the present study, the samples (extracts) showed moderate DPPH radical inhibiting activity at a concentration of 100µg/ml. As can be seen from the graph (fig. 3), activity was increased with the increasing concentration of the samples. But when compared with standard ascorbic acid, it was seen that the extracts of *Kaempferia parishii* exhibited very low DPPH radical inhibiting activity. Higher IC_{50} values of the extracts (leaf extract-99.9µM and rhizome extact-64 µM) indicate its less inhibition capacity against the radical.

Evaluation of antimicrobial activity

The methanolic extracts of *Kaempferia parishii* were examined for their *in vitro* antimicrobial activity using disc diffusion method, MIC and MBC assay against two Gram (+) and two Gram (-) pathogenic bacteria, and two fungus. As can be seen in Table 4, the methanolic extracts possess very weak antimicrobial activities against some microorganisms tested while the extracts had no activity against some microorganisms. The MIC values of the extracts were ranged between 14.44-21.96 mg/ml whereas the MBC values of the extracts were ranged between 25.66-27.83 mg/ml. The zone of inhibition was highest in *K. parishii* rhizome extract (7.9±0.23 mm) against *A. baumannii*.

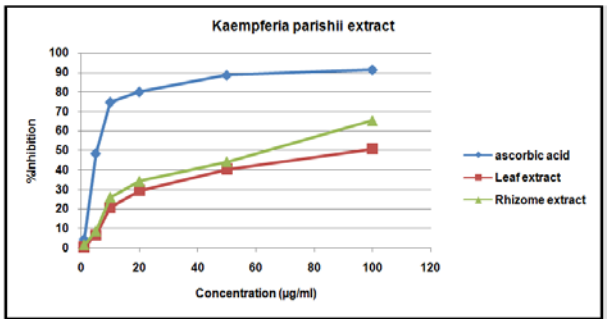

Fig. 3: DPPH radical scavenging activity of methanolic extracts of *Kaempferia parishii*
Each sample were analysed in triplicates

Table 4: Antimicrobial activity of leaf and rhizome extract of *Kaempferia parishii*

Micro-organism	KPL extract (mg/ml)		KPR extract (mg/ml)		IZD of gentamycin at 5 mg in mm	IZD of KPL extract at 5 mg in mm	IZD of KPR extract at 5 mg in mm
	MIC	MBC	MIC	MBC			
E. faecalis	-	-	14.73	**25.66**	19.38±0.13	-	5.52±0.2
S. aureus	19.78	-	15.89	27.83	17±1	3.3±0.3	7.46±0.2
A. baumannii	-	-	-	-	24.67±0.57	1.7±0.5	6.25±0.3
E. coli	21.96	-	14.44	26.78	24.8±0.25	2.9±0.66	7.9±0.23
C. albicans	-	-	-	-	25.67±0.59	-	6.36±0.4
A. niger	-	-	-	-	25±1	-	5.7±0.31

KPL: *Kaempferia parishii* Leaf; KPR: *Kaempferia parishii* Rhizome; IZD: Inhibition Zone Diametre, data given in mean±SD, n=3

DISCUSSION

GCMS analysis of leaf extract revealed phytol (72.55±0.5%), hexadecanoic acid methyl ester (4.94±0.2%), hexahydro farnesyl acetone (3.78±0.2%), dibutyl phthalate (3.31±0.2%) as the major constituents and in case of rhizome extract totarol (74.96±0.86%), cembrene (2.83±0.2%), borneol (1.23±0.15) were the major constituents. Phytol has been found to be a potent antimycobacterial agent. The use of phytol in the fragrance industry and in cosmetics, shampoos, toilet soaps, household cleaners, and detergents has been reported [11, 12]. Totarol exhibits antimicrobial properties [13, 14].

Phytochemical screening of plant extracts provides the necessary information regarding the phytochemical constituents for the discovery of novel drugs. In the present investigation, the phytochemical screening of methanolic extracts of *Kaempferia parishii* revealed the presence of phytochemicals which exhibit various medicinal properties.

The DPPH assay is a universally used method for evaluation of the capacity of plant extracts to scavenge free radicals produced from DPPH reagent [15]. In the presence of hydrogen donating antioxidants, the purple coloured methanolic DPPH solution is

reduced by the formation of yellow coloured diphenyl-picryl hydrazine. In the present study antioxidant activity of *K. parishii* has been evaluated for the first time. The phenolics and flavonoids possess diverse biological activities which might be related to their antioxidant activity [16]. The plant secondary metabolites like phenolics and flavonoids contribute to the antioxidant activity of plant extracts, and consequently, it is quite important to estimate the total phenolic and total flavonoid content [17, 18]. Hence, we can say that lower antioxidant capacity found in this plant may be due to its lower phenolic and flavonoid contents. Though antimicrobial properties of *Kaempferia* species have been reported earlier but there is no published work regarding antimicrobial activities of *K. parishii*. Arambewela (2000) reported that rhizomes and roots of *Kaempferia galanga* showed antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* [19]. Screened the antimicrobial activities of rhizome in *K. galanga* [20].

We found that totarol, an antimicrobial agent [13, 14], was the major constituent of *Kaempferia parishii* extract. But this extract had not shown the significant antimicrobial property. Hence, it has to be noted that the mechanisms of antimicrobial action of plant secondary metabolites are not fully understood. A single compound may not be responsible for the antimicrobial activity but may be caused by a combination of compounds interacting in an additive or synergistic manner [21, 22].

CONCLUSION

In the present investigation, the phytochemical evaluation of methanolic extracts of *Kaempferia parishii* leaf and rhizome have been carried out which revealed the presence of different active phytoconstituents, a group of phytochemicals like alkaloid, flavonoid, and phenolic compounds which exhibit various medicinal properties. Totarol, an antimicrobial agent, was the major constituent of *Kaempferia parishii* rhizome extract. Thus it can be used for the natural source of totarol. Antioxidant and antimicrobial properties of the extracts have been evaluated. This being the first report on *Kaempferia parishii*, further study is required.

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CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Basu SK. Herbal medicine: concepts and perspectives. In: Datta Banik S, Basu SK. ed. Environmental perspectives and human responses. National Service Scheme (NSS) 2000-2001. Govt. of India, S. Graphics, Kolkata, India; 2002;3:27-44.
2. Gill NS, Bajwa J, Sharma P, Dhiman K, Sood S. Evaluation of antioxidant and antiulcer activity of traditionally consumed *Cucumis melo* seeds. J Pharmacol Toxicol 2011;6:82-9.
3. Prajapati J, Prajapati T, Jaipura S. Advances in medicinal plants. In Asian Medicinal Plants and Healthcare. Jodhpur; 2006. p. 23-9.
4. Kokate CK. Practical pharmacognosy. 1st ed (Vallabh Prakashan, New Delhi, India); 1994. p. 107.
5. Harborne JB. Phytochemical methods: A Guide to Modern Techniques of Plant Analysis, Chapman and Hall: London; 1998.
6. Stanly C, Bhatt A, Ali HMD, Keng CL, Lim BP. Evaluation of free radical scavenging activity and total phenolic content in the petiole-derived callus cultures of *Zingiber zerumbet* Smith. J Med Plant Res 2011;5:2210-7.
7. Ordon Ez AAL, Gomez JD, Vattuone MA, Isla MI. Antioxidant activities of *Sechium edule* (Jacq). Food Chem 2006;97:452-8.
8. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature 1958;181:1199-200.
9. Pattnaik S, Subramanyam VR, Rath CC. Effect of essential oils on the viability and morphology of *E. coli* (SP-11). Microbios 1995;84:195-9.
10. Pattnaik S, Subramanyam VR, Bapaji M, Kole CR. Antibacterial and antifungal activity of aromatic constituents of essential oils. Microbios 1997;89:39-46.
11. Mc Ginty D. Fragrance material review on phytol. Food Chem Toxicol 2010;48:559-63.
12. Igwe OU, Abii T. Characterization of bioactive sesquiterpenes, Organic acids and their derivatives from the leaves of Psidium guajava Linn. Int Res J Pure Appl Chem 2014;4:456-67.
13. Mossa JS, El-Feraly FS, Muhammad I. Antimycobacterial constituents from *juniperus procera*, *Ferula communis* and *plumbago zeylanica* and their *in vitro* synergistic activity with isonicotinic acid hydrazide. Phytother Res 2004;18:934-7.
14. Kubo I, Muroi H, Himejima M. Antibacterial activity of totarol and its potentiation. J Nat Prod 1992;55:1436-40.
15. Chung Y, Chien C, Teng K, Chou S. Antioxidative and mutagenic properties of *Zanthoxylum ailanthoides* Sieb and zucc. Food Chem 2006;97:418-25.
16. Chung KT, Wong TY, Huang YW, Lin Y. Tannins and human health: a review. Crit Rev Food Sci Nutr 1998;38:421-64.
17. Mathew S, Abraham TE. *In vitro* antioxidant activity and scavenging effects of *Cinnamommum verum* leaf extract assayed by different methodologies. Food Chem Toxicol 2006;44:198-206.
18. Elzaawely AA, Xuan TD, Koyama H, Tawata S. Antioxidant activity and contents of essential oil and phenolic compounds in flowers and seeds of *Alpinia zerumbet* (Pers.) BL Burtt and RM Sm. Food Chem 2007;104:1648-53.
19. Arambewela L, Perera A, Thambugala R, Wijesundera RLC, Gunatileke J. Investigations on *Kaempferia galanga*. J Natl Sci Found Sri Lanka 2000;28:225-30.
20. Parvez MAK, Khan MHM, Islam ZM, Hasan SM. Antimicrobial activities of the petroleum ether, methanol and acetone extracts of *Kaempferia galanga* L. Rhizome. J Life Earth Sci 2005;1:25-9.
21. Paiva PMG, Gomes FH, Napoleao TH, Sa RA, Correia MTS, Coelho LCBB. Antimicrobial activity of secondary metabolites and Lectins from plants. Curr Res Technol Education Topics Appl Microbiol Microbial Biotechnol 2010;96:396-406.
22. Ulukanli Z, Akkaya A. Antibacterial activities of *Marrubium catariifolium and Phlomis pungens Var. Hirta* grown wild in eastern anatolia, turkey. Int J Agric Biol 2011;13:105-9.