Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Antioxidant and Anticancer Activities of Whole Plant Extracts of *Lepidagathis pungens* Nees: *In vitro* Evaluation

Manoharan Dhanalakshmi, Subramaniam Ananda Thangadurai[1]

Department of Pharmaceutics, Swamy Vivekanandha College of Pharmacy, [1]Department of Pharmaceutical Analysis, J.K.K.Nattraja College of Pharmacy, Namakkal, Tamil Nadu, India

Submitted: 20-Aug-2020 Revised: 12-Oct-2020 Accepted: 23-Feb-2021 Published: 10-Jun-2021

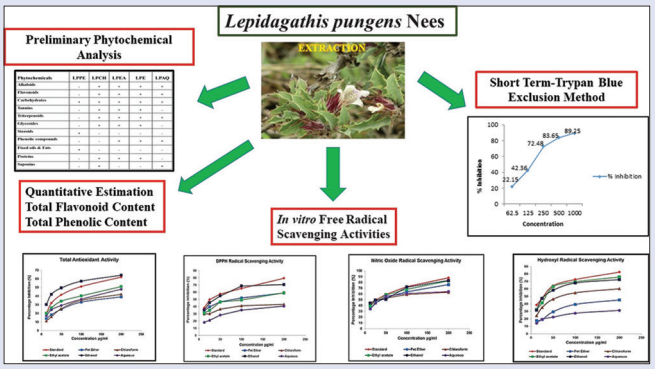
ABSTRACT

Background: *Lepidagathis* genus plants were less explored for its biological activity. *Lepidagathis* genus was utilized traditionally for treating of polyuria, fever, urinary calculi, dysentery, dysuria, and uterine disorders. **Objective:** The present study was planned to evaluate antioxidant activities, anticancer activity of *Lepidagathis pungens* Nees., whole plant (*L. pungens*). **Materials and Methods:** Antioxidants activity of the ethanol extract of *L. pungens* Nees., determined by total antioxidant assay, diphenyl-2-picryl hydrazyl (DPPH) free radical scavenging assays**,** hydroxyl radical scavenging assays, and nitric oxide scavenging activity assay methods. **Results:** Preliminary phytochemical screening showed that the extract of *L. pungens* possesses flavonoids, alkaloids, tannins, saponins, phenolic, and steroid compounds. In the present investigation, quantitative estimation of phenolic compound and flavonoids carried out by spectrophotometric methods, using aluminum chloride and organic solvent separation method, respectively. The extract showed potent activities in all antioxidant assays and it is also comparable to the standard antioxidants in a dose based manner and remarkable properties to scavenge reactive oxygen species which may have been attributed to the high content of hydrophilic phenolics. The IC_{50} values of all parameters were determined and compared with that of the standard. The extract also exhibited potent anticancer activity done by short-term cytotoxicity trypan blue exclusions method with the IC_{50} value of 185 µg/ml. **Conclusions:** The results obtained in the present study indicate that ethanolic extract of *L. pungens* is a potent source of natural antioxidants.

Keywords: Antioxidant activity, cancer, herbal plants, *Lepidagathis pungens* Nees, phytoconstituents

SUMMARY

- Ethanol extract of *Lepidagathis pungens* Nees., provide the antioxidant and anticancer activities.
- Antioxidant and anticancer activities may be expressed due to the presence of phytoconstituents.
- *L. pungens* Nees., is promising natural sources.



Abbreviations used: BHA:Butylated hydroxyanisole;DPPH:2,2-diphenyl-1-picryl-hydrazyl-hydrate;GAE:gallic acid equivalents;HBSS:Hank's buffered salt solution;IC_{50}:Half-maximal inhibitory concentration;Mo:Molybdenum; NO:Nitric oxide

Correspondence:

Dr. Subramaniam Ananda Thangadurai,
Department of Pharmaceutical Analysis,
J.K.K. Nattraja College of Pharmacy,
Kumarapalayam - 638 183, Namakkal District,
Tamil Nadu, India.
E-mail: anands17@gmail.com

DOI: 10.4103/pm.pm_356_20

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

Oxygen containing molecules and one to many unpaired electrons are usually termed as free radicals and they are very reactive for other atoms or molecules. Antioxidants are those moieties that are present in the cells fight against free radicals by donating the free electron there by preventing the reactions. They destabilize themselves and cause the antioxidation. Oxidative-free radicals are causing damage to the tissues and cells by starting the stress leading to lipid peroxidation. This can be more dangerous to the body and can cause cardiovascular diseases, cancer, early ageing, atherosclerotic, and inflammatory diseases.[1,2] The risk of stress-related chronic diseases can be reduced by the intake of definitive types of plants and herbs that enhance the health benefits.

Phytochemicals such as phenolics, alkaloids, flavonoids, and tannins possess free radical scavengers, possessing antioxidant property. Supplementations of antioxidant herbs can reduce the oxidation stress, which includes a nutrient antioxidant such as Vitamin C, Vitamin E, and plant-derived natural antioxidants.[3] Regular antioxidant substances are accepted to show a possible function in meddling the cycle of oxidation and the response to the free radicals and the chelation of synergistic metals to scavenge oxygen in natural system.

Lepidagathis genus plants were less explored for its biological activity. Less scientific literature is available regarding bioactivity.[4] Plants that belong to the *Lepidagathis* class had been utilized customarily for treating

Cite this article as: Dhanalakshmi M, Thangadurai SA. Antioxidant and anticancer activities of whole plant extracts of *Lepidagathis pungens* nees: *In vitro* evaluation. Phcog Mag 2021;17:S63-7.

of polyuria, fever, calculi in the urinary tract, dysentery, uterine issues along with dysuria. Other restorative employments of *Lepidagathis* sp. incorporate skin contaminations, headache, jungle fever, cardiovascular illnesses, and gastric issues. Keeping all these into account, the current study was planned for the evaluation of antioxidant activity, anticancer activity by short-term trypan blue exclusion method as well as it deals with preliminary phytochemical screening, total phenolics, total flavonoid, and tannin content. There is no scientific evidence of biological activity reported in *Lepidagathis pungens* Nees (*L. pungens*).

MATERIALS AND METHODS

Plant collection and authentication

The mature and healthy whole plant of *L. pungens* was collected from Tirunelveli district. Dr. Chelladurai V., Research officer at CCRAS, Tamil Nadu authenticated the sample specimen and the same was deposited to the Pharmacognosy Department at SVCP, Namakkal, Tamil Nadu.

Preparing crude extracts

The entire plant of *L. pungens* was decisively washed with faucet water and dried underneath duskiness in room temperature for about 14 days. At that point, they were squashed into powder and put away in room temperature. The pummeled materials were gone through sifter no. 40 and 80. The squashed materials of indistinguishable size present between those two sifters were gathered and pressed in an impermeable compartment for valuable use. Around 1 kg of concealed and dried entire plant *L. pungens* was removed in soxhlet progressively with oil ether (LPPE), chloroform (LPCH), ethyl acetic acid derivation (LPEA), ethanol (LPE), and water (LPAQ). Each concentrate was vanished utilizing rotating vacuum evaporator. The concentrate acquired with every dissolvable was gauged and the rate yield was determined as far as dried load of the entire plant. The consistency and shade of the concentrate were noted. All the solvents utilized for this work were of expository evaluation.

Preliminary screening for phytochemistry of *Lepidagathis pungens* extracts

Rough concentrates of five extracts were investigated for the detection of phytochemicals following procedures in the course book by Harborne.[5] Immediately arranged concentrates were tried for flavonoids (Alkaline reagent test, Shinoda's test, Lead acetic acid derivation analysis and the Sulfuric analysis), alkaloids were investigated by Dragendorff's test, Hager's method, Mayer's method, Wagner's method along with Tannic basic analysis), steroids were evaluated by Salkowsky's test along with Libermann–Burchard's test), and glycosides (Baljet test, Legal test, Keller-killiani method, and Borntrager's method), Vanillin hydrochloride reagent, fixed oils (Spot test and Saponification test), Lead acetic acid derivation test, saponins (Lead acetic acid derivation and Foam method), tannins were estimated by Ferric chloride reagent and Gelatin, Ammonia test and Potassium dichromate reagent.), phenolic segments, carbohydrates (Fehling's test, Molisch's test, Tollen's test, Benedict's reagent, Seliwanoff reagent, and Bromine water reagent), proteins were estimated by Ninhydrin reagent, Xanthoproteic test, Biuret reagent, and Millon's reagent, and terpenoids by Knoller's test.

Determination of the quantity of phytoconstituents in *Lepidagathis pungens*

Determination of the flavonoid contents

Complete flavonoid content was dictated by aluminum chloride technique utilizing rutin as a norm. 4 ml of water and 1 ml ethanol extract were added all together. Then, 0.3 ml of 5% solution of sodium nitrite and 0.3 ml of 10% aluminum chloride were added after 5 min. After 6 min of incubation in normal temperature, 1 ml of 1M solution of sodium hydroxide were added to response mixture. Quickly last volume was made to 10 ml with double-distilled water. The absorbance of test had been estimated with respect to the blank solution at 510 nm utilizing a UV light. All the examination was rehashed multiple times for accuracy and qualities were communicated in mean ± standard error of the mean in wording flavonoid content (quercetin same) per g of dry extract.[6]

Determination of phenol contents

Complete phenol contents were determined using methods of standard curve technique utilizing gallic-acid as a normal. Twenty percent solution of sodium carbonate, 100 mg of the unrefined extract were added to 0.5 ml double distilled water and afterward 0.25 ml of the Folin-Ciocalteu reagent solution. Then, 1.25 ml solution of sodium carbonate were added and afterwards mixed. The absorbance of standard solution was measured at 725 nm after 40 min of time. Absolute phenol content was determined utilizing the Gallic-acid equal from the standard curve method.[7]

Antioxidant activity of crude extracts of *Lepidagathis pungens in vitro*

Complete antioxidant Profile

The complete antioxidant property of the crude extracts were measured by conversion of Mo(6) to Mo(5) for giving a complex of phosphomolybdenum. Aiquots of 0.2 ml of the extract solutions (10–100 µg/ml) were pooled with 2.0 ml of reaction mixture (0.6M H_2SO_4, 4 mM solution of ammonium molybdate and 28 mM solution of sodium phosphate). These vials were closed with a cap and let to incubate in a water bath under 95°C for 30 min. The aliquots were let to cool in normal temperature and the absorbance was estimated at 695 nm with respect to the blank. The antioxidant potential was represented in relation to ascorbic-acid, used as standard.[8]

Scavenging of diphenyl-2-picryl hydrazyl radicals

Diphenyl-2-picryl hydrazyl (DPPH) is a chemical that is used to measure the scavenging of free radicals activity was measured in terms of radical scavenging ability or hydrogen donating by utilizing the stable DPPH radical, the method described by Blois.[9,10] 1.0 ml of DPPH solutions were added in ethanol to 3.0 ml of the extraction mixture (or standard drugs) and concentrations were made up with distilled water at various concentrations (10–100 µg/ml). After 30 min absorbances were measured at 517 nm. Lowest values of absorbance indicated more scavenging property of free radicals to scavenge. The DPPH free radicals was estimated using the below equation. Standard taken was Ascorbic acid.

$$\% \text{ Inhibition} = \frac{\text{OD of control} - \text{OD of sample}}{\text{OD of control}} \times 100$$

OD refers to Optical densities.

Free radicals scavenging activity (nitric oxide)

Free radical of nitric oxide (NO) scavenging property methodology was reported from reported methodology. Rutin was taken as the standard drug.[11] About 2 ml solution of 10 mM of sodium nitroprusside and 0.5 ml solution of phosphate saline (pH-7.4) was reacted with 0.5 ml of the sample at various concentrations (10–100 µg/ml) and reaction mixtures were incubated under 26°C for 2.5 h. Then 1.5 ml of incubated mixture was mixed to 1.5 ml of griess reagent (1% of sulphanilamide

solution, 2% of O-phosphoric acid, 0.10% of napthylethylenediaminedi hydrochloride). Then again it was reacted at normal temperature for 5 min. The absorbance of the mixture was noted at 546 nm.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance Control}} \times 100$$

Hydroxyl ion free radical inhibition activity

The free radical inhibition activity for hydroxyl radical was determined by fentons reaction and Butylated Hydroxyanisole was used as a standard. The Reaction mixture is composed of 60 µl solution of 1.0Mm $FeCl_2$, 90 µL solution of 1 mM 1, 10-phenanthroline, 2.4 ml solution of 0.2M phosphate saline buffer (pH 7.8), 150 µL of 0.17M solution of H_2O_2, and 1.50 ml of extract in different concentration. Addition of H_2O began the reaction mixture. After the letting to react at normal temperature for 5 min, and the absorbance of the reaction medium at 560 nm (Schimadzu UV-Vis 1601) was measured using a spectrophotometer. The hydroxyl radicals scavenging was estimated as.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

In vitro estimation of anticancer activity

Cytotoxicity of short term estimated by trypan blue exclusion method

Cell viability is most commonly tested using trypan blue exclusion assay. The method starts with the washing of cells using Hank's Buffered Salt Solution (HBSS) and centrifugation at 10000 rpm for about 10–15 min of time. The experiment was repeated for three times. The cells in the EAC were suspended in the already known quantity of the HBSS medium and the cells number is adjusted so that it contains 2 × 106 cells/ml. this suspension was then transferred to the eppendorf tubes. These cells were exposed to the estimated concentrations of the extract in serial dilutions and were incubated at the room temperature for about 3 h. after 3 h the blue dye test meaning the equal quality of the cells that are treated with drug are reacted with the tryphan blue dye and allowed to rest for 1 min. It was then put back in the hemocytometer and the viability and nonviability of the cells was measured in 2 min. The viability of the cells does not allow the cells to take up the dye ad the dead cells with imbibe the color. The onger the reaction as allowed the more the color was absorbed. The percentage inhibition of the growth was estimated using the below formula.[12]

$$\% \text{ Inhibition} = 100 - \frac{(\text{Total no. of cells} - \text{No. of Dead cells})}{\text{Total no. of cells}} \times 100$$

RESULTS AND DISCUSSION

Almost all the phytoconstituents [Table 1] have antioxidant activity that can protect the living cells from free radical damages (oxidative stress) and therefore it can reduce the risk of developing certain degenerative diseases.[13] The phytochemical studies supported that the ethanol extract of whole plant of *L. pungens* resulted presence of phenolic compounds, flavonoids, tannins with 39.28, 12.23, and 5.64 mg/g, respectively, during the quantitative phytochemical screening compared to other extracts [Table 2].

The estimation of the total phenol and flavonoids content resulted in highest content in the ethanol extract suggesting that antioxidant property of plant. Flavonoids and phenols are the compounds that are derived from the plant sources and the major parts of them are sources from food. Thus, most of the edible herbs are rich in antioxidant potential.[14] they also act as by scavenging the different kinds of oxidation species that is hydroxyl and peroxide radicals that also act as for quenching the singlets of oxygen.[15]

The antioxidant potential of the ethanolic extract solution was estimated by the capacity of scavenging DPPH free radical, and were compared to the market standards of the butylated hydroxytoluene . It was noticed that Ethanol extract of *Lepidagathis pungens* (LPE) had the lower percentage inhibition (IC_{50} 49.23 µg/ml). The phenolics that are present in the *L. pungens* extracts might be responsible for the noticed DPPH radicals scavenging mechanisms, since phenol will readily contribute the hydrogen atoms to the radicals.[16]

The total capacity of the antioxidants were represented in terms of ascorbic acid and equal to phospho molybdenum method. The analytical method was dependent on the lowering of the Mo (6) to Mo (5) by the active antioxidant compound and the forming of the green-colored phosphate/Mo (V) complex in acidic pH. LPE showed the high amount of antioxidant capacity 73.04 followed by LPEA, LPAQ, LPCH, and LPPE [Figure 1].

The amount of the flavonoids that are present in the extract are proportional to the free radical activity that the extract process. These compounds have the potential to the prevention of the oxidation[17] carcinogenic and inflammatory effects.[18] Although the DPPH free radicals scavenge capacity of LPE extracts with IC_{50} value equal to 48.89 µg/ml which was comparable to that of standard ascorbic-acid. The

Table 1: Preliminary phytochemical analysis of *Lepidagathis pungens* various extracts

Phytochemicals	LPPE	LPCH	LPEA	LPE	LPAQ
Flavonoids	−	+	+	+	+
Tannins	−	+	+	+	−
Glycosides	−	+	+	+	−
Phenolic compounds	−	−	+	+	+
Proteins	−	+	+	+	−
Carbohydrates	+	+	+	+	+
Alkaloids	−	+	+	+	+
Triterpenoids	−	+	+	+	+
Steroids	+	−	−	−	−
Fixed oils and fats	+	−	−	−	−
Saponins	−	+	−	−	+

+: Present; −: Absent; *L. pungens*: *Lepidagathis pungens*; LPPE: *L. pungens* progressively with oil ether; LPCH: *L. pungens* chloroform; LPEA: *L. pungens* ethyl acetic acid derivation; LPE: *L. pungens* ethanol; LPAQ: *L. pungens* water

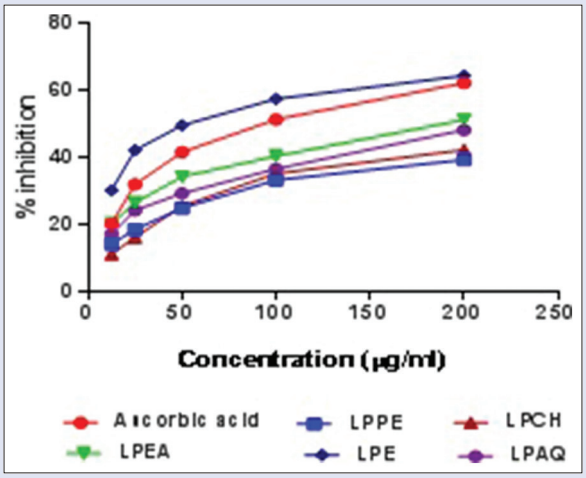


Figure 1: Total antioxidant activity

study demonstrated that the extracts had the proton-donating capacity and could help as free radicals inhibitors and scavengers, acting exactly as potent antioxidants [Figure 2].

NO assumes a significant function in different cycles in the body and their harmfulness duplicates just when they respond with the oxygen radicals to frame peroxynitrite atom, which makes further the harm cell molecules like lipids, and nucleic acids.[19] The concentrate of *L. pungens* hindered the nitrite development by rivaling the oxygen particle to respond with NO shaped by the response of sodium nitroprusside in physiological arrangement in the fixation subordinate way. The LPE showed the high NO activity at with an IC_{50} value; of 31.19 µg/ml [Figure 3] followed by LPEA, LPPE, LPCH, and LPAQ. The OH radical is profoundly receptive oxygen focused radical shaped from the response of different hydroperoxide with change metal particles. It assaults DNA, proteins, polyunsaturated unsaturated fat in films, and the greater part of the organic particles. LPEA remove showed focus subordinate rummaging action against hydroxyl radical created in a Fenton's response framework. The IC_{50} value of LPE was found to be 44.82 µg/ml comparable the standard Gallic acid, followed by LPE, LPCH, LPPE, and LPAQ [Figure 4]. The potent antioxidant property of the medicinal plants may be due to the appearance of significant level of phenolic compound.[20]

Trypan blue is a stain utilized in the ID of dead tissue or suitable cells. As the color is not assimilated through the unblemished cell film, practical cells or tissues with flawless cell layer are not hued. In any case, it

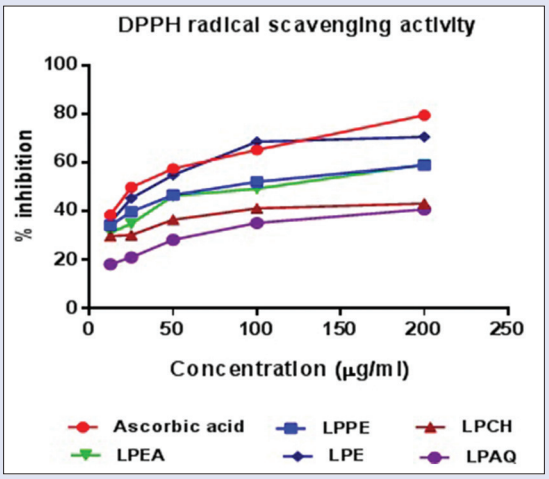


Figure 2: Diphenyl-2-picryl hydrazyl radical scavenging activity

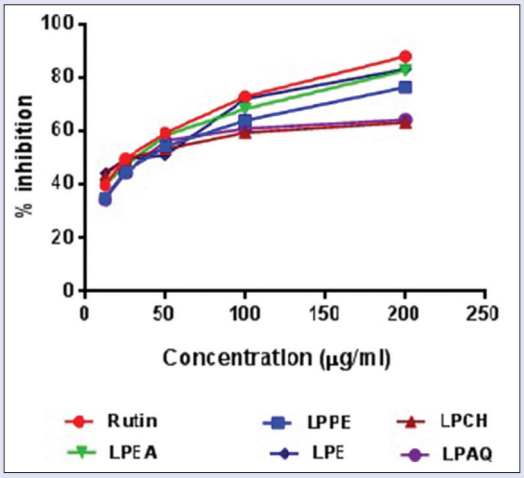


Figure 3: Nitric oxide radical scavenging activity

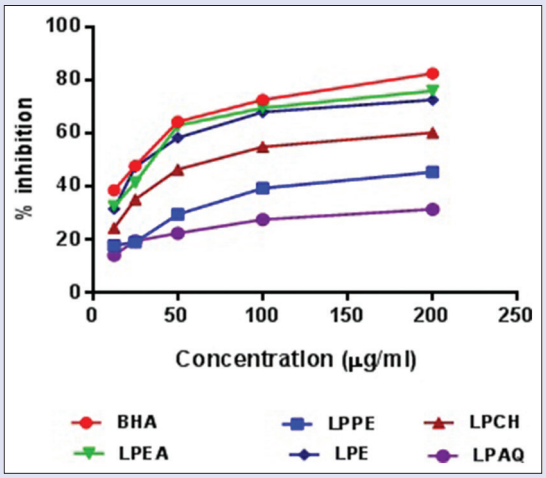


Figure 4: Hydroxyl radical scavenging activity

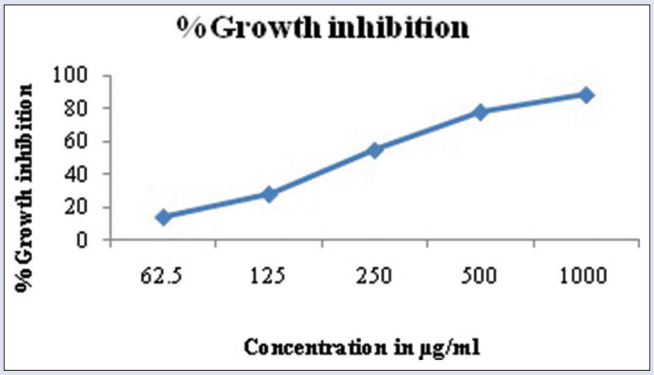


Figure 5: Short term-Trypan blue exclusion method

Table 2: Quantitative estimation of phytoconstituents present in various extracts of *Lepidagathis pungens*

Extract	Percent of yield	Total phenol content (mg of GAE/gm of the plant extract)	Total flavonoids content (mg of QE/g of the plant extract)
LPPE	25.00	11.71±0.08[a]	3.14±0.26[a]
LPCH	9.30	21.25±0.32[b]	7.58±0.02[b]
LPEA	6.60	38.34±0.64[c]	10.64±0.09[c]
LPET	15.00	39.28±0.18[d]	12.23±0.31[d]
LPAQ	19.30	37.56±0.05[b]	11.86±0.22[b]

All values are represented as mean±SEM (n=3). [a-d]Columns wise value with various superscripts of above type indicates significant differences at (P<0.05). SEM: Standard error of mean; GAE: Gallic acid equivalents; *L. pungens*: *Lepidagathis pungens*; LPPE: *L. pungens* progressively with oil ether; LPCH: *L. pungens* chloroform; LPEA: *L. pungens* ethyl acetic acid derivation; LPE: *L. pungens* ethanol; LPAQ: *L. pungens* water

navigates the layer of dead cells. Subsequently, dead cells are appeared in an unmistakable blue shading when seen under the magnifying lens. In the trypan blue avoidance test, there is a portion subordinate inhibitory impact on both malignancy cell lines treated with the LPE remove at expanding concentrations (62.5–1000 µg/mL) for 30 min. After hatching with extricate fundamentally influenced by cytotoxic qualities at the greatest concentration of 1000 µg/ml [Figure 5].

Overall, the finding of this study reveals that ethanolic extract of *L. pungens* has free radical scavenging activity. This high antioxidant activity and potent cytotoxic activity may be due to noticeable quantity of polyphenolic compounds is also reason that *L. pungens* as a beneficial herb to be used as an effective natural antioxidant for health and food industries.

CONCLUSION

The results of this present study confirmed that the ethanol extract of *L. pungens* possessed remarkable anticancer and antioxidant activities when they are tested against different methods *in vitro* models. The antioxidant potency may be expressed due to the presence of phenolic compounds, flavonoids which possess high antioxidant potency. Thus, *L. pungens* extract found to have a non-compromising natural resources of antioxidants and anticancerous constituents, can be used in pharmaceutical fields or nutritional for the prevention of various free radicals mediated diseases.

Acknowledgements

The authors would like to thank the Department of Pharmaceutics, Swamy Vivekanandha College of Pharmacy, Namakkal District for facilitating the research work.

Financial support and sponsorship

Nil.

Conflict of interest

There are no conflict of interest.

REFERENCES

1. Braca A, Sortino C, Politi M, Morelli I, Mendez J. Antioxidant activity of flavonoids from Licania licaniae flora. J Ethnopharmacol 2002;79:379-81.
2. Kumar V, Lemos M, Sharma M, Shriram V. Antioxidant and DNA damage protecting activities of Eulophia nuda Lindl. Free Rad Antioxid 2013;3:55-60.
3. Halliwell B. Antioxidants and human disease: A general introduction. Nutr Rev 1997;55:S44-9.
4. Devkar RA, Chaudhary S, Adepu S, Xavier SK, Chandrashekar KS, Setty MM. Evaluation of antiurolithiatic and antioxidant potential of Lepidagathis prostrata: A Pashanbhed plant. Pharm Biol 2016;54:1237-45.
5. Harborne AJ. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd ed. London: Springer Science and Business Media; 1998. p. 302.
6. Sakanaka S, Tachibana Y, Okada Y. Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha). Food Chem 2005;89:569-75.
7. Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant activity phenol and flavonoid contents of some selected Iranian medicinal plants. Afri J Biotech 2006;5:1142-5.
8. Mitsuda H, Yasumoto K, Iwami K. Antioxidative action of indole compoundsduring the autoxidation of linoleic acid. Nihon Eiyo Shokuryo Gakkai-Shi 1966;19:210-4.
9. Blois M. Antioxidant determinations by the use of a stable free radical. Nature 1958;26:1199-200.
10. Marcocci L, Maguire JJ, Droy MT. The nitric oxide scavenging properties of Gingo biloba extract EGb 761. Biochem & Biophy Res Communi 1994;15:748-55.
11. Garrat DC. The Quantitative Analysis of Drugs. Japan: Chapmanand Hall; 1964. p. 456.
12. Unnikrishnan MC, Kuttan R. Cytotoxicity of extracts of spices to cultured cells. Nutr Cancer 1988;11:251-7.
13. Yu W, Zhao Y, Shu B. The radical scavenging activities of radixpuerariae isoflavonoids: A chemiluminescence study. Food Chem 2004;86:525-9.
14. Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Fazelian M, Eslami B. *In vitro* antioxidant and free radical scavenging activity of Diospyros lotus and Pyrus boissieriana growing in Iran. Pharmacog Mag 2009;4:123-7.
15. Das NP, Pereira TA. Effects of flavonoids on thermal autoxidation of palm oil: Structure activity relationships. J Amer Oil Chem Society 1990;67:255-58.
16. Tung YT, Wu JH, Huang CY, Ku YH, ChangST. Antioxidant activities and phytochemical characteristics of extracts from Acacia confuse bark. Biores Tech 2009;100:509-14.
17. Oki T, Masuda M, Furuta S, Nishibha Y, Terahara, Suda I. Involvement of anthocyanins and other phenolic compounds in radical- Scavenging activity of purple- fleshed sweet potato cultivars. J Food Sci 2002;67:1752-6.
18. Huang WY, Cai YZ, Zhang Y. Natural phenolic compounds from medicinal herbs and dietary plants: Potential use for cancer prevention. Nutr Cancer 2010;62:1-20.
19. Gouthamchandra K, Mahmood R, Manjunatha H. Free radical scavenging, antioxidant enzymes and wound healing activities of leaves extracts from Clerodendrum infortunatum L. Environ Toxicol Pharmacol 2010;30:11-8.
20. AnandakumarAM, Paulsam S, Sathishkumar P, Senthilkumar P. Preliminary phytochemical studies for the quantification of secondary metabolites of medicinal importance in the plant Acalypha fruticosa Forssk. J App Nat Sci 2009;1:41-3.