



***In vitro* screening of antioxidant and antiproliferative effect of *Pogostemon quadrifolius* extracts on cultured MCF-7 cell line**

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ABSTRACT

The aim of this study was to determine the antioxidant and antiproliferative activity of *Pogostemon quadrifolius* medicinal plant extract from Lamiaceae family. Crude leaf extracts, of methanol, ethyl acetate, aqueous and chloroform were evaluated for *in vitro* antioxidant activity using 2,2-diphenyl-2-picrylhydrazyl (DPPH) and reducing power assay. Antiproliferative activity of the plant extract was evaluated in breast cancer cell lines of MCF-7 using 3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium (MTT) assay. The investigation of antioxidant activity by reducing power assay showed that the methanolic extract had a higher activity followed by ethyl acetate, chloroform and aqueous extracts. Antioxidant assay for the different extracts by DPPH assay gave percentage levels of inhibition of various concentrations, among which ethyl acetate extract and methanol extract had relatively higher IC₅₀ values of 88.5 and 80 µg/ml respectively, indicating relatively high antioxidant activity. The results of MTT assay revealed that methanol extract of *P. quadrifolius* is effective, with 50 µg/ml as minimal lethal dose in killing approximately 50% of cells. Planned future investigations should involve the purification, identification, determination of the mechanisms of action, and molecular assay of *P. quadrifolius* plant extracts.

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INTRODUCTION

Cancer is a health problem of global concern (Graidist et al., 2015). Cancer statistics project that by 2030, there will be 17 million deaths and 26 million new cases per year (Thun et al., 2009). Chemotherapy, the most common mode of intervention in cancer, is associated with adverse effects, from nausea to bone marrow failure. Pain and drug resistance are the most frequent and disturbing challenges experienced by patients in all stages of cancer (Miaskowski et al., 2005; Raguz and Yague, 2008). Therefore, the search for effective therapeutics for cancer is an ongoing process (Fadeyi et al., 2013). Alternative and complementary medicines

such as Ayurveda are playing an important role in the treatment of cancer. Currently, about 65% of drugs used in chemotherapy are of natural origin (Nurhanan et al., 2008). Medicinal plants have a long history in both traditional and modern cancer treatments (Conforti et al., 2008; Jain and Jain, 2011) and have been used to treat human diseases for centuries (Sharma et al., 2011; Nisa et al., 2011). Thus, it is possible that traditional medicinal plants can serve as potential sources for developing new drugs and more effective anti-cancer agents for future therapy (Caamal-Fuentes et al., 2011).

One of the plants that was believed to possess potential anticancer activity and is currently under investigation in our laboratory is *Pogostemon quadrifolius* (family: Lamiaceae). The Lamiaceae contains about 236 genera and 6,900 to 7,200 species (Hao et al., 2015) in

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this *Pogostemon* is one of the important genera and a large widespread genus having 114 species in India. Both roots and leaves are mostly used by tribal people in this genus like the fresh root or poultice of the leaves is applied on the snake bites particularly Phursa (*Echis carinatus*) snake bite, uterine haemorrhage, leaf extract used as an insect repellent, treatment of food poison, vomiting and stomach troubles, respiratory tract infection and pollen and nectar used as a source of Panagol honey in Maharashtra (Muthuraj et al., 2015). Recent phytochemical examination of plants which have a suitable history of use in folklore for the treatment of cancer has often resulted in the isolation of principles with anti cancer activity studied by (Afolabi et al., 2007).

The present study aims to investigate antioxidant potential of plant extracts of *P. quadrifolius* using different solvents methanol, ethyl acetate, aqueous and chloroform and also antiproliferative potential of the extracts on cultured MCF-7 cell line.

MATERIALS AND METHODS

Plant materials

The plant samples were collected from uncultivated area of Vazhayoor Hills, Malappuram District, Kerala, South India. The plant samples were preserved as herbarium specimen, identified and authenticated by a taxonomist. The fresh plant was collected and washed thoroughly in distilled water. The leaves were allowed to dry in shade for two weeks. Well dried leaf samples were powdered by conventional methods and stored at 4°C until further use. Crude extracts (10% w/v) were made using 4 solvents; methanol, chloroform, ethyl acetate and aqueous. The extraction was done by rotary shaker at 10,000 rpm for 24 h. Then each extract was filtered through fine muslin cloth and the clear filtrate was evaporated to dryness to form the crude extract and stored at 4°C for further use.

Antioxidant activity by DPPH radical scavenging assay

Free radical scavenging activity of the crude leaf extracts was measured using DPPH radical scavenging assay (Mathew and Abraham, 2006). The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. The absorbance decreases as a result of a colour change from purple to yellow as the radical is scavenged by antioxidants through donation of hydrogen to form the stable DPPH-H molecule.

Various concentration of plant extract (4.0 ml) was mixed with 1 ml of methanol solution containing DPPH (1 mM) radicals to get a final volume of various

concentrations from 40-120 µg/ml. An equal amount of respective solvents were added to the control and finally the mixture was shaken vigorously and left to stand for 30 min followed by measurement of absorbance at 517 nm. Ascorbic acid was used as standard. The percentage of DPPH inhibition of the sample was calculated according to the equation:

$$\% \text{ of inhibition} = \frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}} \times 100$$

The percentage of inhibition was plotted against the sample extract concentration, and a logarithmic regression curve was established in order to calculate the inhibitory concentration 50 µg/ml (IC₅₀) which is the amount of sample necessary to decrease by 50% the absorbance of DPPH.

Antioxidant activity by reducing power assay

Reducing power activity was determined according to the method of (Yildirim et al., 2001). Different concentrations of plant extracts (40, 60, 80, 100, 120 µg/ml) of the study species were mixed with 1 ml of 200 mM sodium phosphate buffer (pH 6.6) and 1 ml of 1% potassium ferric cyanide followed by incubation at 50°C for 20 min. After adding 1 ml of 10% trichloroacetic acid, the mixture was centrifuged at 3000 rpm for 10 min. The supernatant was taken out and mixed with 2 ml of distilled water and 0.5 ml of 1% ferric chloride. Ascorbic acid was used as a reference standard for the test.

Cell lines

MCF-7 breast cancer cell lines were purchased from NCCS Pune and maintained in Dulbecco's modified eagles media (HiMedia) supplemented with 10% fetal bovine serum (FBS) (Invitrogen) and grown to confluency at 37°C in 5% CO₂ in a humidified atmosphere in a CO₂ incubator (NBS, EPPENDORF, GERMANY). The cells were trypsinized [500 µl of 0.025% Trypsin in PBS/ 0.5 mM EDTA solution (HiMedia)] for 2 min and passaged in T flasks in complete aseptic conditions. Extracts were added to grown cells at a final concentration of 6.25, 12.5, 25, 50 and 100 µg/ml from a stock of 1 mg/ml and incubated for 24 h. The percentage difference in viability was determined by standard MTT assay after 24 h of incubation.

In vitro cytotoxicity by MTT assay

The assay was performed as per the procedure

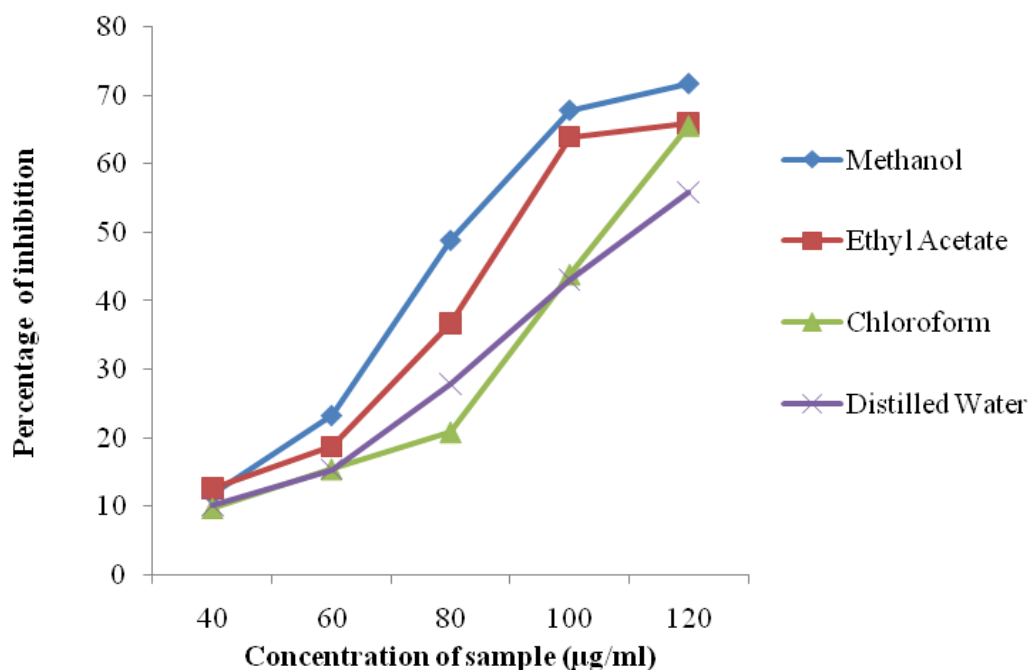


Figure 1. DPPH scavenging activity in different solvents.

Table 1. DPPH absorbance rate in different extracts.

Concentration (µg/ml)	Percentage of inhibition				
	Standard	Methanol	Ethyl acetate	Chloroform	Distilled water
40	13.65±0.30	11.81±1.2	12.7±0.69	9.7±1.6	10.26±1.45
60	33.53±0.2	23.3±1.4	18.8±0.93	15.4±1.03	15.5±0.63
80	61.7±0.51	48.9±0.69	36.7±0.12	20.8±0.9	28±1.42
100	72.92±0.3	67.9±0.1	63.9±0.64	43.8±0.89	43.1±0.92
120	80.04±0.8	71.8±0.68	65.9±0.67	65.5±0.85	55.9±1.03

described previously (Arung et al., 2006). MTT is a colorimetric assay that measures the reduction of yellow MTT by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilised with an organic solvent dimethyl sulfoxide (HiMedia) and the released, solubilised formazan product was measured at 540 nm. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells.

The cells was washed with 1x PBS and then added 30 µl of MTT solution to the culture (MTT -5 mg/ml dissolved in PBS). It was then incubated at 37°C for 3 h. MTT was removed by washing with 1x PBS and 200 µl of dimethyl sulfoxide (DMSO) was added to the culture. Incubation was done at room temperature for 30 min until the cell

got lysed and colour was obtained. The solution was transferred to centrifuge tubes and centrifuged at top speed for 2 min to precipitate cell debris. Optical density was read at 540 nm using DMSO as blank in a micro plate reader (ELISASCAN, ERBA).

RESULTS AND DISCUSSION

DPPH assay

Antioxidant assay for different extracts by DPPH assay gave different percentage level of inhibition for various concentration as shown in Figure 1 and Table 1, and IC_{50} was calculated for each extract, among which methanol extract and ethyl acetate extract showed high IC_{50} value of 80 and 88.5 µg/ml respectively, indicating high

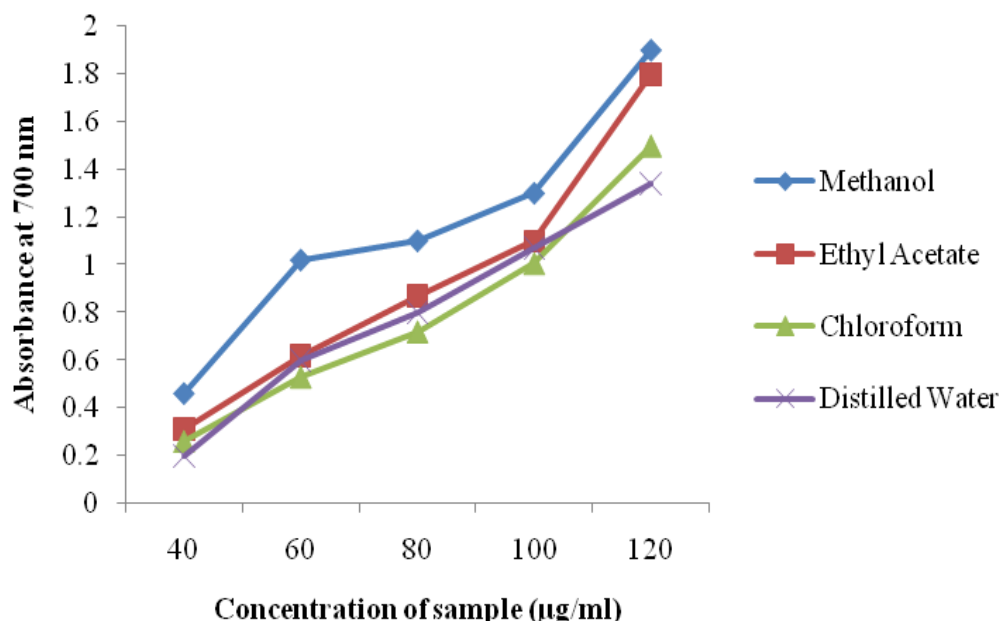


Figure 2. Reducing power assay in different solvents.

Table 2. Reducing power assay rate in different solvents.

Concentration (µg/ml)	Absorbance-700 nm				
	Standard	Distilled water	Chloroform	Ethyl acetate	Methanol
20	0.58±0.09	0.2±0.11	0.26±0.13	0.31±0.09	0.46±0.07
40	1.23±0.08	0.6±0.21	0.53±0.15	0.62±0.07	1.02±0.01
60	1.31±0.11	0.8±0.14	0.72±0.10	0.87±0.11	1.1±0.09
80	1.57±0.09	1.07±0.08	1.01±0.11	1.1±0.12	1.3±0.11
100	2.9 ±0.12	1.34±0.07	1.5±0.12	1.8±0.13	1.9±0.12

antioxidant activity followed by distilled water and chloroform extract with IC_{50} value 104.1 and 110 µg/ml respectively. DPPH radical scavenging activity of *P. quadrifolius* extracts with ascorbic acid as positive control were evaluated based on their ability to quench DPPH and H_2O_2 radicals. The DPPH radical scavenging effect percentage accelerated with increase in the concentration of the extract (40-120 µg/ml). Similarly, appreciable antioxidant and radical scavenging activities have been reported in *P. quadrifolius* (Wojdylo et al., 2007) and in some Lamiaceae members (Cheriyamundath et al., 2015).

Reducing power assay

Reducing power activity is often used to evaluate the ability of natural antioxidant to donate electron (Pavithra and Vadivukkarasi, 2015). The investigation of

antioxidant activity by reducing power assay showed activity in the range (Methanol > Ethyl acetate > Chloroform > Distilled water). Methanol extract showing good activity followed by ethyl acetate, chloroform and distilled water extracts. There is a direct correlation between antioxidant activities and reducing power of certain plant extracts (Hossain et al., 2014). In this study, the methanol extracts exhibited significant reducing power activity in the plants extracts, the reductive capabilities for the methanol extracts of *P. quadrifolius* is presented in Figure 2 and Table 2. According to the results, it can be said that methanol extracts of plants have been significant activities on the reducing power.

MTT assay of *P. quadrifolius* with MCF-7 cell lines

In studied cell lines, the *P. quadrifolius* extract decreased cell viability in longer time exposure in a dose dependent

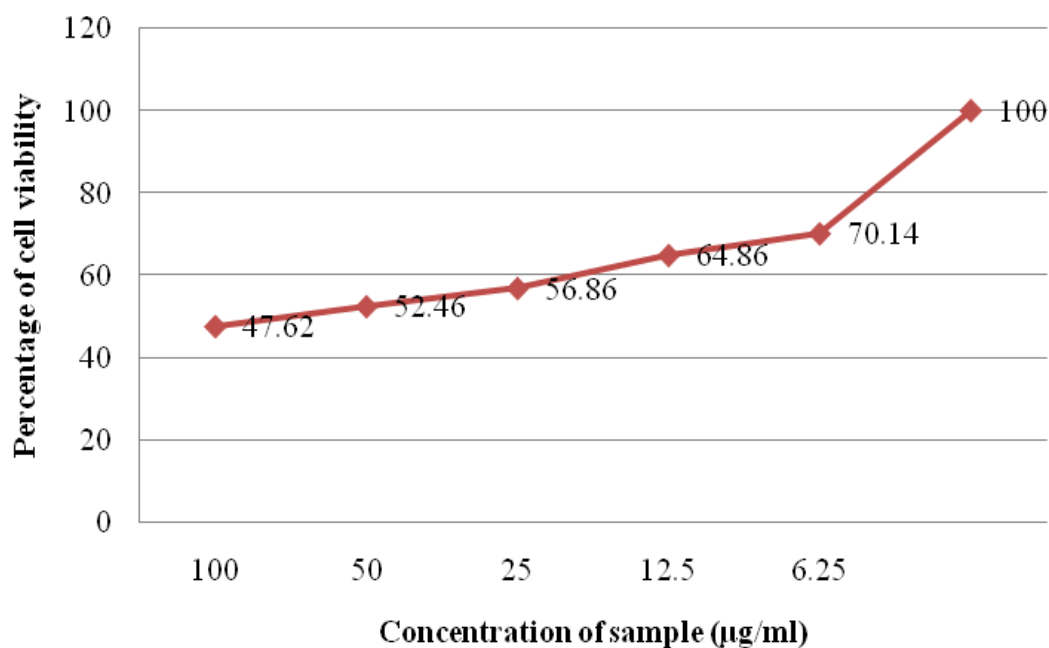


Figure 3. Graphical representation of antiproliferative effect of *P. quadrifolius* (methanol extract) on MCF-7 cell line.

Table 3. Effect of *P. quadrifolius* (methanol extract) on MCF-7 cell line.

Sample concentration (µg/ml)	Average OD (540 nm)	Percentage viability
Control	0.59	100
6.25	0.41	70.14
12.5	0.39	64.86
25	0.33	56.86
50	0.31	52.46
100	0.28	47.62

manner. The more concentrated extract led to higher motility of cell line. Although, MCF-7 cell line required longer exposure time to reach the motility. Both DPPH and reducing power assay showed higher activity for methanol extract comparing to other extracts. So the MTT assay was conducted using methanol extract. For methanol leaf extract it was observed that 50 µg/ml concentrations is the minimum lethal dose that kills approximately 50% of cells (Figure 3 and Table 3). The morphological observation confirmed the apoptosis nature of *P. quadrifolius* extracts on cells as their membrane kept intact and no membrane permeabilization was observed. The results revealed that the leaf (methanol extract) significantly decreased the growth rate and cell survival MCF-7 cell line. The extract induced cell death regarding natural cell growth rate.

MCF-7 cell line naturally has a higher growth rate, thus higher growth inhibition of MCF-7 cell line by the plant extract was confirmed from the Figure 4.

Conclusion

In conclusion, the methanol and ethyl acetate extract of *P. quadrifolius* had cytotoxic effect. The plant extracts, showed a significant decline in the growth rate and survival of breast cancer cell lines. On the basis of generated information on the cytotoxicity and antiproliferative activity of *P. quadrifolius* plant extracts, it may lead to the development of potential herbal formulation to treat cancer, and will require further extensive studies.

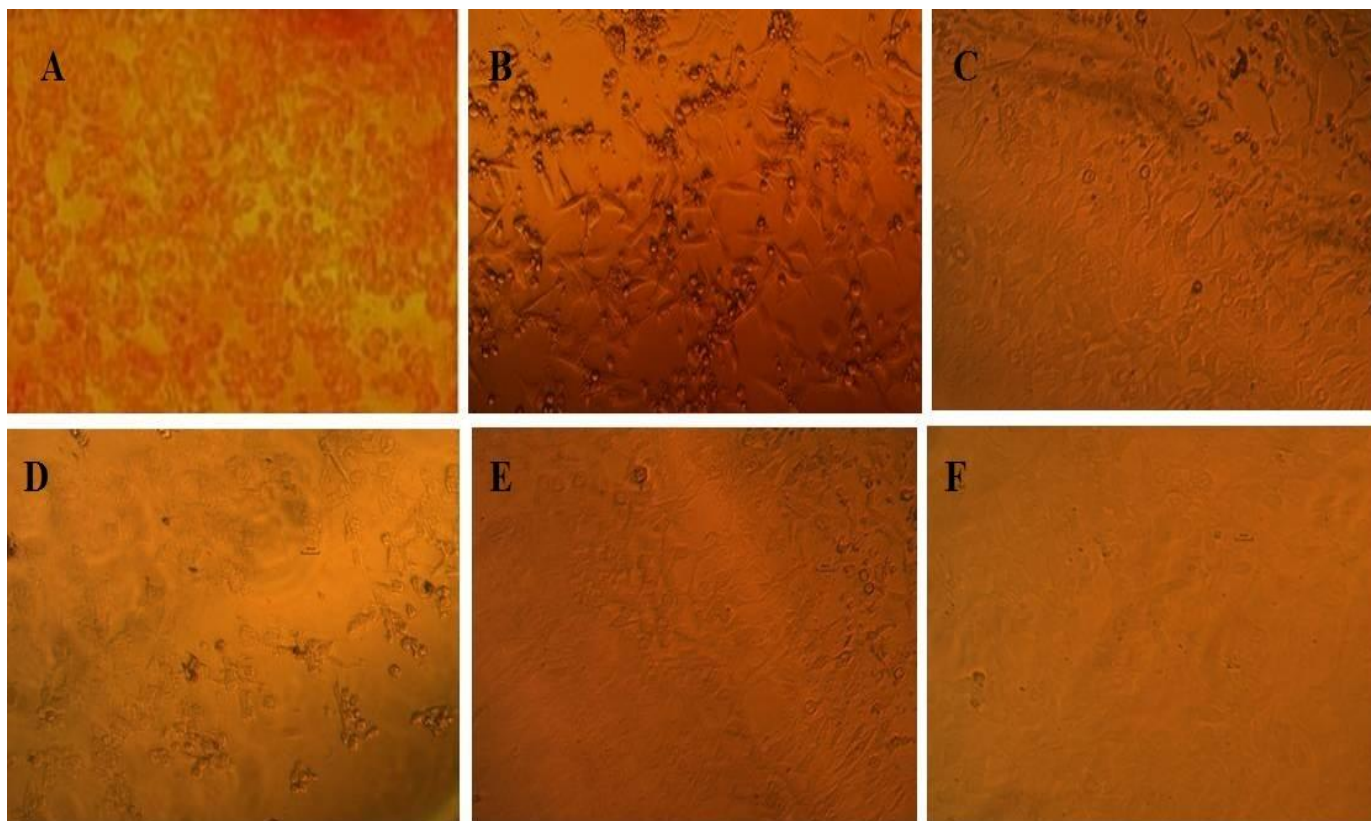


Figure 4. Effect of *P. quadrifolius* (methanol extract) on MCF-7 cell line with toxicity. **A**, Control; **B**, 6.25 µg/ml; **C**, 12.5 µg/ml; **D**, 25 µg/ml; **E**, 50 µg/ml; **F**, 100 µg/ml.

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