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DPPH Radical Scavenging Property of Methanol Leaf Extract from *Pogostemon quadrifolius* (Benth.)

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ABSTRACT

The study was conducted to evaluate the DPPH scavenging property of leaf extracts of plant *P. quadrifolius* (Benth.). In this study, plant leaves were solvent extracted by soxhlet method using petroleum ether, chloroform and methanol sequentially. The extracts were then analyzed for DPPH scavenging properties. Among the three extracts used, only methanol extract has exhibited the DPPH scavenging property. The results showed that the methanol extract of *P. quadrifolius* (Benth.) leaf exhibit DPPH scavenging property with IC_{50} 14.5±1.05 μg mL^{-1}. The phytochemical analysis indicated that the methanol extract is a rich source of phenolic compounds and this may be the reason for DPPH radical scavenging property of this extract.

Key words: Lamiaceae, phytochemical analysis, DPPH, thin layer chromatography, phenol

INTRODUCTION

During the past many decades the researches are going on to identify the health benefits of plants. Many plants are using as folk medicine or as herbal medicine in India and other countries for treatments. It was identified that the phytochemical compounds present in the plants are linked with the reduction of many human chronic diseases like diabetes, Alzheimer's disease, cataract, age related diseases, cancer, cardiovascular diseases etc. (Blomhoff *et al*., 2006; Liu, 2003). The human body is exposed to many oxidizing agents and body itself produces free radicals by metabolic reactions. The free radicals can cause damages to the human body or cells by damaging the DNA, proteins or lipids. The antioxidant systems of human are not completely effective to scavenge and minimize the formation of these oxygen derived species (Blomhoff *et al*., 2006; Halliwell, 1994). Phenolic phytochemicals are most abundant secondary metabolites present in plants and most of them exhibit antioxidant properties. The major categories of phenolic compounds like simple phenol, poly phenol, flavonoids, coumarins, lignans, phenolic acids, xanthones, etc. help to enhance our health benefits (Vattem *et al*., 2005).

Pogostemon quadrifolius (Benth.) is a shrub distributed in India, Bangladesh and Myanmar (Bhatti and Ingrouille, 1997; Lansdown, 2011). The plant is used as folk medicine in India and Bangladesh for the treatment against chicken pox worms and also as a blood purifier (Biswas *et al*., 2010; Padal and Chandrasekhar, 2013; Padal *et al*., 2013; Padal and Raju, 2013; Raju *et al*., 2014). The plant also exhibits mosquito larvicidal and antimicrobial property. (Thoppil *et al*., 2003; Trivedi, 2006). Our studies explored the antiproliferative property of *P. quadrifolius* (Benth.) leaf extracts. It was also found that the reason for antiproliferative property is due to the presence of a new compound (Z)-ethylidene-4,6-dimethoxycoumaran-3-one

and it induces apoptosis in cancer cell line (Cheriyamundath *et al*., 2015; Klika *et al*., 2014). Even though the plant possesses all these medicinal properties, till now there are no reports available on the antioxidant property of this plant. Therefore the present study was conducted to identify its DPPH radical scavenging property of *P. quadrifolius* (Benth.) leaf extracts.

MATERIALS AND METHODS

Preparation of solvent extracts: The plant leaves were collected from the University of Calicut campus. The plant name was authenticated and a voucher specimen (Accession No. 6597, 6598) has been deposited in the herbarium of the Department of Botany, University of Calicut, India. The dried leaves were powdered and extracted sequentially using petroleum ether, chloroform and methanol in increasing order of polarity using soxhlet apparatus until all the constituents were completely eluted. The extracts were then filtered and evaporated to dry. The dried solvent extracts were used for the present study.

DPPH assay: The assay was performed as described earlier (Enujiugha *et al*., 2012). About 0.1 mL of the test extracts was added to 1.9 mL methanol solution of DPPH with concentration of 0.1 mM. The mixture was mixed thoroughly and kept it for 30 min incubation in the dark. After incubation the OD readings of the samples were taken at 517 nm, where methanol was used as blank. The increasing concentration of methanol extract ranging 10-100 μg mL^{-1} was used for this study. Ascorbic acid (Vitamin C) was used as positive control. The IC_{50} was calculated using the ED50 V1.0 freeware tool. The percentage of DPPH scavenging of the sample was calculated according to the equation:

$$\text{DPPH scavenging}\,(\%) = \left(1 - \frac{\text{Sample}}{\text{Control}}\right) \times 100$$

Phytochemical screening by Thin Layer Chromatography (TLC): Preliminary phytochemical analysis of the methanol fraction was evaluated using Thin Layer Chromatography (TLC). The TLC plates were prepared manually and activated in an oven for 1 h at 110°C prior to use. Ethyl acetate-methanol-water (30:5:4) mixture was used as the solvent system for TLC. The TLC plates were sprayed with Dragendorff's reagent followed by 10% aqueous sodium nitrite for the detection of alkaloids, fast blue B salt solution was sprayed for the detection of phenolic groups was determined using, amino acids and biogenic amines were detected using ninhydrin, vanillin-phosphoric acid was used for the detection of terpenoids (Wagner and Bladt, 1996). The antioxidant property of the methanol extract was identified by spraying DPPH (Bhattarai *et al*., 2008). Shinoda's test was performed to detect the presence of flavonoid (Krishnaswamy, 2003). The R_f values of the compounds were identified by the equation:

$$R_f \text{ value} = \frac{\text{Distance from baseline travelled by the solute}}{\text{Distance from the baseline travelled by solvent (Solvent front)}}$$

RESULTS

DPPH assay: Among the three extracts used for the analysis, only methanol extract has exhibited the DPPH radical scavenging property. The petroleum ether and chloroform extracts failed to exhibit DPPH scavenging property. The DPPH scavenging action of the methanol at various concentrations from 10-100 μg mL^{-1} was analyzed. The result displayed the remarkable DPPH

scavenging property of methanol extract with IC_{50} 14.5±1.05 µg mL^{-1}. In this experiment Vitamin C was used as a positive control, where Vitamin C exhibited the IC_{50} of 0.37±0.2 µg mL^{-1}. The graph presenting the DPPH radical scavenging property of methanol extract is shown in the Fig. 1.

Phytochemical analysis (TLC): Phytochemical analysis of the methanol extract was carried out to identify the phytochemical compounds responsible for this activity. The TLC results obtained for the methanol extract in the visible light and UV 365 are shown in the Fig. 2. Visibly identifying

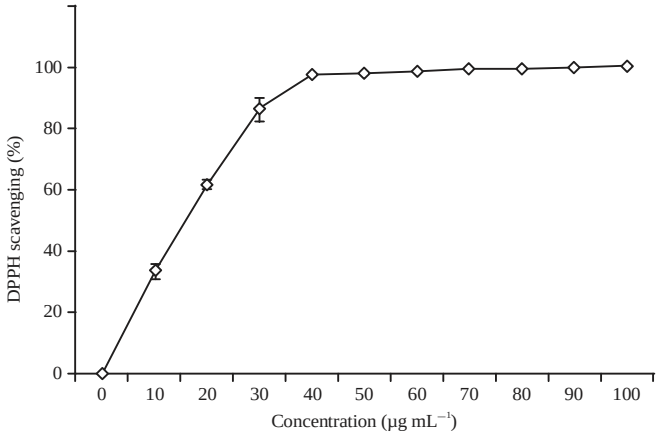

Fig. 1: Graph showing the DPPH radical scavenging property of *P. quadrifolius* (Benth.) leaf methanol extract

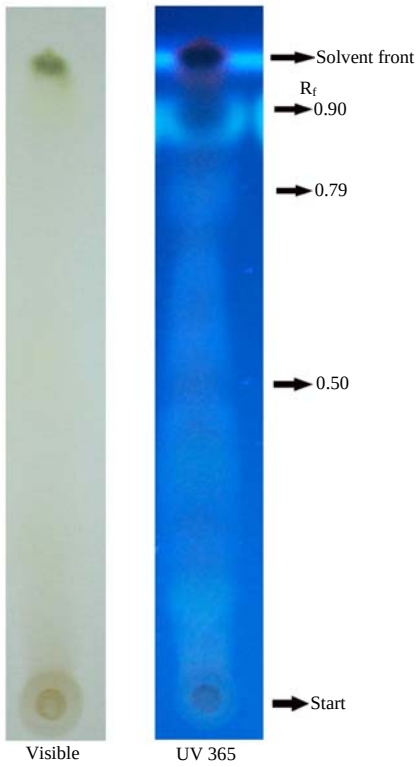

Fig. 2: TLC of methanol extract in visible light and UV 365

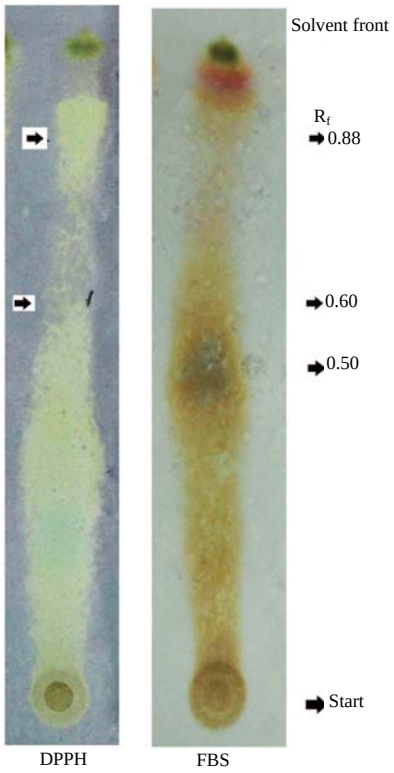

Fig. 3: TLC of methanol extract sprayed with FBS and DPPH reagents

Table 1: Phytochemical screening of methanol extract of *P. quadrifolius* (Benth.) leaves

Spraying reagents/test	Methanol
Dragendorff's	-
Fast blue B	++
Ninhydrin	+
Vanillin-phosphoric acid	+
Shinoda's	-
DPPH	++

compounds under normal light and brightly fluorescing compounds under UV-365 were absent in the methanol extract. The identification of the chemical nature of the compounds present in the extract was further analyzed by spraying the reagents on TLC. The results indicated the presence of high content of phenol in the methanol extract. The spraying reagents also indicated the absence of alkaloid in extract as well as the presence of trace of terpenoid and amino groups just above the starting point of TLC (Fig. 3). Comparing the TLC results of DPPH and FBS spray on TLC, it is understood that the scavenging property of the extract is due to the presence of the phenols in the extract (Fig. 4). Phenols were distributed in the TLC from the starting point to the R_f 0.60. The presence of high content of phenolic compound was observed at the R_f 0.50. Another phenolic compound with DPPH scavenging property was also detected at the R_f 0.88. The phytochemical screening result of methanol extract is shown in the Table 1.

DISCUSSION

At present condition there is an increased interest of identifying antioxidants from the natural product rather than from synthetic sources. The antioxidant compounds reduce the harmful effect

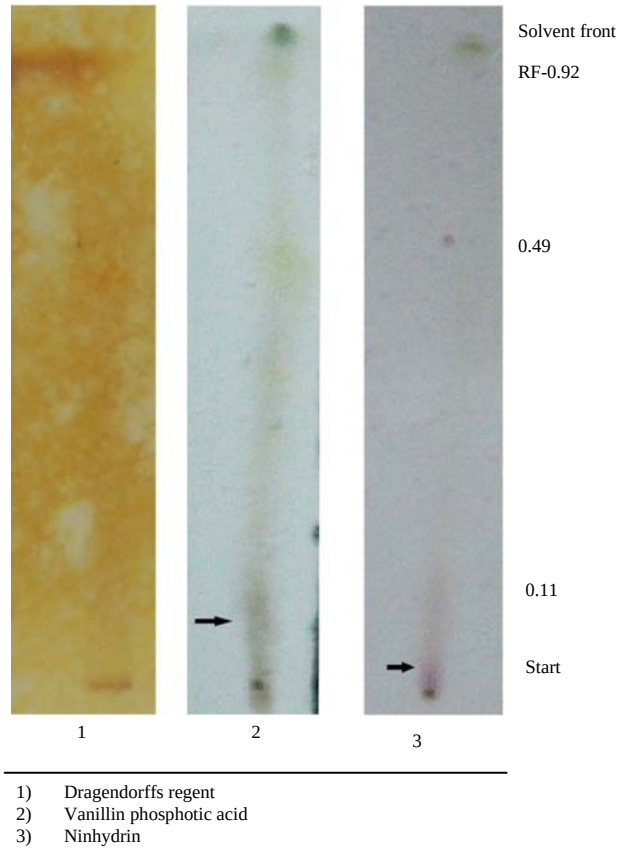

Fig. 4: TLC of methanol extract sprayed with Dragendorff's, Vanillin-phosphoric acid and Ninhydrin reagents

of free radicals or oxidants produced in the human body. Antioxidant also used in the foodstuffs to reduce the worsening of fats and other contents also to improve the health benefits (Blomhoff *et al*., 2006; Molyneux, 2004). Till now there was no studies conducted to identify the antioxidant potential of *P. quadrifolius* (Benth.). *Pogostemon cablin* is a well-studied plant of this genus, which exhibits antioxidant and ROS scavenging property. The major compounds in the plant oils were identified to be sesquiterpenes such as patchouli alcohol, α-bulnesene, α-guaiene , γ-patchoulene, β-patchoulene (Hussain *et al*., 2011; Kim *et al*., 2010). To detect the presence of phytochemical compounds responsible for the antioxidant property of methanol extract, the extract chromatographed TLC plates were sprayed with DPPH. This has revealed the antioxidant property of the methanol extract as shown in the Fig. 4. The discoloration of DPPH spray was due to the scavenging properties of the compounds present in the extract (Bhattarai *et al*., 2008). Comparing the phytochemical analysis results and DPPH sprayed TLC of the extracts confirmed that the DPPH radical scavenging property is due to the presence of high content of phenols in the methanol extract. It was found that most of the phenolic compounds present in the plants possess antioxidant properties due their redox properties, which make the phenol as reducing agent, hydrogen donator and singlet oxygen quencher (Kahkonen *et al*., 1999). Still now, no report is available which indicates the presence of phenolic compounds with antioxidant property from *P. quadrifolius*

(Benth.). Therefore, further experiments for the isolation and identification are yet to be conducted to completely elucidate the structure of the major phenolic compound present in the methanol extract.

CONCLUSION

The present study helped to identify the DPPH radical scavenging property of *P. quadrifolius* (Benth.) leaf methanol extract. The presence of phenolic constituents in the methanol extracts are considered to be the major reason for this property.

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