Original Article

Comparison of Antioxidant activity of in vivo and in vitro leaf explants of Piper longum

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ABSTRACT

Piper longum is a medicinal plant which has antioxidant property. Antioxidant property of plants can scavenge free radicals and protect the cell from oxidation. It helps to cure myocardial ischemic disease, a serious cardiac problem. Hot methanolic extract from leaf explants of plant was prepared for in vivo and in vitro studies and its antioxidant activity was determined by estimating Total phenolic content (TPC) and 2,2- diphenyl 1-2-picryl hydrazyl (DPPH) radical scavenging activity. As compounds responsible for the antioxidant effects are phenolic, hence, a preliminary assessment was done with total phenolic assay. Oxidative stress which can be relieved by antioxidant, is resultant from free radicals. Thus, it is important to measure free radical scavenging activity using DPPH. Comparison of in vivo and in vitro results showed the effect of tissue culture on extent of antioxidant activity of plant which is informative in terms of medicinal value of the plant. Further, the percentage of TPC and percentage of DPPH radical scavenging capacity were evaluated.

Keywords: Piper longum, TPC, DPPH, antioxidant activity, methanol

Introduction

Antioxidant are inhibitors of oxidation,which prevents the oxidation and protect the cell from damage.Plant possessing antioxidant activity are of great use in treatment of various human related ailments.Oxidative stress results due to oxidants or free radicals and this stress is major cause of many diseases.It can cause damage that can result in cancer, ischemia, aging, rheumatoid arthritis, etc.

Antioxidant property of *Piper longum* was determined by performing two methods TPC and DPPH radical scavenging capacity.Hot methanolic leaf extract of the explants was prepared and its antioxidant activity was evaluated. Comparison of *in vivo* and *in vitro* leaf explants yields different results which further provide information about medicinal value of the plant in each case.

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Objective

To estimate antioxidant activity of leaf extract of the medicinal plant Piper longum by two methods namely, TPC and DPPH radical scavenging capacity. Further there was comparison of in vivo and in vitro activity of hot methanolic leaf extract by calculating percentage of TPC and percentage of DPPH from optical densities.

Material and methods

Piper longum plant was procured from botanical garden of National Research Institute of Basic Ayurvedic Sciences, Nehru Garden, Pune, Maharashtra, India.Chemicals and reagents used were Gallic acid, sodium carbonate, 2, 2 – diphenyl 1-2-picryl hydrazy1 (DPPH), Folin-Ciocalteu reagent, concentrated sulphuric acid.

Preparation of Hot Extracts

10 gm leaf powder of Piper longum was taken and mixed with 100 ml methanol it was then heated at 50°C and kept on shaker overnight, next day it was dried in rotavapour, then filtered using whatman filter paper and was preserved at 4°C.

Table 1. Antioxidant property for TPC

Tube number	OD (*in vivo*)	OD (*in vitro*)
1.(Blank)	0.7	0.64
2.(0.1ml gallic acid)	1.25	1.25
3.(0.2ml gallic acid)	1.72	1.68
4.(0.3ml gallic acid)	1.88	1.81
5.(0.4ml gallic acid)	1.94	1.97
6.(0.5ml gallic acid)	1.98	1.93
7.(200µg/ml plant sample+1ml gallic acid)	1.85	1.69
8.(400µg/ml plant sample+1ml gallic acid)	1.65	1.53
9.(800µg/ml plant sample+1ml gallic acid)	1.60	1.48

Table 2: Antioxidant property for DPPH radical scav-enging capacity

Tube number	OD (*in vivo*)	OD (*in vitro*)
1.(Blank) (5ml metha-nol+5ml DPPH)	1.25	0.64
2. 1ml methanolic leaf extract +4ml methanol+5ml DPPH	1.12	1.25
3. 2ml methanolic leaf extract +3ml methanol+5ml DPPH	1.13	1.68
4. 3ml methanolic leaf extract 2ml methanol+5ml DPPH	1.20	1.81
5. 4ml methanolic leaf extract +1ml methanol+5ml DPPH	1.21	1.97
6. 5ml methanolic leaf extract +0ml methanol+5ml DPPH	1.23	1.93

For in vivo analysis

For TPC: Experiment was carried out using 9 test tubes .1st test tube was kept as blank. Gallic acid solution was pipetted in following order 0.1 ml, 0.2 ml, 0.3 ml , 0.4ml, 0.5 ml in 2nd , 3rd , 4th ,5th and 6th test tube , and 1 ml each in 7th,8th and 9th test tube respectively. Plant sample was pipetted in following order in 200 µg/ml, 400 µg/ml and , 800 µg/ml in 7th 8th and 9th test tubes respectively . Then DW was pipetted in following order in 2.5 ml,2.4 ml, 2.3 ml, 2.2 ml, 2.1 ml, 2 ml in 1st, 2nd, 3rd, 4th ,5th and 6th test tube , and 1.5 ml each in 7th 8th and 9th test tube respectively, , further 0.5 ml of folin reagent was added to each tube and were kept for 3 min at room temperature there after 1 ml of 20% sodium carbonate was added and incubated at room temperature for 90 min and absorbance of blue colour developed was read at 760 nm using spectrophotometer[1].

For DPPH: Experiment was carried using 6 test tubes (sterile). 1stt tube was marked as blank, methanolic extract of plant leaf was pipetted in following order 1 ml, 2 ml, 3 ml, 4 ml,5 ml in 2nd ,3rd ,4th ,5th and 6th test tube respectively. Methanol was added in following order 5 ml, 4 ml, 3 ml, 2 ml, 1 ml in 1st ,2nd ,3rd ,4th ,5th test tubes respectively. No methanol was added to the last tube, then DPPH quantity 5 ml was added to each test tube and was kept for 20 min at a temperature of 27°C. Using methanol as blank OD of sample was measured at 517 nm[1].

For in vitro analysis

Callus was grown by tissue culture method using three hormonal combination i.e, Indole acetic acid (IAA), Benzyl amino purine (BAP) and kinetin (KIN) to carry in vitro study.

In vitro grown callus were cut into pieces and crushed with the help of motor and pestle with methanol and then 10 ml of solution was added to conical flask containing 90 ml of water. The steps for preparation of hot extract and protocol for detection of antioxidant activity (TPC and DPPH radical scavenging capacity) were same as in vivo.

Results

In the case of TPC, it was 43.75% for in vivo and 44.75% for in vitro leaf extract whereas in the case of DPPH, it was 1.60% for in vivo and 5.10% for in vitro leaf extract. It was concluded from the calculations that in vitro results were better than in vivo results.

In case of TPC positive control was gallic acid. The percentage TPC for gallic acid was 40.69% for in vivo case for leaf. The percentage TPC for gallic acid was 51.32% for in vitro for leaf. Percentage TPC for test sample in vivo was 43.75% for leaf and for in vitro case it was 44.75% for leaf.

In the case of DPPH positive control was considered in which there was no methanol but only sample. The Percentage of positive control in case of in vivo was 1.60% for leaf and in case of in vitro it was 5.10% for leaf.

Discussion

Antioxidant property was determined by estimation of Total Phenolic Content (TPC)[2]. In the present work, control sample in case of TPC was gallic acid and for DPPH radical scavenging capacity last test tube was considered as control in which there was no methanol. In present work there was dose dependent increase in percentage TPC for hot methanolic leaf extract.Optical densities evaluated in ELISA reader plate are shown in table 1. In previous studies which was carried on fruit extract there was also increase in percentage TPC[1]. Antioxidant property was also determined on the basis of the ability of DPPH to scavenge free radicals[3]. In present work, at low concentration of leaf extract, percentage DPPH radical scavenging capacity is high and vice versa. Optical densities evaluated in ELISA reader plate are shown in table 2.Comparitive analysis between percentage TPC and percentage DPPH in vivo and in vitro shows that in vitro results were better than in vivo results , but results were different in case of previous studies carried on fruit extract of *Piper longum*, in which there was dose dependent increase in percentage DPPH radical scavenging capacity[4,5].

$$\text{Percentage TPC} = \frac{\text{Actual Concentration}}{\text{Observed Concentration}} \text{X} 100$$

$$\text{Percentage DPPH} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \text{X} 100$$

Conclusion

In vitro results were better as compared to *in vivo* results for leaf explants in case of TPC and DPPH radical scavenging capacity for determination of anti oxidant activity which is informative in terms of medicinal value of the plant that can be proved beneficial in treatment of diseases related to oxidative stress.

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