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Full Length Research Paper

Observation of antioxidant activity of leaves, callus and suspension culture of *Justicia gendarusa*

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Antioxidant activity and phenolic compound was found in *Justicia gendarussa* via total phenolic content (TPC) and α,α-diphenyl-β-pycrilhydrazil hydrate (DPPH) radical scavenging assays. The assays were applied on aqueous and methanolic extracts of leaves, callus culture and cell suspension culture of *J. gendarussa*. Callus was induced from green and matured leaves of *J. gendarussa* cultured on Murashige and Skoog (MS) basal medium supplemented with different concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) or 1-naphthaleneacetic acid (NAA) and benzylaminopurine (BAP). Callus induced on NAA showed higher phenolic content and antioxidant activity as compared to the one induced on 2,4-D. The highest total phenolic content (88 mg GAE/g) was observed in callus culture induced with NAA and BAP, while the highest antioxidant activity (lowest IC_{50}; 8.46 × 10^{-3} mg/ml) was observed in the cell suspension.

Key words: Anti-oxidant, callus, *Justicia gendarusa*, suspension culture.

INTRODUCTION

Justicia gendarussa is one of the therapeutic plants which is considered as native to China and it is frequently grown in Indian gardens as hedge or border plant (Krishna et al., 2009). A study showed that the *J. gendarussa* leaves contain beta-sitosterol, antispasmodic, carmiative, alkaloid, lupeol, friedelin and four simple *0*-substituted aromatic amines and its antioxidant is widely used in the treatment of rheumatism, inflammations, bronchitis, vaginal discharges, dyspepsia, eye diseases and fever (Mruthunjaya and Hukkeri, 2007). However, they evaluated the antioxidant and free radical scavenging potential of *J. gendarussa* in leaves only. Besides, it is known that the antioxidant properties of plant extracts cannot be evaluated by one single method due to the complex nature of phytochemicals. So, a detailed antioxidant assay of plant extracts should be developed with different possible sources such as callus and suspension culture by plant tissue culture technique. Johnson et al. (2004) had worked on *in vitro* multiplication of *J. gendarussa*. However, none of them precedes their study by combining both observations. Moreover, no study has been done on the antioxidant activity of callus and suspension culture of *J. gendarussa*. Thus, this study aimed to observe the antioxidant activity of callus and suspension culture of *J. gendarussa* and then compare results with antioxidant activity of its intact leaves.

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Abbreviations: TPC, Total phenolic content; **DPPH,** diphenyl-β-pycrilhydrazil hydrate; **MS,** Murashige and Skoog; **2,4-D,** 2,4-dichlorophenoxyacetic acid; **NAA**, 1-naphthaleneacetic acid; **BAP,** benzylaminopurine.

MATERIALS AND METHODS

Induction of callus, cell suspension and subculture

This method was adapted and modified from Khanam and Khoo, (2000) and Pretto and Santarem (2000). The callus was induced from young leaves of the *J. gendarussa* plant by incising it into small pieces (9 to 10 mm) and cultured aseptically on callus induction media (CIM) supplemented with 1.0 mg/L 2,4-D or NAA and 0.5 mg/L BAP. The cultures were kept in the dark at 26 ± 3°C

Table 1. Results of total phenolic content.

Sample	Total phenolic content (mg GAE/g of extracts)	
	Methanolic extract	Aqueous extract
Leaves	12.68	7.41
Callus culture (2,4-D + BAP)	48	0.78
Callus culture (NAA + BAP)	88	1.43
Cell suspension culture	3.098	0.04

and subcultured every 2 weeks interval onto a fresh CIM plates as the initiation medium (Hasan et al., 2008).Cell suspension culture was initiated by transferring friable callus into a 250 ml conical flask containing 100 ml of optimized liquid CIM. The culture was agitated on a rotary shaker (100, 125 and 150 rpm) in the dark at 26 ± 3℃ and subcultured (Kawazu et al., 1998) in intervals of 10 days into fresh liquid CIM.

Extraction of *J. gendarussa* samples (leaves, callus and cell suspension culture)

The whole antioxidant evaluation method was adapted from Ling et al. (2009). Leaves of *J. gendarussa* were cut into small pieces. Then, the leaves were dried in oven for 16 h at 40 to 50℃. Next, the *J. gendarussa* leaves were ground into powder form using dry blender for further extraction step. Ground sample (leaves of explants, 4 weeks callus and 4 weeks cells suspension culture) and solvent were added into conical flask. Then, the solutions were agitated at 100, 125 and 150 rpm for 24 h at room temperature (25℃). Next, the solutions were filtered using gravity filtration to separate the liquid from solid form. Then, the filtrate was evaporated (applicable for methanolic extract) using dry-rotary evaporator at 40℃ while for water extract, the method was obtained as described by Bidchol et al. (2009) with slight modifications. First, the solution was freezed in freezer for few hours at -20℃ and then kept at -80℃ overnight. Then, the frozen filtrate was lyophilized in lyophilizer (freeze drier) at 5 µm Hg pressure at -50℃.

Total phenolic content (TPC)

TPC was measured by Folin-Ciocalteau method as described by Bidchol et al. (2009) with slight modification. One milliliter of extracts was added to the mixture of 5 ml of deionized distilled water, 1 ml 95% ethanol and 1 ml 5% Na_2CO_3 followed by the addition of 0.5 ml 50% Folin-Ciocalteau reagent. The reaction mixture was incubated for 30 min at 25℃, the absorbance was measured at 765 nm in spectrophotometer, and the phenolic content was measured using Equation 1 with gallic acid as standard and expressed as gallic acid equivalent per gram dry weight.

$$TPC = \frac{concentration\left(\mu\frac{g}{ml}\right) x\ dilution\ (ml)}{weight\ of\ sample\ (g)} \quad (1)$$

DPPH radical scavenging activity

The DPPH test was carried out as described by Bidchol et al. (2009) with slight modification. About 0.2 ml extracts were mixed with 6 ml DPPH solution (0.1 mmol/L, in 95% ethanol (v/v)) and shaken thoroughly. Next, the reaction mixture was incubated for 30 min at room temperature. The absorbance was read at 517 nm in a spectrophotometer against a blank. Gallic acid was used as a positive control. The radical scavenging activity was measured as decrease in the absorbance of DPPH and calculated using the following equation:

$$\%\ activity = \frac{abs(blank) - abs\ (sample)}{abs\ (blank)} \quad (2)$$

RESULTS AND DISCUSSION

Table 1 shows results on TPC assay. The total phenolic content of methanolic extract of leaves of *J. gendarussa* was found to be 12.68 mg GAE/g. In methanolic extract of callus culture with 2,4-D and BAP, the TPC was found to be 48 mg GAE/g, while in NAA and BAP, it was 88 mg GAE/g. Phenolic content was found to be 3.47 mg GAE/g in suspension culture. Aqueous extraction of leaves callus of 2,4-D and BAP, callus of NAA and BAP and suspension culture had shown lower amount of phenolic content than methanolic extraction which are 7.41, 0.78, 1.43 and 0.04 mg GAE/g.

Phenols are very important plant constituents because of their radical scavenging activity resulting from their hydroxyl group.

The phenolic compounds may contribute directly to the antioxidative action (Bidchol et al., 2009). It is known that polyphenolic compounds have inhibitory effects on human body like carcinogenesis and mutagenesis. In this study, it is clearly shown that there is a correlation between phenolic content and antioxidant activity of *J. gendarussa* plant. Methanolic extract of leaves is low in phenolic content as compared to callus culture (2,4-D and BAP) but have high ability for DPPH radical scavenging activity. It shows that in methanolic extract, leaves can scavenge more callus culture and cell suspension culture, which may be due to the presence of nonphenolic compounds in the extract. Bidchol et al. (2009) reported that antioxidant activity could also be caused by nonphenolic compound.

DPPH is a free radical compound. It has been used to test for the free radical scavenging activity of various samples which can be used as reducing agent. The scavenging activity of leaves, callus culture and cell suspension culture of *J. gendarussa* plant was best determined by the DPPH assay. The DPPH radical scavenging assay is a sensitive antioxidant assay and is independent of substrate polarity (Ani et al., 2006). DPPH is a stable free radical that can accept an electron or

Table 2. Summary of IC_{50} for all samples measured by DPPH assay.

Sample	IC_{50} (mg/ml)
Gallic acid (positive control)	0.1
Methanolic extract	
Leaves	26.84
Callus culture (2,4-D + BAP)	15.89
Callus culture (NAA + BAP)	39. 32
Cell suspension culture	8.46×10^{-3}
Aqueous extract	
Leaves	141.49
Callus culture (2,4-D + BAP)	15.81
Callus culture (NAA + BAP)	40.75
Cell suspension culture	10.76×10^{-3}

hydrogen radical to become a stable diamagnetic molecule. The maximum absorption of a stable DPPH radical for methanolic and aqueous extraction was reported (Amarowiz et al., 2000) to be 517 nm. The absorption at this characteristic wavelength disappears as the reaction between the antioxidant molecules and radicals progresses. The decrease in the absorbance depends on the concentrations of the antioxidant and the radical, the molecular structure of the antioxidant, and its kinetic behavior. Therefore, the decrease in absorbance at the characteristic wavelength is a measure of the radical scavenging activity of the antioxidant employed (Pretto and Santar´em, 2000). Table 2 shows the concentration of extract that had 50% free radical scavenging activity (IC_{50}). The initial ratio of solvent and sample is important to begin the extraction process for this DPPH assay. In this study, the ratio of solvent to sample for leaves is 50 ml : 1 g, callus (both 2,4-D and NAA) is 15 ml : 0.3 g and for cell suspension culture is 10 ml : 0.05 mg. This ratio has effects on the amount of extraction yield. The scavenging effects of each sample was expressed in terms of IC_{50} (1 mg/ml or mM required to inhibit DPPH radical formation by 50%) and calculated from the log dose inhibition curve.

J. gendarussa extract exhibited a dose-dependent scavenging of DPPH radicals (Ani et al., 2006) and 26.84 mg/ml of methanolic extract from leaves was sufficient to scavenge 50% of DPPH radicals. The radical scavenging effect of *J. gendarussa* leaves was found to be 2.6 times less potent than the standard gallic acid (IC_{50} 0.1 mg/ml). However, the callus culture (induced with 2,4-D and BAP) extract gave 15.89 mg/ml, while callus culture of NAA and BAP extract was sufficient to scavenge 50% of DPPH radical at 39.32 mg/ml. This suggested that *J. gendarussa* callus induced on NAA and BAP is the least effective free radical scavenger or hydrogen donor and contributes insignificantly to the antioxidant capacity of *J. gendarussa*. Methanolic extract of cell suspension culture seems to be very weak to scavenge DPPH radical with IC_{50} value of only 8.46×10^{-3} mg/ml.

DPPH radical reacts with suitable reducing agents and loses color stoichiometrically with the number of electrons consumed which is measured using spectrophotometer at the wavelength of 517 nm. Gallic acid is a potent free radical and also known as natural antioxidant. The IC_{50} value of DPPH radical scavenging activity of leaves, callus culture and cell suspension culture seems to be higher as compared to gallic acid. This shows that *J. gendarussa* is a good free-radical scavenger. Whereas, the DPPH radical scavenging activity of aqueous extract for all leaves, callus culture of 2,4-D and BAP, callus culture of NAA and BAP and also cell suspension somehow shows lower antioxidant activity as compared to methanolic extract with IC_{50} value of 141.49, 15.81, 40.75 and 10.76×10^{-3} mg/ml, respectively. This might be due to the presence of more phenolics and flavonoids components which are responsible for free-radical scavenging activity in methanolic extract as compared to aqueous extract. It also suggested that methanol is the suitable solvent.

Conclusion

Callus is the most suitable sample that shows highest antioxidant activity by TPC and DPPH assay and methanol is the suitable solvent for antioxidant extract from *J. gendarussa*.

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REFERENCES

Amarowiz R, Naczk M, Shahidi F (2000). Antioxidant Activity of Crude Tannins of Canola and Rapeseed Hull. JAOCS 7:957-961.

Ani V, Vadaraj MC, KAN (2006). Antioxidant and Antibacterial Activities of Polyphenol Compounds from Bitter Cumin (*Cuimium nigrum* L.). Eur Food Res Teschnol 224:109-115.

Bidchol AM, Wilfred A, Abhijna P, Harish R (2009). Free radical scavenging activity of aqueous and ethanolic extract of Brassica oleracea L. var. Italic. Food and Bioprocess Technology. DOI: doi:10.1007/s11947-009-0196-9.

Hasan MF, Das R, Rahman MS, Rashid MH, Hossain MS, Rahman M (2008). Callus Induction and Plant Regeneration from Shoot Tips of Chakunda (*Cassia obtusifolia* L.). Int. J. Sustain. Crop Prod 3:6-10.

Johnson M, Manickam VS, Sonali D, Nikhat Y, Andal N (2004). *In Vitro* Multiplication of Two Economically Important and Endangered Medicinal Plants – Justicia gendarussa Drum and Adenia hondala (Gaertn) De Wilde. Malaysian J Sci. 23:49-53.

Kawazu K, Marwani E, Kobayashi A, Nitoda T, Kanzaki H (1998). Production of Antibacterial Triterpene Acids Not Detected inTectona grandis the Native Plant by Cell Suspension Culture of Tectona grandis, Okayama Univer. pp. 9-12.

Khanam N, Khoo CAGK (2000). Effects of cytokinin/auxin combinations on organogenesis, shoot regeneration and tropane alkaloid production in Duboisia myoporoides. Plant Cell, Tissue Organ Cult. 62:125-133.

Krishna KL, Mruthunjaya K, Patel JA (2009). Antioxidant and Hepatoprotective Activity of Leaf Extract of Justicia gendarussa Burm. Int. J. Biol. Chem 3:99-110.

Ling LT, Yap SA, Radhakrishnan AK, Subramaniam T, Cheng HM, Palanisamy UD (2009). Standardised Mangifera indica extract is an ideal antioxidant. Food Chem 113:1154-1159.

Mruthunjaya K, Hukkeri VI (2007). Antioxidant and Free Radical Scavenging Potential of *Justicia gendarussa* Burm. Leaves *in vitro*. Nat. Prod. Sci. 13:199-206.

Pretto FR, Santar´em ER (2000) Callus Formation and Plant Regeneration from Hypercium perforatum Leaves. Plant Cell, Tissue Organ Cult. 62:107-113.