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Research Article

EVALUATION OF PLANT AND CALLUS EXTRACTS OF *JUSTICIA GENDARUSSA* BURM. F. FOR PHYTOCHEMICALS AND ANTIOXIDANT ACTIVITY

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ABSTRACT

The *in vitro* grown stem and leaf derived calli of *Justicia gendarussa* Burm. f. obtained on solid MS medium supplemented with NAA+BAP (1+0.1mg/l) were cultured on solid and liquid MS medium for 30-35days. The, stem, leaf and their respective calli samples were extracted in ethanol, methanol and ether. A significantly highest on par concentrations of 183.33 ± 57.73mg/g of phenolics was observed in the solid grown stem callus extracted in methanol and solid grown leaf callus extracted in ethanol. Ethanol found to be a better solvent for the extraction of flavonoids with a highest on par concentrations of 17.00 ± 7.31mg/g, 17.39 ± 1.50mg/g and 14.23 ± 0.28mg/g in liquid grown leaf callus, solid grown leaf callus and liquid grown stem callus respectively. Highest DPPH scavenging activity of methanol extracts of stem derived callus cultured on solid medium was observed at the concentrations of 145.00 ± 5.00µg/ml. The highest reductive capacity was observed for ethanol extract of stem derived callus cultured on solid medium (136.66 ± 2.88mg AAE/g of dried extract).

Keywords: *Justicia gendarussa*, *In vitro* culture, Phenolics, Flavonoids, Antioxidant activity

INTRODUTCION

The over production of reactive oxygen (ROS) and nitrogen species (RNS) in the system damage to DNA, lipids and proteins which is associated with an increased risk of cardiovascular disease, cancer and other chronic diseases[1]. The natural antioxidants of late, considered to be important due to their fewer side effects are the compounds that can neutralize free radicals by donating hydrogen thus, reduce the risk of vascular diseases, some forms of cancer and oxidative stress responsible for DNA, protein and membrane damage[2]. Antioxidant properties of sesame (*Sesamum indicum*) cake extract used in the stabilization of sunflower and soybean oils[3], antioxidant and free radical scavenging activity of Korean medicinal plants[4] due to the presence of phenolics and flavonoids were reported. The phytochemicals produced in the *in vitro* cultured cells were also reported to show the antioxidant properties in *Tecoma stans* (L.) Juss. ex Kunth.[5], *Brassica nigra* L.[6], *Gardenia jasminoides* Ellis[7].

Justicia gendarussa Burm. f. (Acanthaceae) is well known for its medicinal properties such as larvicidal, antioxidant, immunosuppressive, antinociceptive, ametic, anti-arthritic and anti-inflammatory activities[8-15]. The plant was also reported to contain phenolics, alkaloids and sterols[16-20]. The present work is aimed at the maintenance of stem and leaf derived callus on solid and liquid nutrient media, extraction of phytochemicals in different solvents and determination of phenolics, flavonoids and antioxidant activity followed by comparison with the plant samples.

MATERIALS AND METHODS

Plant material

Justicia gendarussa was collected from natural forests of Dakshina Kannada District, Karnataka, India, and identified following the Flora of Udupi and Dakshin Kannada[21] and the voucher specimen (MU/AB/BN-02) were deposited at the herbarium of Department of Applied Botany.

The healthy stem and leaf samples were collected from the plant, cleaned, dried at 40°C and powdered using Cyclotech lab mill for the extraction of phytochemicals.

Callus induction and harvesting

The surface sterilization of the stem and leaf samples were carried out as reported by Bhagya and Chandrashekar[22]. The stem and leaf explants were washed thoroughly under running tap water for 20-30min to remove the surface debris and treated with a systemic fungicide Bavistine for 30-45min. The explants were then sterilized with 70% alcohol (2min) and 0.1% $HgCl_2$ (8 min). After each step, the explants were washed using sterile distilled water. The callus was induced on MS medium with NAA + BAP (1+0.1mg/l) under 25±2°C with 16 hrs of photoperiod and 40.0±3.0µmol $m^{-2}s^{-1}$ light intensity. The cultures maintained for 320-360d were inoculated to both solid and liquid MS medium. The cells were harvested at the stationary phase i.e. between 30-35th day based on the growth analysis (Unpublished data), washed in distilled water and freeze dried.

Chemicals and reagents

All the chemicals used were of analytical grade, purchased from Merck, Himedia and SRL. DPPH was purchased from Sigma Aldrich, USA.

Extraction and spectrophotometric estimation of phytochemicals

The phenolics and flavonoids found in the stem, leaf, stem derived callus and leaf derived callus was extracted in methanol, ethanol and ether as per Harborne[23]. Total phenolic and total flavonoid contents in the crude extract was determined as per Malic and Singh[24] and Jia *et al*[25]respectively. Free radical scavenging activities of the extracts were determined using 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) as per Mensor *et al*[26] and the reducing power of the samples by the method of Oyaizu[27].

Statistical analysis

The data presented are the average of three replicates and expressed as Mean±SD. The statistical analysis of all the data were carried out using SAS 9.0 version and the treatment means were compared using DMRT at a level of 5% significance.

RESULTS AND DISCUSSION

Spectrophotometric estimation of phenolics

The spectrophotometric estimation of phenolics in the test samples showed a significantly highest on par concentrations of 183.33 ± 57.73mg/g in the solid grown stem callus extracted in methanol and solid grown leaf callus extracted in ethanol (alcohol) followed by 133.33 ± 14.43mg/g and 100.00 ± 0.00mg/g in solid grown stem callus in ethanol (alcohol) and liquid grown stem callus in methanol respectively (Fig. 1).

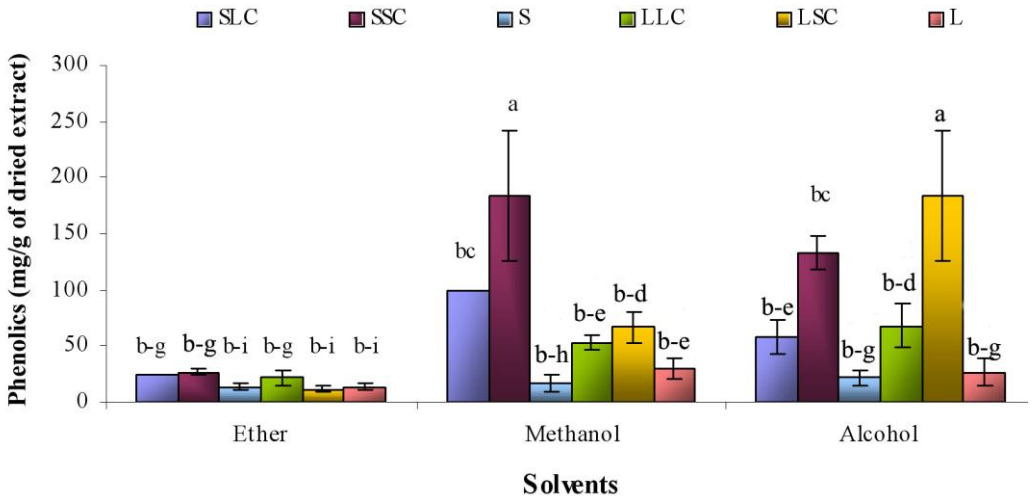

Fig. 1: Concentration of phenolics in different extracts of *J. gendarussa*

S.E/plot = 21.21, C.V. (%) = 36.26

Values represent the Mean±SD. Means with different letters indicate significant differences at 5% level.

SLC = stem callus in liquid medium, SSC = stem callus on solid medium, S = stem, LLC = leaf callus in liquid medium, LSC = leaf callus on solid medium, L = leaf

The concentration of total phenolics accumulated in the cultured tissue was higher in the callus of *Tecoma stans* (L.) Juss. ex Kunth.[5] and in the hypocotyl explants derived callus of *Brassica nigra*[6]. Even in the present study, the spectrophotometric estimations of phenolics in *J. gendarussa* showed the highest concentration in solid grown callus of stem explant extracted in methanol and solid grown callus of leaf extracted in ethanol. Amid *et al.*[28]estimated the phenolic content in the suspension culture from the leaf derived callus of *J. gendarussa* grown in MS medium supplemented with NAA + BAP (1 + 0.5mg/l). The phenolic content of 88mg/g observed by them was very low compared to the total phenolic content of 183.33 ± 57.73mg in the solid grown callus of stem and leaf explants extracted in methanol and ethanol respectively in the present study.

Spectrophotometric estimation of flavonoids

Ethanol (alcohol) was a better solvent for the extraction of flavonoids with a highest on par concentrations of 17.00 ± 7.31mg/g, 17.39 ± 1.50mg/g and 14.23 ± 0.28mg/g in liquid grown leaf callus, solid grown leaf callus and liquid grown stem callus respectively (Fig. 2).

Flavonoids were reported from the leaf derived callus of *Saussurea medusa* Maxim.[29] and callus cultures of *Saussurea involucrata*[30]. The cell suspension cultures from leaf, fruit and root explants of Indian Mulberry (*Morinda citrifolia*) in MS medium supplemented with NAA, BAP and Kn led to the increased production of flavonoids[31]. Similarly, in the present study the suspension grown leaf callus, solid grown leaf callus and suspension grown stem callus showed the highest on par concentrations of flavonoids in *J. gendarussa*.

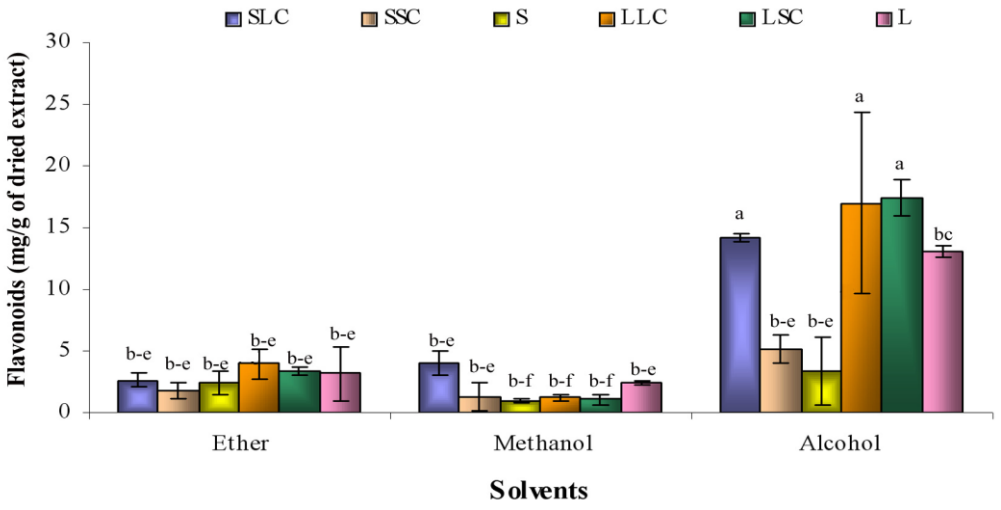

Fig. 2: Concentration of flavonoids in different extracts of *J. gendarussa*

S.E/plot = 2.04, C.V. (%) = 37.29

Values represent the Mean±SD. Means with different letters indicate significant differences at 5% level.

SLC = stem callus in liquid medium, SSC = stem callus on solid medium, S = stem,

LLC = leaf callus in liquid medium, LSC = leaf callus on solid medium, L = leaf

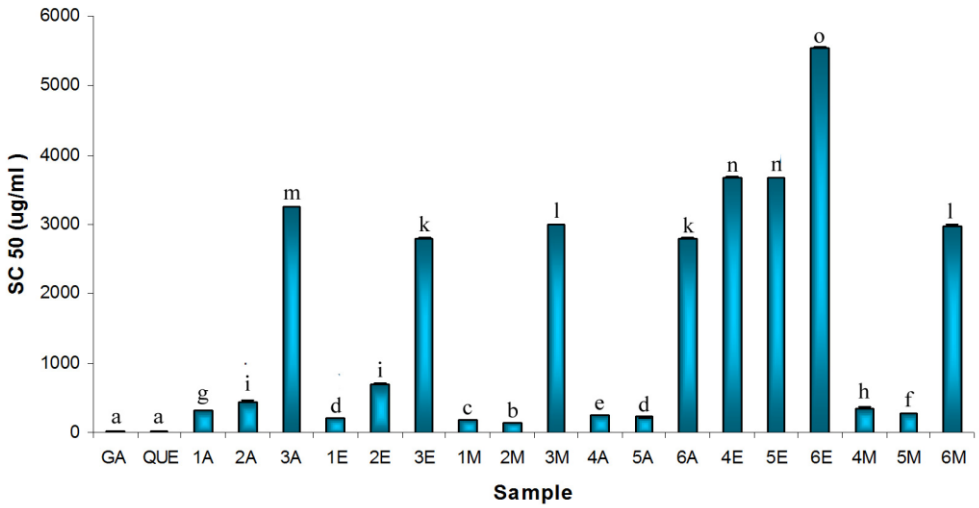

Fig. 3: DPPH activity of different extracts of *J. gendarussa*

S.E/plot = 7.01, C.V (%) = 0.45

Values represent the Mean±SD of three experiments. Means with different letters indicate significant differences at 5% level.

1 - Liquid callus of stem; 2 - Solid callus of stem; 3 - Stem; 4 - Liquid callus of leaf; 5 – Solid callus of leaf; 6 - Leaf; A - Ethanol extract; E - Ether extract; M - Methanol extract

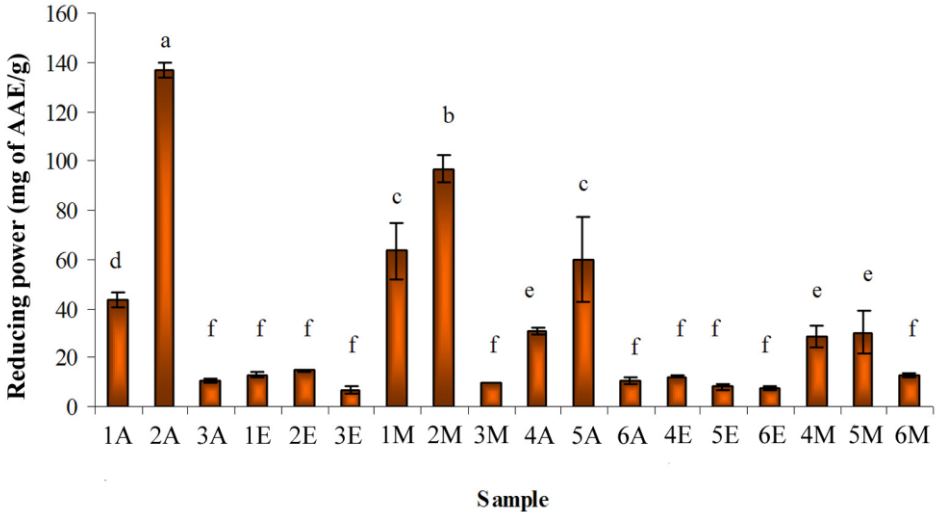

Fig. 4: Reducing power assay of different extracts of *J. gendarussa*

S.E/plot = 5.72, C.V (%) = 17.32

Values represent the Mean±SD of three experiments. Means with different letters indicate significant differences at 5% level.

1 - Liquid callus of stem; 2 - Solid callus of stem; 3 - Stem; 4 - Liquid callus of leaf; 5 – Solid callus of leaf; 6 - Leaf; A - Ethanol extract; E - Ether extract; M - Methanol extract

Determination of antioxidant activity

Highest DPPH scavenging activity of methanol extracts of stem derived callus cultured on solid medium was observed at the concentration of 145.00 ± 5.00µg/ml followed by methanol extract of stem derived callus cultured in liquid medium at the concentration of 185.00 ± 8.66µg/ml (Fig. 3).

However, the scavenging activity was lower than the gallic acid and quercetin standards used in the present study. The reducing power assay showed a significant reducing capacity of the extracts and the absorbance of the sample increased with increase in the concentrations. The highest reductive capacity was observed for ethanol extract of stem derived callus cultured on solid medium (136.66 ± 2.88mg AAE/g of dried extract) followed by methanol extracts of stem derived callus cultured on solid medium (96.66 ± 5.77mg AAE/g of dried extract) (Fig. 4).

The higher antioxidant activity of the solid grown callus samples of *J. gendarussa* observed in the present study may be due to the type and concentrations of chemical components produced *in vitro*. In solid medium the cultures will grow under stress compared to suspension grown callus in terms of nutrient and oxygen supply. The present study also confirmed the presence of phenolics and flavonoids in the test samples. All these compounds are responsible for the antioxidant properties as reported by Dimitrios[2], Jayaraj and Punja[32]. Amid *et al*[28] reported the highest antioxidant activity of 8.46×10^{-3}mg/ml (8460µg) in the leaf derived callus of *J. gendarussa* cultured in liquid medium supplemented with NAA + BAP (1 + 0.5mg/l). However, in the present study, the antioxidant activity of *J. gendarussa* illustrated a higher scavenging activity at145.00 ± 5.00µg/ml in methanol extract of stem derived callus cultured on solid medium. Uddin *et al*[20] reported the antioxidant activity (IC50 = 18.8µg/ml) in chloroform fraction of whole plant

extract of *J. gendarussa*. The enhanced production of phenolics content and free radical scavenging activity was reported in gamma irradiated leaves of *Justicia adhatoda*[33]. Methanol extract of stem and leaf samples of 2 months old *in vitro* grown seedlings of *Lycium barbarum* (goji) showed the highest antioxidant activity[34]. The antioxidant activity of *Justicia spicigera* and also the presence of phenolics and flavonoids in the aqueous and methanolic extracts of the leaf, stem and flowers were reported by Sepulveda-Jimenez *et al*[35]. However, in the present study, the scavenging activity was higher in ethanol and methanol extracts of solid grown stem callus (350.00±86.60µg/ml and 446.67±50.33µg/ml).

The *in vitro* culturing of stem and leaf derived calli of *J. gendarussa* on the solid and liquid medium for the production of bioactive compounds and a comparative study of the concentrations produced *in vitro* with their respective plant parts is the first report. The phytochemicals detected in the present study may have their role as antidiabetic, anti-inflammatory, hepatoprotective, anticarcinogenic agents as these properties of *J. gendarussa* were already reported from various laboratories as mentioned before. The present study forms an alternative method for the extraction of bioactive compounds from the *in vitro* grown cultures and also to isolate and characterize many more bioactive compounds from the cultures.

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