Production of coloured callus in *Orthosiphon stamineus* Benth and antioxidant properties of the extracted pigments

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Abstract

**Purpose**

The purpose of the present study is to understand the role of auxin and cytokinin in stimulating the production of pigmented callus in *Orthosiphon stamineus* and to gain correlation between the callus colours with their antioxidant capacity and bioactive constituents.

**Design/methodology/approach**

In this study, plant tissue culture was used to induce production of callus of various colours from leaf explants of *O. stamineus*, via manipulation of plant hormones (0-2.0 mg L⁻¹ indole-3-acetic acid [IAA] and Kinetin [Kin]). The coloured callus was subjected to solvent extraction and used for quantification of its carotenoid, chlorophyll, anthocyanin and phenolic contents. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of the extracts was also evaluated, before and after four weeks of storage at –20°C.

**Findings**

The highest mean (per cent) explants that produced roots (93.33 ± 0.05 per cent) were observed when the cultures were supplemented with 2.0 mg L⁻¹ IAA. The colour of the callus changed with time, from green to cream to brown after two and four months of culture, respectively. Optimum production of green callus was achieved with addition of 2.0 mg L⁻¹ Kin plus 1.0-2.0 mg L⁻¹ IAA to the media, while cream callus in 0.5 mg L⁻¹ Kin plus 2.0 mg L⁻¹ IAA and brown callus in 0.5 mg L⁻¹ Kin plus 1.5 mg L⁻¹ IAA. Green callus was found to contain the highest amount of chlorophylls, carotenoid and anthocyanin, while cream callus contained the highest amount of phenolic compounds. The amount of pigments and secondary metabolites in the callus extracts decreased after four weeks of storage, except anthocyanin. The antioxidant potential of the extracts also increased after storage.

**Research limitations/implications**

The major compounds identified in the methanolic extracts of *O. stamineus*-coloured callus are chlorophylls, carotenoids, flavonoids and phenolic acids. Future research work should include improvements in the extraction and identification methods which may lead to detection of other compounds that could contribute to the antioxidant capacity, to complement the findings of the current study.

**Practical implications**

This analysis provides valuable information on the application of IAA and Kinetin (Kin) to manipulate the content of major pigments with medicinal benefits in *O. stamineus* by using the plant tissue culture system.

**Originality/value**

A comparative study on antioxidant capacity and bioactive constituents of pigmented callus from *O. stamineus* leaves is original. To the best of the authors’ knowledge, this is the first attempt of comparative evaluation on antioxidant potential of *O. stamineus*-coloured callus produced using IAA and Kin.

**Keywords** Pigments, Coloured callus, Indole-acetic acid, Kinetin, *Orthosiphon stamineus*

**Paper type** Research paper

Introduction

Misai Kucing (*Orthosiphon stamineus*) is a well-known medicinal herb that belongs to the *Lamiaceae* family. It is also known as Kumis Kucing and Java Tea. This species can be found throughout Southeast Asia and tropical Australia.