Phytochemical Screening, DNA Fingerprinting, and Nutritional Value of *Plectranthus amboinicus* (Lour.) Spreng

Seham S. El-hawary, Rabie H. El-sofany, Azza R. Abdel-Monem*, Rehab S. Ashour

Department od Pharmacognosy, Faculty of pharmacy, Cairo University, Cairo 11562, Egypt.

ABSTRACT

In this study, *Plectranthus amboinicus* (Lour.) Spreng was subjected to phytochemical and genetic investigation, so that it could be properly identified. The results of phytochemical screening of the different plant organs (leaves, stems and roots) revealed the presence of steam volatile substances, sterols and/or triterpenes, flavonoids, carbohydrates and/or glycosides, catechol tannins in the three organs under investigation. A DNA sample of the plant was analyzed by Random Amplified Polymorphic DNA (RAPD) technique using eleven oligonucleotide primers. The analysis of RAPD data achieves the use of B-19 and B-6 primers for selective discrimination of this plant. Also, the nutritional value, including the total carbohydrates, total soluble sugars, proteins, amino acids and vitamin content, of the leaves, stems and roots was determined. The roots recorded the highest values of total carbohydrates, total soluble sugars and proteins content (66.04, 23.33 and 17.58 g%, respectively), followed by the leaves (48.12, 4.74 and 16.45 g%, respectively) and the stems (44.62, 3.10 and 9.52 g%, respectively). The three organs under investigation contain essential amino acids in moderate amounts compared to the WHO daily recommended doses. They are rich in vitamins and can be used as a vitamins supplement.

Key words: *Plectranthus amboinicus*, DNA fingerprinting, phytochemical screening, nutritional value

INTRODUCTION

Genus *Plectranthus* belongs to family Lamiaceae and comprises about 350 species cultivated as ornamental plants or as sources of essential oils.[1] *Plectranthus amboinicus* (Lour.) Spreng (synonyms include *Plectranthus aromaticus* Roxb., *Coleus aromaticus* Benth. and *Coleus amboinicus* Lour.), is a perennial herb, native to Indonesia and is distributed in Tropical Africa, Asia and Australia. It is used as food, additive and fodder, and as medicine in treating a wide range of diseases.[1] The leaves extract is used to treat inflammatory disease,[2,3] chronic cough and urinary disease[2]. It is also used as an aromatic carminative and anthelmintic.[2] It was reported to have antimicrobial,[2,4] cytotoxic[2,3] and antioxidant activities[2,5]. Essential oils, diterpenes, flavonoids and phenolic acids are the main constituents isolated from the different *Plectranthus* species.[6,7] Because of taxonomic similarities of the different *Plectranthus* species, the same species of *Plectranthus* usually shows a number of synonyms.[1] To help in solving this terminology problem, in this study, the different organs (leaves, stems and roots) of *Plectranthus amboinicus* (Lour.) Spreng growing in Egypt were subjected to a phytochemical screening aiming for chemical identification of this plant and the DNA profile of the plant was analyzed for genetic identification. The total carbohydrates, total soluble sugars, proteins, amino acids and vitamin content were determined to evaluate the nutritional value of this plant as a food.

***Address for correspondence:**
Azza R. Abdel-Monem
Faculty of Pharmacy, Cairo University, Cairo, Egypt
E-mail: azzaramy@yahoo.com

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MATERIALS AND METHODS

Plant material

Plectranthus amboinicus (Lour.) Spreng were collected over the years (2008-2010) from El-Orman garden. The plant was identified by Dr. Mohamed el Gebaly and Madam Treze (Taxonomist). A voucher specimen was kept in the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Cairo University.

Preliminary Phytochemical Screening

The powdered air-dried leaves, stems and roots of *Plectranthus amboinicus* (Lour.) Spreng were tested for the presence of steam volatile substances,[8] sterols and/or triterpenes,[9,10] flavonoids,[11,12] crystalline sublimate,[13] carbohydrates and/or glycosides,[14,15] tannins,[16] saponins,[17] alkaloids and/or nitrogenous bases,[18] anthraquinones,[19] cardiac glycosides,[8,20-23] and oxidase enzyme[8]. The results are given in table 1.

DNA fingerprinting

DNA fingerprinting was performed in Agricultural Genetic Engineering Research Institute using the Random Amplified Polymorphic DNA (RAPD) technique.[24] DNA was extracted using CTAB extraction buffer (1% N-cetyl-N,N,N trimethyl ammonium bromide). Eleven oligonucleotide primers (Operon Technologies Inc., Almeda, California, USA) were used for analysis. Amplification of DNA was carried out in thermal cycler, programmed as follows: first cycle at 94 ºC for 5 min. (initial strand separation), followed by one cycle for 40 seconds at 94 ºC (denaturation), 1 min. at 36 ºC (annealing), forty cycles each for 1 min at 72 ºC (elongation) and the last cycle for 7 min at 72 ºC (final extension).

PCR reactions were performed in polypropylene tubes containing 2.5 µl reaction buffer, 2 µl $MgCl_2$, 2.5 µl of each dNTPs (Pharmacia, Sweden), 3µl primers, 0.5 µl Taq DNA polymerase (Perkin-Elmer/Cetus, USA; advanced Biotechnologies, UK), 3 µl template DNA, and enough sterilized water to obtain 25 µl.

PCR material was separated by horizontal electrophoresis in a 1.5% agarose gel plate (Sigma Co.). 10 µl of each PCR product was mixed with 3 µl loading buffer and loaded onto wells of the gels. The gels were run at 95 volts.

After electrophoresis the RAPD pattern was visualized by staining the gel with ethidium bromide solution (0.5 µg/ml), visualized under UV light, and photographed using a gel documentation system. RAPD molecular weight markers (Biolab Co.) were used. The banding profile produced by the eleven decamer primers is given in table 2.

Table 1: Results of phytochemical screening of the different organs of *Plectranthus amboinicus* (Lour.) Spreng

Constituent	Leaves	Stems	Roots
Steam volatile substances	++	+	±
Sterols and/ or triterpenes	++	+	+
Free aglycones	++	+	+
Flavonoids	+	+	+
Crystalline sublimate	-	-	-
Carbohydrates and/or glycosides	+	+	+
Catechol tannins	+	+	++
Pyrogallol tannins	-	-	-
Saponins	-	-	-
Alkaloids and/or nitrogenous bases	-	-	-
Free anthraquinones	-	-	-
Combined anthraquinones	-	-	-
Cardiac glycosides	-	-	-
Oxidase enzyme	-	-	-

+: Present, ++: Strongly positive, -: Absent, ± : Traces

Determination of total carbohydrates and total soluble sugars

The total carbohydrate and total soluble sugars were determined in the leaves, stems and roots by colorimetric method according to Duboies et al, (1956).[25]

Determination of protein content

The total protein content was determined, in the three organs under investigation adopting Micro-Kjeldahl method according to A.O.A.C. (1995).[26]

Determination of amino acids

The amino acids content in the three organs was determined by spectrophotometric method using amino acids analyzer (AAA 400, INGOS Ltd) after acid hydrolysis.[27] The results obtained are shown in table 3.

HPLC analysis of vitamins

HPLC analysis of vitamins[28-31] was carried out on Agilent 1100 apparatus equipped with Hypersil-BDS-C_{18} column (4.6 × 250 mm). The injection volume was 5 µl, the mobile phase was methanol at a flow rate of 1 ml/min. Detection was carried out with UV detector. The results are shown in table 4.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

The results of phytochemical screening showed the presence of steam volatile substances, sterols and/or triterpenes, flavonoids, carbohydrates and/or glycosides, catechol tannins in all organs of *Plectranthus amboinicus* (Lour.) Spreng. Crystalline sublimate, saponins, alkaloids and/or nitrogenous bases, cardiac glycosides and oxidase enzyme are absent in all organs.

Genetic profiling

The RAPD electrophoretic profile of the DNA sample amplified with the eleven decamer primers generated 58 fragment patterns, produced by B-19 (9 bands), B-6 (9 bands), D-20 (8 bands), E-4 (6 bands), A-6 and G-5 (5 bands each), A-17 and G-2 (4 bands each), A-18 and G-17 (3 bands each) and G-19 (2 bands). Thus, primers B-19 and B-6 could be selected for discrimination of *Plectranthus amboinicus* (Lour.) Spreng.