MORPHOLOGICAL STUDIES AND CHARACTERSTIC OF ALKALOID CONTENTS OF THE SUDANESE PLANT KNOWN AS (WINCA)

AWATIF AHMED MOHAMMED AHMED[1] AND GAAFAR MOHAMMED EL HASSEN[2]

ABSTRACT

A plant grown in Sudan locally named (winca) was studied for its morphological features and its characteristic alkhaloids that determine its classification. It was found that this plant morphologically belongs to the genus *Catharanthus*. Its chemical constituents indicate that it contains the dimmeric indole alkaloid called *vinblastine* which is not found in the other plant species, confirming that it belongs to the species roseus, hence this plant which is locally grown is *Catharanthus roseus*.

الملخص

أجريت دراسة لمعرفة الجنس والنوع الذى ينتمى إليه نبات الونكا الذى ينمو في السودان، وذلك عن طريق دراسة الوصف النباتي والتركيب الكيميائى له.

جمعت عينات من النباتات ذات الزهرة الحمراء والبيضاء من منطقة شمبات (الخرطوم)، أثبتت نتائج الدراسة الوصفية أن نبات الونكا الذى ينمو في السودان ينتمي لجنس الكاثارانسس (*Catharanthus*)، كما أثبتت نتائج التحاليل الكيميائية أن هذا النبات يحتوي على قلويد الفنبلاستين (Vinblastine) والذى لايوجد إلا في عينة (*Catharanthus roseus*)، مما يثبت أن نبات الونكا الذى ينمو في السودان هو (*Catharanthus roseus* L.).

INTRODUCTION:

Catharanthus roesus (L.) G.Don; formerly known by various names such as *Vinca rosea* L., V.speciosa (L.) Salisb, *Provinca rosea* (L.)

[1]Medical and Aromatic Plants Research Institute (MAPRI), National Center for Research(NCR) Khartoum..

[2]Faculty of Agriculture, Department of Horticulture, Univ. of Khart.

Moench, *Ammocallis rosea* (L.) Small and *Lochnera rosea* (L.) Reichenbach, belongs to the family Apocynaceas (1). It contains more than 100 alkaloids among them are the powerful anti-tumor drugs (*Vincristine and Vinblastine*), and the monomeric indole alkaloid Ajmalicine which is used to improve cerebral circulation (2,3).

The genus *Catharanthus* G. Don; a genus in the subtribe Catharanthine of the Apocynaceae, is widely spread as ornamental and comprising eight species; seven of which are endemic to Madagascar. The best-known species of those of Madagascar is *C.roseus*, hence the name "Madagascar periwinkle". The genus is closely allied to the genera *Amsonia* and *Vinca* and that is why the species is wrongly designated in the past as Vinca rosea. Most of the diffferences are found in the floral struture. Differences in characters between these genera and their similarities are cited by Van Bergen et al 1996 (1).

Vinca series as *V.minor*, *V.major*, and *V. herbacea* are found in temperate zones, whilst *Catharanthus* genus such as (*C.corianceus*, *C. Lanceus*, *C. trichophyllus*, *C. longifolius* *C. ovalis*, *C. scitulus* and *C. corianceus*,)) are indigenous to Madagascar. And *C.pusillus* is indigenous to India, they occur in warm dry areas, mainly in tropical and subtropical countries (1). Moreover, *Catharanthus roseus* L.; contains the toxic antineoplastic dimmeric indole alkaloids; *Vinblastine* and *Vincristine* which are not present in *Vinca* species and other *Catharanthus* species (4). *Vinblastine* content varies with locality, part and age of the plant, it was reported that it ranges between 0.0045-0.0007% in the leaf(5,6,7), while Hirata et al 1989 (8) in Japan found that it is about 1.4% of the dry weight of leaves. Ajmalicine content in roots ranges between 0.63-1.2% in roots of one year old(6).

The objectives of this study were to investigate the morphological features and the characteristic alkaloids of the plant grown in Sudan, locally named as (*winca*), to clear up the confusion of whether it belongs to genus *Catharanthus* or *Vinca*.

EXPERIMENTS:

PLANT MATERIAL: Samples of 9 months old plants of red flowered, white flowered and white with red eye flowered of winca plant were uprooted from the field of the experimental farm at Shambat (Khartoum North). The age of leaves was three months after the last cut, observations of the vegetative and floral parts of the plant were noted. The various vegetative parts were examined with (X10 magnification handlens, whereas the structure of floral parts were carefully examined using a binocular dissecting microscope (X40) magnification.

CHEMICAL ANALYSIS: The dried samples of leaves and roots of the white and red flowered plant were analyzed by (Gorlaeus Laboratories) Division of Pharmacognosy-Center of Drug Research-Leiden.

The plant material was extracted with methanol by grinding three times for 5 minutes using an Ultra Turrax. The extracts were concentrated under reduced pressure and dissolved in a mixture of 1ml of methanol, 1ml of internal standard solution (50 g/ml dihydroquinone) and 8ml of 0.05M sodium phosphate buffer solution (pH 7.0). After filtration the filterate was applied to an RP-8 preconcentration column, previously prepared by washing with 3ml of methanol and 3ml of 0.05 M sodium phosphate buffer solution (pH 7.0). After application, the column was washed with 1.2ml of sodium phosphate buffer solution (pH9.5)-isopropanol (70:30) and 1ml of 0.05 M sodium phosphate buffer solution (pH9.5)-isopropanol (95:5). The alkaloids were diluted with 1.5ml of 0.05 M sodium phosphate buffer solution (pH 2.0)-isopropanol (60:40). The dilute was evaporated to dryness, the residue was dissolved in 0.5 ml of HPLC mobile phase and 25-100ml of this solution were injected in the HPLC system. A suspension culture medium was adjusted to (pH 7.0) by adding 2ml of 0.05 M sodium phosphate buffer solution (pH 7.0). After adding 1ml of internal standard solution the medium was filtered and applied to an RP-8 pre-concentration column. The washing and dilution steps were the same as described above.

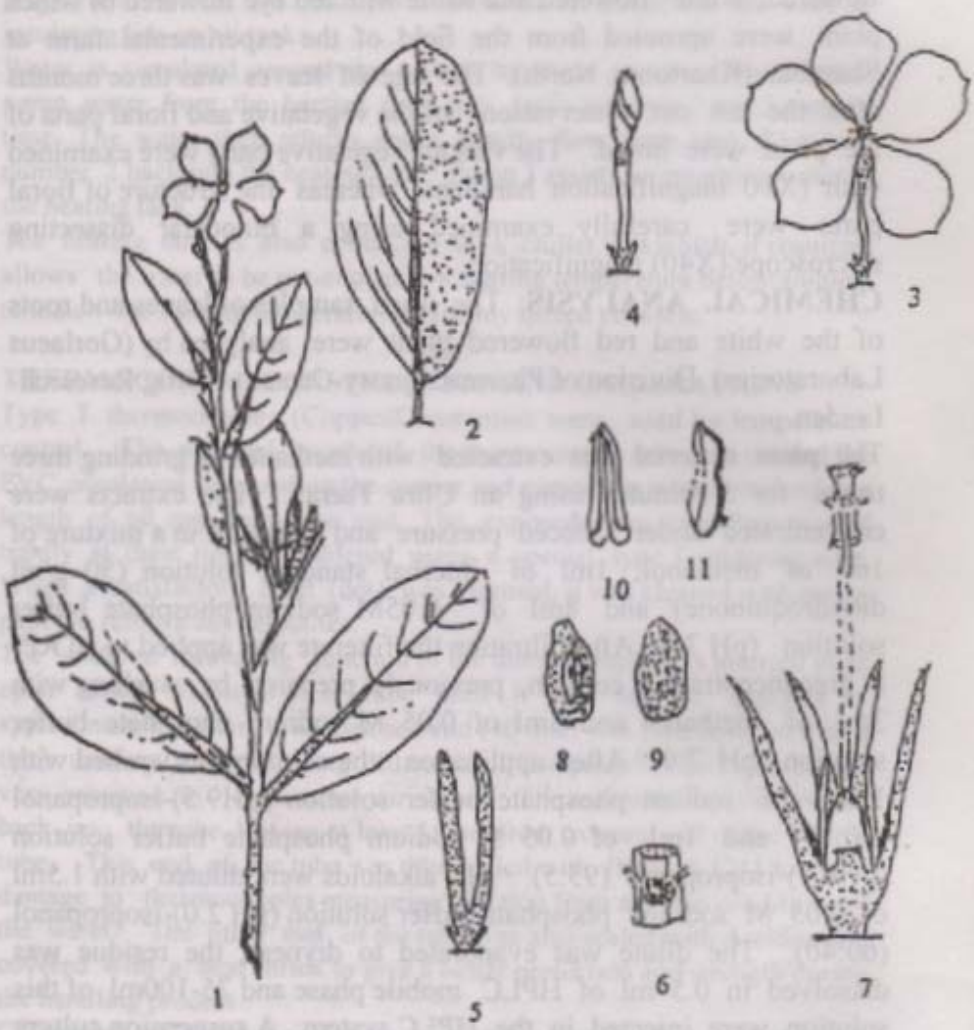

Fig. 1. *Catharanthus roseus.*
1, habit; 2, leaf; 3, flower; 4,bud; 5, fruit; 6, stem; 7, pistil; 8 &9, seed; 10&11, anther.

HPLC:

The extracts were analyzed by HPLC for alkaloids. The apparatus consists of an LKB 2150 pump (LKB, Bromma, Sweden) combined with a WISP 710B automated injection system (Waters Assoc., Milford, MA, U.S.A.), two LKB2158 Uvicord fixed-wavelength detectors operating at 275 and 313 nm, a Shimadzu Chromatopac C-R3A computing integrator (Shimadzu, Kyoto, Japan) and two-channel recorder (Kipp and Sons, Delf, The Netherlands). The column was a Waters Assoc. μ Bondapak-Phenyl (300X 3.9mm I.D) and eluent was of 0.05 M sodium dihydrogen phosphate solution (6.8 g/L Na H2PO4 H2O, pH adjusted to 3.9 2-methoxyethanol (80:15:5) at a flow-rate of 2ml/min. Peak areas were used for calculation of the ejected amounts of alkaloids. The identities of the analytes were established by photodiod-array detection of their UV spectra (9).

RESULTS AND DISCUSSION

GENERAL MORPHOLOGY:

The plant is a perennial herb, 50-120 cm high, erect or decubment, with white latex and a somewhat unpleasant smell. *Trunk* up to 1 cm in diameter, pale gray. *Stems terete, narrowly winged, green, pink or pale red.* Leaves glossy, decussate, petiole green or pink; blade dark green with paler main veins on both sides, oblong or obovate, acute or rounded or slightly retuse at the apex, cuneate at the base, apiculate, margin entire. *Flowers* axillary, solitary or paired. *Sepals* green. *Corolla* lobes pink with a darken eyes, white with yellowish eyes or white with red-purple eyes, paler or whitish outside; tube pale green, throat pale green, apex very pale green-white, bud white. *Stamens* with apex below the mouth of corolla tube, anthers subsessile. *Pistil* pale green surrounded by ring of hairs. *Ovary* ovoid. Style filiform. *Fruit* green follicles forming a V shaped. Seeds small black (Fig 1.)

These morphological features indicate that the plant under study belongs to genus *Catharanthus.*

CHEMICAL CONSTITUENTS:

The results of the chemical analysis of the plant samples as shown in (table 1) indicate that: in the leaf material, *vinblastine* and *Ajmalicine* was about (0.7 & 0.66mg/g dry weight), respectively, in the Red flowered plant, and about (0.57 &0.64mg/g dry weight), respectively, in the White flowered plant. The detection of *Vinblastine* in the leaves confirms that this plant is *Catharanthus* roseus L. (4,10) who declared that C. roseus is the only plant that contains *Vinblastine*. The presence of 3, 4- *Anhydro-vinblastine* - which is the key intermediate in the coupling of *Catharanthine* and *Vindoline* to give *Vinbastine* - is a preliminary indicator for *Vinblastine* (11,12).

On the other hand, the roots were devoid of *vinblastine* and contain about (4.94&6.03 mg/g dry weight) of *Ajmalicine*. in the Red and White flowered plants respectively. These results were in conformity with the previous result(6).

CONCLUSION:

The results of this study indicate that the (Red and White flowered) plants grown in Sudan and locally Known as (*winca*) do not belong to the genus *Vinca* but to the genus *Catharanthus*, species *roseus*. Synonym *Vinca rosea* L.

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Table 1. Alkaloid content of the Red and White flowered *Catharanthus roseus* plants grown in Sudan (mg/g DW)

Strains		Alkaloids (mg/g DW)		
		Ajmalicine	Vinblastine	AVLB
Red flowered	Leaf	0.66±0.11	0.73±0.03	7.67±1.43
	Root	4.96±0.96	nd	nd
White flowered	Leaf	0.64±0.11	0.57±0.02	9.34±1.02
	Root	6.03±0.96	nd	nd

Notes:

nd: not detected
AVBL= 3,4-anhydro-vinblastine
DW=Dry weight

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