International Research Journal of Plant Science (ISSN: 2141-5447) Vol. 2(11) pp. 323-327, November 2011
Available online http://www.interesjournals.org/IRJPS

Full length Research Paper

Phytochemical and Pharmacognostic Standardization of the Leaf of *Hypoestes rosea* P. Beauv Acanthaceae

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Accepted 19 September, 2011

The leaves of the antimalarial wonder plant *Hypoestes rosea* P. Beauv of the Acanthaceae family was subjected to phytochemical and pharmacognostic investigations to determine its microscopical features, secondary metabolite content and also some of its physical constants. Phytochemical studies of the powdered leaf revealed the presence of terpenes, sterols, balsam, monosaccharides, reducing sugars, tannins, flavonoids and carbohydrates. Moisture content, total ash and acid-insoluble ash values, alcohol soluble and water soluble extractive values were determined and found to be 11.99%, 11.13%, 0.64%, 12.70% and 22.17 % w/w, respectively. The leaf microscopy, reported here for first time, showed wavy walled epidermal cells on both surfaces with abundant distribution of paracytic stomata on the lower surface and short stalked glandular trichomes on the upper surface; wostly oblong cystoliths on both surfaces. Transection of the leaf across the midrib is characterized by meristele consisting of proto and metaxylem vessels preceded by a bundle of phloem cells. The results of the study could be useful in setting some diagnostic indices for the identification and preparation of a monograph of the plant.

Keywords: *Hypoestes rosea,* microscopy, chemical composition, monograph, bioactivity, antimalarial.

INTRODUCTION

Standardization of herbal medicinal plants is a complex task due to their heterogeneous composition. To ensure reproducible quality of herbal products, proper quality control of starting material is essential. The first step towards ensuring quality of starting material for the production of any herbal medicinal preparation is authentication of the plant(s). Thus, in recent years there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance (Reddy et al., 1999; Venkatesh et al., 2004). According to the World Health Organization (WHO, 1998), the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such plant materials and should be carried out before any other tests are undertaken.

Hypoestes rosea, an antimalarial wonder plant, is an evergreen shrub or small tree reaching 1m high, abundantly available in Western Cameroun and in Southern Nigeria. The reported medicinal uses of *H. rosea* by indigenous people in different parts of the world show considerable similarities. In a broad sense, preparations were used largely as anti-inflammatory, anticancer and antimalaria, (Pulai et al., 2005; Ojo-Amaize et al., 2007a). The dried leaf powder of *H. rosea* at 10 mg/kg in mice against *Plasmodium berghei* suppressed parasitemia by 75% and this supports the herbal use of the plant material for the management of malaria by Nigerian natives (Ojo-Amaize et al., 2007a). In spite of its abundant medicinal uses, the pharmacopoeia standards of *H. rosea* leaves have not been reported.

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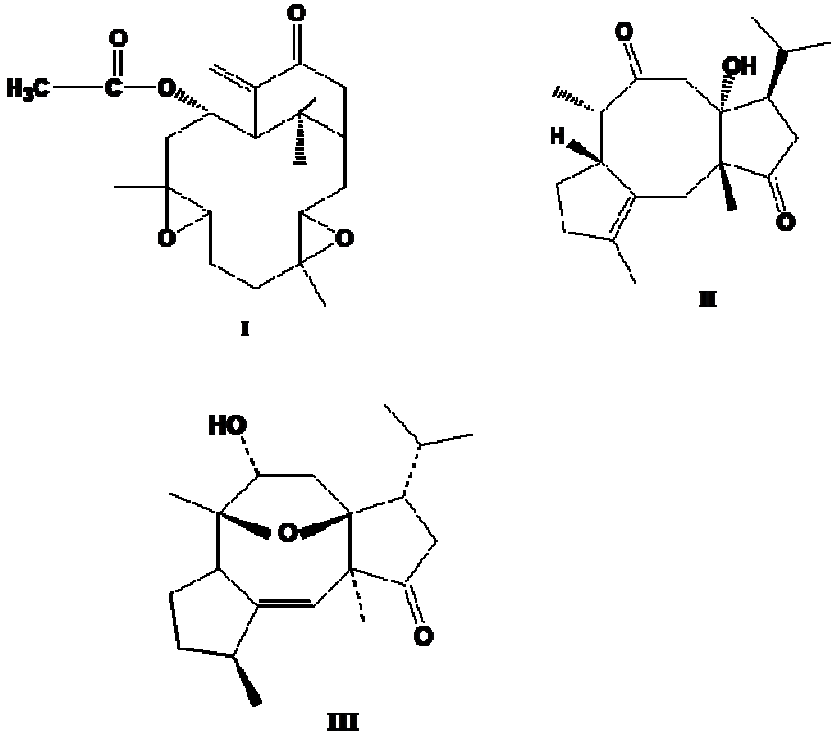

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Some bioactive chemical constituents of *H. rosea leaf*

Hence, the aim of this work to pursue a study on the phytochemical and pharmacognostic standardization of *H. rosea* leaves, as this plant is widely used in indigenous system of medicine.

The leaves of *H. rosea* contain bioactive chemical compounds one of which is hypoestoxide (Adesomoju et al., 1983a), a diterpene, with yield of 0.1% by dry weight of leaves. Due to its complex chemical structure, hypoestoxide cannot be synthesized in the laboratory. This is no doubt a robust natural synthetic process, considering the fact that taxol, a related natural diterpene, is 0.001% by dry weight of the Yew tree bark which needs up to 20 years to attain full growth. *H. rosea* is a hardy shrub which reproducibly provides hypoestoxide, a natural product of interest. Hypoestoxide features a bicyclo-(9, 3, 1)-pentadecane ring system and contains two epoxide moieties, an α, β-unsaturated ketone, an acetylated alcohol group, and seven stereo-centers. Compounds containing epoxides and α, β-unsaturated ketone functionalities are often associated with alkylator properties and therefore are usually toxic. However, hypoestoxide is non-toxic based on the results obtained from several studies (Ojo-Amaize et al., 2002). Ojo-Amaize et al., 2007b reported hypoestoxide as inhibitor of tumor growth in the mouse CT26 colon tumor model and as a novel anti-inflammatory agent, with IkappaB kinase inhibitory activity (Ojo-Amaize et al., 2001. In addition to hypoestoxide(**I**), roseadione (**II**) (Adesomoju et al. 1983b) and roseanolone (**III**) (Okogun et al., 1982) have been isolated from the leaves of *H. rosea* (Stoessl et al., 1988).

Immune Modulation Inc, IMI, Nigeria Limited is the manufacturing company harvesting and processing *H. rosea*. IMI Nigeria Limited has successfully cultivated and domesticated *Hypoestes rosea* outside its natural wild habitat. This ensures conservation of the plant. The Federal Government of Nigeria, through the Federal Ministry of Environment and other relevant bodies, has granted IMI Nigeria Limited, the Biodiversity Access Permit for the commercial exploitation of *Hypoestes rosea.*

MATERIALS AND METHODS

Hexane, ethyl acetate and methanol were AnalaR grade from BDH Chemical Ltd (Poole, England). The plant was collected from the garden of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development, Idu Industrial Layout, Abuja, Nigeria. The plant was identified and authenticated at the Institute herbarium in the above Department where a voucher specimen has

Table 1: Result of phytochemical screening of the *Hypoestes rosea* leaves

Test	Results
Flavonoids , Terpenes and Sterols	
Balsam, Carbohydrates, Monosaccharides Reducing sugars, Tannins and Saponins	Positive
Resins, and Glycosides	Negative

Table 2: Result of pharmacognostic analysis of the *Hypoestes rosea* leaves

Parameters	Value (% w/w)
Moisture content	11.99
Total ash	11.13
Acid-insoluble ash	0.64
Alcohol soluble extractive value	12.70
Water-soluble extractive value	22.17

been deposited.

Pharmacognostic and phytochemical analyses

The pharmacognostic parameters determined include: moisture content, total ash and acid-insoluble ash values, alcohol soluble and water soluble extractive values.

The phytochemical screening and pharmacognostic analysis were carried out on the powdered leaves using standard methods (Evans 2002, Ayurvedic Pharmacopia of India 1990, and Sofowora 2008).

Leaf microscopy

Fresh sample of the leaves were detached into a petri dish, and sodium hypochlorite solution TS was added enough to cover the surface and left for about 24 hours when the leaf was completely bleached. Micrsocopic studies were carried out on the upper and lower leaf surfaces; and transection of the leaf across the midrib.

RESULTS AND DISCUSSION

The leaves have characteristic odor and astringent taste. The results of the evaluation of phytochemical and pharmacognostic standards of the leaf of *H. rosea* are presented in Tables 1 and 2. Different chemical compounds such as, terpenes, sterols, balsam, carbohydrates, monosaccharides, reducing sugars, saponins, tannins and flavonoids among others were detected in the plant, which could make the plant useful for treating different ailments and having a potential of providing useful drugs and drug leads for human use.

Glycosides and resins were not detected. The pharmacological activities of any plant are usually traced to particular chemical compound(s) present in the plant. The presence of alkaloids, tannins and saponins in the plant suggest it might possess anti-bacterial activity. Isaac and Chinwe (2001) reported that alkaloids along with tannins and saponins are responsible for anti-bacterial activity in *Fetracarpidium Conopheoum;* and Favel (1994) reported antifungal activities of saponins. Onoruvwe and Olorunfemi (1998) also attributed the anti-bacterial effect of the root extract of *Dichrostachys cinerea* to alkaloids, saponins and flavonoids. The presence of tannins could also be responsible for the astringency, wound healing and anti-parasitic properties (Odukoya et al., 2007 and Bose et al., 2007). Adesomoju et al. (1983) isolated a diterpene, hypoestoxide (**I**), from *H. rosea*, which had been reported by Ojo-Amaize et al. (2007a) as a more potent antimalarial agent compared to standard antimalarial drugs. Hypoestoxide was found to reduce parasitemia by 90% in *Plasmodium berghei*-infected mice (SD_{90} = 250 µg/kg for hypoestoxide, versus 5 mg/kg for chloroquine and 150mg/kg for artemisinin).

The quantitative determination of some pharmacognostic parameters is useful for setting standards for crude drugs. The physical constants evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The moisture content of the drug is not high, thus it could discourage bacterial, fungal or yeast growth. Equally important in the evaluation of crude drugs is the total ash value and acid-insoluble ash value determination. The total ash is particularly important in the evaluation of

purity of drugs, for the presence or absence of foreign inorganic matter such as metallic salts and/or silica. Since the plant is useful in traditional medicine for the treatment of some ailments, it is important to standardize it for use as a drug plant.

Microscopical analyses

The anatomy of the leaf indicated the presence of wavy walled epidermal cells on both surfaces; with abundant distribution of paracytic stomata on the lower surface, as compared to the few on the upper epidermis; a fair distribution of short stalked glandular trichomes, with a lesser distribution on the lower surface; specialized and usually localized and wostly oblong cystoliths distributed on both surfaces; occasional trichomes scar (cicatrix) or stump on upper epidermis, roughly found on the lower epidermis; lignified spiral vessels making up the veinlets.

The transection of the leaf across the midrib showed the presence of a meristele made up of an arc consisting of proto and metaxylem vessels preceeded by a bundle of phloem cells; an isobilateral leaf arrangement with no palisade cells; an abundant distribution of large oil globules scattered throughout the mesophyll and transversing the midrib and cutting out two separate characteristic bundles of collenchyma cells toward the upper epidermis separate from the main meristele at the junction between the lamina and the midrib.

CONCLUSION

This work is the first in a series on the phytochemical and pharmacognostic standardization of the leaf of the antimalarial wonder *H. rosea* plant. The pharmacognostic constants for the leaves of this plant and the phytochemical screening results reported in this work could be useful for the compilation of a suitable monograph for its proper identification and authentication. Further work is on-going in our laboratories toward establishing its chemomicroscopical characteristics and quantitative microscopy so as to provide a standard reference for the identification and authentication of the drug plant of *H. rosea.*

ACKNOWLEDGEMENTS

The authors thank Mr. John Atogwe for collecting the plant, Mr. John Apev and Mr. Andrew Sule for their assistance with the laboratory work.

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