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GC-MS metabolite profiling, antinociceptive and antipyretic activities of methanol stem bark extract of *Tabernaemontana pachysiphon* Stapf. (Apocynaceae)

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Abstract

Purpose: The study was designed to determine the GC-MS metabolite profile and evaluate the antinociceptive and antipyretic activities of the methanol stem bark extract of *Tabernaemontana pachysiphon*.
Methods: Metabolite profile was assessed using GC-MS technique while the antinociceptive and antipyretic activities were evaluated using the acetic acid induced writhes, hot plate and baker's yeast induced pyrexia models.
Results: GC-MS profiling revealed the presence of forty metabolites in the extract, with n – Hexadecanoic acid (27.49%), Oleic acid (14.60%) and Octadecanoic acid (6.38%) being the most predominant metabolites. In the acetic acid induced writhes, hot plate and baker's yeast induced pyrexia models; the extract exhibited a dose – dependent activity that was comparable to the reference drugs (Acetylsalicylic acid, pentazocine and paracetamol).
Conclusion: The methanol extract of *T. pachysiphon* possess useful metabolites, antinociceptive and antipyretic activities.

Keywords: *Tabernaemontana pachysiphon*, stem bark, GC–MS, antinociceptive, antipyretic

Indexing: Index Copernicus, African Index Medicus

Background

Medicinal plants have been of great importance to the healthcare needs of individuals and their communities. The use of herbal preparations made from medicinal plants is widespread in developing countries. In these local communities where medical care is not so easily accessible due in part to lack of healthcare facilities and the high cost of orthodox treat, recourse to traditional medicine offers the only hope of staying healthy and alive. Crude drugs obtained from medicinal plants have been used to treat all manner of aliments.

Tabernaemontana pachysiphon Stapf. (Apocynaceae) is a glabrous shrub or medium sized tree occurring in the tropics. It grows well in Nigeria where, particularly the stem bark is a popular herbal medicinal material for general pains, fever, malaria, dysmenorrhea, gastrointestinal infections and hyperglycaemia (personal communication). It is called "Giant Pin wheel flower" [English]; "Ibu"[Edo]; "pete – pete" [Igbo] and "abododo" [Yoruba] [1,2].There are reports on the ethno-medicinal uses and biological activities of this plant [3-5]; no report on the gas chromatography – mass spectrometry (GC-MS) metabolite profile, antinociceptive and antipyretic activities of the stem bark was however found. For this reason, the stem bark sample of this plant was studied in this research.

Aim/Objectives

The aim of this study was to evaluate the antinociceptive and antipyretic activities of the methanol stem bark extract of *T. pachysiphon*.

Specific objectives were to: a) collect, identify and authenticate the plant as *T. pachysiphon*, b) determine the metabolite profile of the methanol extract using gas chromatography – mass spectrometry (GC-MS), c) evaluate the antinociceptive activity of the extract in mice, and d) evaluate the antipyretic activity of the extract in mice.

Materials and Methods

Fresh parts (fruits, flowers, stem barks and leaves) of *T. pachysiphon* were collected from Ekiadolor forest in Ovia North – East Local Government Area of Edo State, Nigeria; to which Herbarium voucher specimen number FHI 110375 was assigned at the Forest Herbarium, Forest Research Institute of Nigeria, Ibadan.

The stem barks were properly rinsed with distilled water, cut into small pieces, air - dried for twelve (12) days under shade, powdered and stored in air tight containers until required for use. The powdered stem bark (200 g) was exhaustively extracted with methanol (1.5 L) using Soxhlet apparatus and then concentrated using a rotary evaporator to obtain a yield of 18.05% extract. The dried extract was stored in a refrigerator at 4oC and used for the various analyses.

GC - MS analysis was conducted using GCMS-QP2010 Plus Shimadzu Japan with column oven temperature of 60°C. Oven temperature programming was from 60 – 280°C, held at 60°C for 0 min, 120 °C for 2 min (rate 15/min) and then at 280°C for another 2 min (rate 15/min). The injection temperature was 200°C. The carrier gas was helium, with a pressure of 21.9 kPa and 6.2 mL/min, column flow was 1.61 mL/min, injection mode was split, flow control mode was linear velocity, purge flow was 3.0 mL/min and split ratio was 1.0.

Also, ion source temperature was 200°C, interface temperature was 250°C, equilibrium time was 1.0 min, solvent cut time was 1.50 min., detector gain was 0.96 kV + 0.00 kV, detector gain mode was relative and the threshold was 1000. For the mass spectrometry, start time was 2.0 min., end time was 17.0 min, event time was 0.5 s, scan speed was 1428, and start m/z was 45 while end m/z was 700. The mass spectrum was also equipped with a computer fed mass spectra data bank. Using computer searches on a NIST Ver. 2.1 MS data library and comparing the spectrum obtained via GC-MS, compounds present in the methanol stem bark extract were identified. Antinociceptic activity was evaluated using acetic acid induced writhes model [6] and hot plate model [7]. Antipyretic activity of the methanol extract was evaluated using the Baker's yeast induced pyrexia model [8].

This research on animals was carried out in accordance with internationally accepted laws governing the use of laboratory animals and ethical approval was sought from the Ethics Committee of the Faculty of Pharmacy, University of Benin, Benin City, Edo State

Results

GC-MS metabolite profiling of the methanol stem bark extract of *T. pachysiphon* revealed the presence of forty (40) metabolites in the extract, with n – Hexadecanoic acid (27.49%), Oleic acid (14.60%) and Octadecanoic acid (6.38%) being the most predominant metabolites. In the acetic acid induced writhes model, the methanol extract caused a dose – dependent reduction in the number of writhes observed. This was comparable to results obtained with the reference drug, Acetylsalicylic acid, and was significantly different ($p < 0.05$ at a dose of 125 mg/kg and $p < 0.01$ at doses of 500 and 1000 mg/kg) when compared to the control (5 mL/kg Tween 80 solution).

Also, with increase in dose, the methanol extract was found to increase the pain reaction time (PRT) in the hot plate model. At 30 min post administration, the extract caused a significant effect ($p < 0.01$) which lasted for the duration of the test (180 min), when compared to the control (except the 125 mg/kg dose which had duration of action of 150 min). This effect was comparable to that produced by the reference drug, pentazocine (10 mg/kg). In the antipyretic test, the extract significantly ($p < 0.01$) reduced pyrexia induced by baker's yeast when compared to control. The effect of the extract was comparable to that of the reference drug (paracetamol).

Conclusion

This study has shown that the methanol extract of *T. pachysiphon* contains useful metabolites which may be responsible for its antinociceptive and antipyretic activities.

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