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# Standardization of *Andrographis paniculata*, *Mitracarpus scaber* and *Nauclea latifolia* Herbal Preparations as per European and Nigerian Drug Regulations

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author SJA designed the study, performed the statistical analysis and wrote the protocol and the first draft of the manuscript. Author NI managed the literature searches and revised the first draft. Author AA managed the GC-MS study. Authors OO, MA, MG, HC and KSG approved the study. All authors read and approved the final manuscript.*

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## ABSTRACT

**Background:** Herbal drug standardization (HDS) is multidisciplinary with botany and chemistry working together to facilitate decisions on production of herbal medicines. The common reasons for HDS are: i) it creates the need for establishing botanical identity; ii) it is necessary for establishing dosage and iii) it facilitates industrial production and good

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manufacturing practice (GMP).
**Aims:** To outline the strategies being used to standardize Conavir, Niprd-AM1 and Niprifan and to show that HDS is the ideal strategy for herbal drug development (HDD) from traditional medicines (TMs).
**Methodology:** Relevant data on: i) the regulatory requirements of Europe's Medicines Evaluation Agency (EMEA) and Nigeria's National Agency for Food and Drug Administration and Control (NAFDAC) and ii) on *Andrographis paniculata* (AP), *Mitracarpus scaber* (MS) and *Nauclea latifolia* (NL) were reviewed. Crude herbal drugs (CHDs) from aerial parts each of AP and MS and from roots of NL and the active crude extracts (ACEs) derived from them were studied using standard botanical, phytochemical and physicochemical techniques with the aim of standardizing them for production. The ACEs from AP (Conavir) and from NL (Niprd-AM1) were dry water extracts. The ACE from MS (Niprifan) was a dry ethylacetate extract.
**Results:** The regulatory provisions of NAFDAC for herbal preparations were broadly similar to those of EMEA but the latter proved more explicit in many respects. Furthermore, the results on the CHDs and ACEs adequately meet the requirements of the two agencies.
**Conclusions:** The results here provided and those reported elsewhere collectively furnish the data needed for drawing-up the registration dossiers of AP/Conavir, NL/Niprd-AM1 and MS/Niprifan as per EMEA and NAFDAC requirements. But for purposes of further work, it is needful for the GC-MS studies to be amplified and combined with others, so as to facilitate identification of suitable markers and pave the way for studies requiring bioassays.

*Keywords: Herbal drug standardization (HDS); Crude herbal drug (CHD); Active crude extract (ACE); Traditional medicine (TM); Herbal drug development (HDD); Conavir; Niprid-AM1; Niprifan.*

## 1. INTRODUCTION

### 1.1 The Rising Popularity of Standardized Herbal Preparations

The reasons for the ever increasing popularity of standardized herbal preparations (SHPs) as opposed to highly purified chemical entities (PCEs) since 1978 have been described [1-4]. SHPs are minimally processed but botanically defined. Plant parts may, on account of their minimal process status, be called "crude". Thus, a crude herbal drug (CHD) is either a freshly harvested plant part or one that has only been dried and cut to usable particle size. Examples of CHDs are: i) the fresh or dried and cut aerial parts of *Andrographis paniculata*; ii) the fresh or dried and cut root of *Nauclea latifolia* and iii) the fresh or dried and cut aerial parts of *Mitracarpus scaber*. Similarly, an active crude extract (ACE) is either a mixture or solution derived from a CHD or the dry residue from such a solution. Examples of ACEs are: i) "Abiku Mixture" - the clear, brownish yellow, orange or red liquid resulting from mixing prescribed quantities of black pepper, clove, "osun", sorghum leaf stalk and trona in a pint bottle filled to mark with "ogogoro" (local gin distilled from palm wine) and used in managing sickle cell crisis (SCC); ii) Niprisan – the dry water extract made from mixing prescribed quantities of *Piper guineense* seeds, *Eugenia caryophyllata* flower buds, *Pterocarpus osun* stem, *Sorghum bicolor* leaf stalk and sodium sesquicarbonate [5,6] used in managing SCC; iii) Conavir – the dry water extract of aerial parts of *Andrographis paniculata* [7,8]; iv) Niprd-AM1 – the dry water extract of the root of *Nauclea latifolia* [9] and v) Niprifan – the dry

ethylacetate extract of aerial parts of *Mitracarpus scaber* [10,11]. Conavir, Niprd-AM1 and Niprifan are used as antiviral/ immunostimulant, antimalarial and topical antifungal drug respectively. The three ACEs were developed at the Nigerian National Institute for Pharmaceutical Research and Development (NIPRD). Herbal drug standardization (HDS) is the subject of this article, which posits that HDS is the primary strategy for herbal drug development (HDD) from proven traditional medicines (TMs).

## 1.2 The Relevance of Botany in Herbal Drug Standardization

Once ethnobotanical survey or some other accepted practice (such as a valid observational study) has confirmed the merit of a given plant for a stated indication and preliminary *in vitro* or *in vivo* biological data are consistent with that indication and the material has passed WHO's acute and chronic toxicity tests, the next logical action should be to standardize the plant material involved. This involves: i) establishing the plant's taxon, including the name of the authority behind the classification system used; ii) establishing the part of the plant used and how it was collected and iii) taking due cognizance of the herbalist's method for preparing the clinical sample.

## 1.3 Requirements for Herbal Drug Registration in Europe and Nigeria

The requirements of EMEA and NAFDAC for herbal drug registration are summarized in Table 1. These requirements were borne in mind in planning this study and are in the following areas:

### 1.3.1 Product information/legal status of applicant

Both EMEA and NAFDAC require product information but EMEA was more explicit in its requirements for technical data on "product characteristic" than was NAFDAC, which harped more on the "legal status of the applicant".

### 1.3.2 Quality control data/analytical status of the product for registration

EMEA requirements were more comprehensive and explicit and were hinged on "GMP compliance". By contrast NAFDAC requirements were less explicit but touched on all key issues pertaining to GMP.

### 1.3.3 Safety data requirements/ pre-registration inspection of premises

Both EMEA and NAFDAC adequately addressed safety. EMEA was more detailed on safe use of the product, while NAFDAC was more concerned with the storage, distribution and integrity of the product.

### 1.3.4 Traditional use evidence/ post-marketing surveillance

While EMEA harped on the importance of traditional use evidence, NAFDAC overlooked it, but discussed on post-marketing surveillance.

**Table 1. Requirements for herbal drug registration in Europe and Nigeria**

| | European union (EU) | Nigeria | |
|---|---|---|---|
| **Type of data** | **Details of data required** | **Regulatory aspect** | **Requirement** |
| Product information: Summary of product characteristics | These include: name, strength and dosage form, list of excipients, shelf life, posology, indications, contraindications, and special precautions. These are used as basis for inserts or advertisement, which must undergo a process called “readability”. | Legal status of applicant - manufacturer or marketer | Applicant must be certified by the Corporate Affairs Commission as a business. A marketer must show evidence of Power of Attorney. |
| Quality control data: Refer to GMP requirements for production. | These include: production must be in a GMP compliant, product must be produced with validated formula and method, there must be a product specification, stability studies must be carried out in the container proposed for marketing for purposes of storage/ shelf-life, and dossiers must be provided for starting materials and finished product. | Analytical status of the product for registration. | The product must have: certificate of analysis, dossier containing data on ingredients, method of analysis, stability, dosage and safety precautions. |
| Safety data requirements | The data may be assembled from: animal or human studies, review of potential drug-drug interactions, side effects and contraindications. Others include: recognized monographs, data special groups - children, the elderly and mothers. | Pre-registration inspection of premises. | Manufacturing, storage and distribution premises must be GxP compliant. Marketers must provide convincing evidence of GXP |
| Traditional use evidence | Evidence that the product has been in use as medicine for 30 years or more (the last 15 must be in the EU. Notably, there is no requirement to prove efficacy (De Smet, 2005). | Post marketing surveillance plan/ report | Applicant may be required to provide a plan for reporting on the use of the product and of any adverse reactions. |

*The table is based largely on Ann Godsell Regulatory [12] and various NAFDAC leaflets, including Akunyili [13]. The requirements for registration in Nigeria appear scanty yet demanding but are not necessarily less tasking, although their lack of explicitness can hinder transparency.*

## 2. METHODOLOGY

This study aimed to promote the thesis that HDS is the ideal strategy for developing herbal medicines from proven folk medicine and for regulating the use of drugs developed from TMs. The methodology is based essentially on WHO documents [14,15].

### 2.1 Macroscopic Examination of Freshly Harvested Plant Parts

Fresh samples were harvested, treated and examined for shape, size, color, texture, appearance of cut surfaces, odor and taste as per WHO [14].

### 2.2 Preparation of Crude Herbal Drugs (CHDs)

NIPRD's department of Medicinal Plants Research & Traditional Medicine (MPRTM) routinely prepares CHDs as described below for use by all other departments of the Institute.

#### 2.2.1 *Andrographis paniculata* and *Mitracarpus scaber*

Aerial parts of *Andrographis paniculata* or *Mitracarpus scaber* were harvested in the mornings by cutting plants at least 1 cm above ground level with a pair of scissors. The parts were briskly shaken to remove dust, dead parts and foreign matters. Subsequently, the parts were air-dried by placing them on stainless steel mesh maintained in an airy shade for drying herbal materials. The materials were considered sufficiently dry when they became brittle and amenable to grinding with an electrically powered grinding machine. The coarsely ground drugs were packed in sacks, labeled with batch numbers and date and stored in a cool, dry room. Coarsely ground aerial parts of *Andrographis paniculata* were labeled (AP). Coarsely ground aerial parts of *Mitracarpus scaber* were labeled (MS).

#### 2.2.2 *Nauclea latifolia*

Root parts of *Nauclea latifolia* were harvested in the mornings by digging out the roots, and cutting off portions thereof with a sharp axe. The parts were routinely treated with running potable water to remove soil particles. Subsequently, the parts were cut into manageable bits and dried by placing them on stainless steel mesh in an airy shade for drying herbal materials. The materials were considered sufficiently dry when they became brittle and amenable to grinding with an electrically powered grinding machine. The coarsely ground drugs were packed in sacks, labeled with batch numbers and date, and stored in a cool, dry room. Coarsely ground *Nauclea latifolia* was labeled (NL)

### 2.3 Preparations of Active Crude Extracts (ACEs)

NIPRD's department of Medicinal Plants Research & Traditional Medicine (MPRTM) routinely prepares ACEs as described below for use by all other departments of the Institute.

#### 2.3.1 Conavir and niprd-AM1

Suitable quantities of coarsely ground *Andrographis paniculata* (AP) or *Nauclea latifolia* (NL) were exhaustively extracted with boiling. The extract from AP was filtered and freeze-dried to give a dark greenish-brown granular powder called Conavir. The extract from NL was also filtered and freeze-dried to give a yellowish-brown granular powder called Niprd-AM1.

### 2.3.2 Niprifan

Suitable quantities of coarsely ground *Mitracarpus scaber* (MS) were exhaustively extracted with ethylacetate. The resulting extract was then concentrated using a rotary evaporator to yield a thick, greenish black liquid, which was evaporated to constant weight on a water-bath to yield a dark, waxy or sticky mass called Niprifan.

## 2.4 Sampling

### 2.4.1 Size of cut

Cut materials, where necessary, were graded according to the aperture size of sieve through which the materials passed, as follows: coarse cut, 4.00 mm sieve; medium cut, 2.80 mm sieve; and fine cut, 2.00 mm sieve.

### 2.4.2 Sampling of materials in bulk

Each batch of consignment was inspected to ascertain conformity with prescribed packaging and labeling as prescribed by WHO [14].

### 2.4.3 Quartering

Quartering consisted of the following steps:

a. A pooled sample from original samples was mixed carefully and thoroughly, and constituted it into a square-shaped heap.
b. The heap was divided diagonally into 4 equal parts and any 2 diagonally opposite parts were selected and mixed carefully.
c. The process was repeated as necessary until the required quantity of sample was obtained.
d. Any remaining material was returned to the sack.

### 2.4.4 Final samples

Final samples were obtained from an average sample by quartering, as described above. This means that an average sample gave rise to 4 final samples. Each final sample was divided into 2 portions. One portion was retained as reference material, while the other was tested.

## 2.5 Physicochemical Characterization

The physicochemical parameters listed or described below were determined as per WHO [14] as described previously [16]. The results were expressed in percentage weight/ weight (% w/w) and as mean values ± standard deviations (Means ± SDs). Where necessary, Student's "t" Test was used as a test of the null hypothesis in the computation of P values.

### 2.5.1 Loss on drying

Loss on drying (LOD) to constant weight was carried out in a Lindberg/Blue M gravity-convention oven maintained at 105-110$^{o}$C as described previously [16].

### 2.5.2 Ash values

Total ash (TA) and Acid insoluble ash (AIA) values were determined as described previously [16-18], using a minimum of 0.5 – 1.0 g of material and a furnace (Vecstar Furnace) heated gradually to the ignition temperature of 650 – 700 $^0$C.

### 2.5.3 Determination of extractive values in water and other solvents

About 4g of air-dried and coarsely powdered sample were utilized in the test. The sample was accurately transferred into a glass-stoppered, 250-ml reflux conical flask and followed by the addition of 100 ml of water or other solvents as described previously [8].

### 2.5.4 Determination of solubility in water or other solvents – methods I and II

Solubility in water was determined at room temperature (c. 25ºC) and expressed in terms of "parts", representing the number of milliliter of solvent, in which 1 g of the material is soluble. Vials of appropriate sizes: c. 4-ml, c. 12-ml and c. 20-ml capacities were used. The mixtures were thoroughly shaken for at least 30 min before inspection for un-dissolved solute as previously described [17,18].

### 2.5.5 Determination of pH of preparations

The pH of an aqueous preparation of CHD or ACE was determined with a pH meter (Jenway). Standard pH buffer solutions of 4, 7 and 10 were used to calibrate the equipment.

### 2.5.6 Determination of bitterness value

Bitterness values were determined as per WHO [14] as described previously [7] to serve as a means of monitoring effect of storage.

### 2.5.7 Determination of foaming index

Foaming indices were determined as per WHO [14] as described previously [18] as a means of monitoring effect of storage.

### 2.5.8 Light absorption of preparations

UV-VIS Spectrophotometer (Jenway or Shimadzu) and Quartz 1-cm cells or Glass 1-cm cells were used in the study as described previously [19]. Filtered preparations of the plant in water or select organic solvents or dilute acids and alkalis were appropriately diluted to obtain absorbance readings of at least 0.050 at the wavelength showing maximum absorption (λmax) using quartz (for wavelengths below 300 nm) or glass cells (for wavelengths above 400 nm) respectively. The dilution factor and the absorbance reading for each test sample were noted for use in calculations of A1% 1cm. For AP/ Conavir, the conditions for the light absorption experiments were adapted from Suo et al. [19]

### 2.5.9 Thin layer chromatography (TLC)

Solutions of the analytes in ethanol were prepared and applied as follows: To about 1 mg of the analyte, 2 drops of ethanol were added and swirled until dissolved (this is equivalent to about 1%w/v solution of the analyte).The analyte solution was applied to the plate as a 1 µl

droplet from a micro-syringe. The spot was allowed to dry before developing the plate. In order to saturate the chamber of the developing tank with the vapor of the solvent system to be used as mobile phase, the inner surfaces were padded with pieces of filter paper soaked in the solvent. The level of the solvent in the developing tank was adjusted to a level 2 to 3 mm below the line of origin on the plate. The plate was considered as developed when the distance between point of origin and the distance travelled by the solvent front was not less than ¾ of the length of the plate and no further than 5 mm below the top of the plate. The plate so developed was allowed to air dry before visualizing, using a viewing cabinet (CAMMAG) and a UV-lamp (CAMMAG – equipped to emit light at 254 or 366 nm). The retention factor (Rf) value for a given spot, defined as the distance moved by the spot divided by the distance moved by the solvent front, was calculated for each spot on the plate.

#### 2.5.10 Stability of CHDs and ACEs

Where feasible, the following real time stabilities tests were carried out on the CHDs and ACEs, as a means of gauging their stabilities in capped glass bottles, under room conditions of temperature and humidity. The parameters included: loss on drying, appearance; extractability or solubility, pH, light absorption, TLC characteristics, bitterness value and foaming index.

### 2.6 Metallic Content Analysis/Limit Test for Heavy Metals

#### 2.6.1 Requirements

- Atomic absorption spectrophotometer
- Vitreous silica crucible, with matching lid (this is the digestion vessel)
- Nitric acid (1000 g/l)
- Perchloric acid (1170 g/l)
- Lead nitrate reference standard
- Suitable reference material
- Sample

#### 2.6.2 Preparation of digestion mixture

Two parts by weight of the nitric acid were mixed with 1 part by weight of the perchloric acid. Alternatively, the volumes of the acids were calculated from their wt/ml, and then delivered with a burette.

#### 2.6.3 Procedure for the test

The following steps were taken:

- All items to be used were scrupulously cleaned with the nitric acid, washed and rinsed with water; and dried at 120ºC.
- About 250 mg of the coarsely powdered, air-dried sample was accurately transferred into the digestion vessel, and 1 ml of the digestion mixture was added. The crucible was covered and the mixture was placed in an oven, slowly heated to 100ºC and maintained for 3 hours.

- The Heating was slowly stepped-up to 120ºC for 2 hours then gradually increased to 240ºC and maintained for 4 hours.
- The digest or residue was dissolved in 2.5 ml of the nitric acid and its absorption was determined with an atomic absorption spectrophotometer using nitric acid as blank solution, and lead nitrate standard solution made with the same nitric acid as reference solution.
- Calculation was based on the air-dried material.

## 2.7 Phytochemical Tests

The following phytochemical tests, previously described [20,21] were carried out on the CHDs and ACEs:

- Dragendorff's test for alkaloids
- Frothing test for saponins
- Salkowski's test for terpenoids
- Keller-Kiliani's test for cardiac glycosides
- Sodium picrate paper test for cyanogenic glycosides
- Borntrager's test for anthraquinone derivatives

## 2.8 Application of Gas Chromatography-Mass Spectrometry (GC-MS)

### 2.8.1 Requirements

Requirements were: SHIMADZU GCMS-QP2010 PLUS equipment; a GC column; 99% Helium gas and GC or HPLC grade ethanol supplied by Sigma Chemical Company or Aldrich. The column was a 30-m capillary tubing (internal diameter: 0.25mm) with a special interior coating of 5% phenyl siloxane polymer/95% dimethyl siloxane polymer as the stationary phase.

### 2.8.2 Preparations/ operating conditions

Samples were dissolved in GC grade ethanol so as to contain 0.1µg per µl of the solution. Operating conditions (appearing as "comments" under the chromatogram generated for each test sample) were selected automatically/ instrumentally as stated in the Operating Manual.

## 3. RESULTS AND DISCUSSION

## 3.1 Herbal Drug Standardization (HDS) and Herbal Drug Development (HDD)

HDS consists of botanical, physicochemical and phytochemical characterizations, in combination with select bioactivity and toxicity studies, as prescribed by WHO [14,15]. In our opinion, a thorough familiarity with ethnobotany, access to an institutional herbarium, and a team of botanists are a cardinal prerequisite for HDS and HDD. Second, and also in our experience, a laboratory equipped to the standard prescribed by WHO [14,15] and supplemented with a GC-MS and/ or an HPLC system would be adequate for HDS. HDS is a critical step in HDD because it embraces the totality of actions taken to facilitate GMP production of an herbal medicine, and its dispensing for use in accordance with good clinical practice (GCP). It is important to bear in mind that the definition of an herbal drug or medicine does not include products of fractionation [15,22]. For example, the European

Pharmacopoeia [23] stated as follows: “Herbal drugs are mainly whole, fragmented or cut, plants, parts of plants, algae, fungi, lichen in an unprocessed state, usually in a dried form”. Nigeria’s NAFDAC on the other hand, defined “Herbal Preparations” as “regulated products of plant origin consumed or used by man or on animals, examples are phytomedicines, herbal medicines, dietary supplements, nutraceuticals and phytocosmetics” [13]. Both of these definitions are borne in mind in the discussion.

## 3.2 Botanical Characterization as a Key Step in HDS and HDD

The three plants and their ACEs are described in Table 2. The data in Table 2 are useful for identifying the plants and their derivatives. Figs. 1-3 are intended to convey the rigor attached to identification and the critical relevance of botany in HDS and HDD. Table 2 also shows the plants identified up to the Family level, while Figs. 1-3 show their key features.

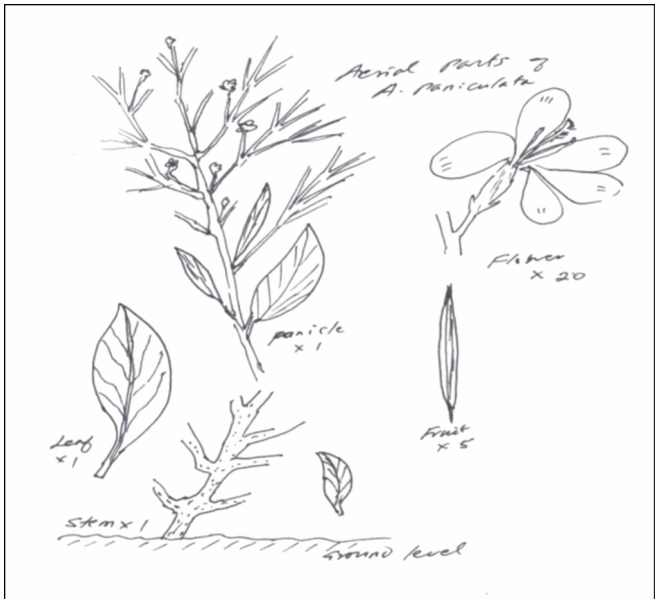



**Fig. 1. Sketches of the aerial parts of *Andrographis paniculata***

*The flowers are tiny inflorescences with minute white petals bearing purplish spots; and occurring on lax spreading auxiliary and terminal racemes. The fruits bear numerous tiny brown seeds (less than 0.25 mm diameter) and occur in linear-oblong capsules that taper at the ends, measuring up to 2.0 cm long and 0.3 cm wide. The leaves are glabrous, varying in shape and size as shown above; and have a prominent midrib from which arise four radiating veins. The mature leaves measure 3-9 cm long x 0.7-2.0 cm wide. The herb is a perennial, mostly but not always grows erect to a height of 25-110 cm. The stem is deep green with diameter measuring 2 – 6 mm; is quadrangular with longitudinal furrows and wings at the axils of younger parts; and is slightly enlarged at the nodes. All the parts, except the flowers and the seeds, were odorless and taste intensely bitter. The flowers and seeds were not examined organoleptically*

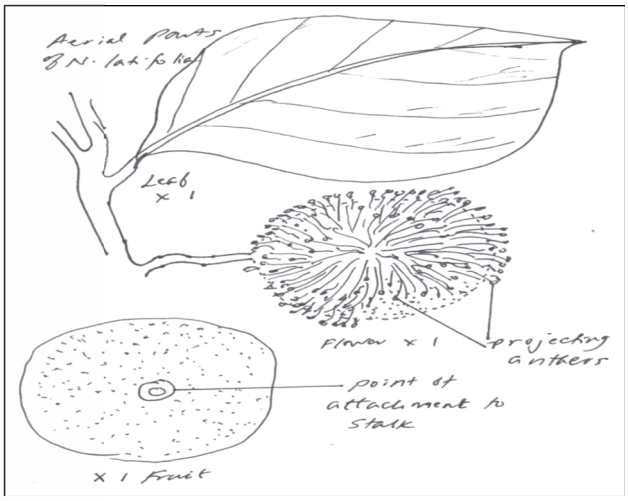



**Fig. 2. Sketch of the leaf, inflorescence and fruit of N. latifolia**

*N. latifolia is a multi-stemmed straggling tree with an open canopy. The leaves are obovate, with a main midrib and 4-8 veins, as depicted above. The mature leaves range in size from less than 13cm x 7cm to over 26cm x 14cm (Length x Width). The inflorescence is a cyme in which a large terminal spherical structure bears small projecting yellow-white structures as flower parts. The N. latifolia fruit is a syncarp.*

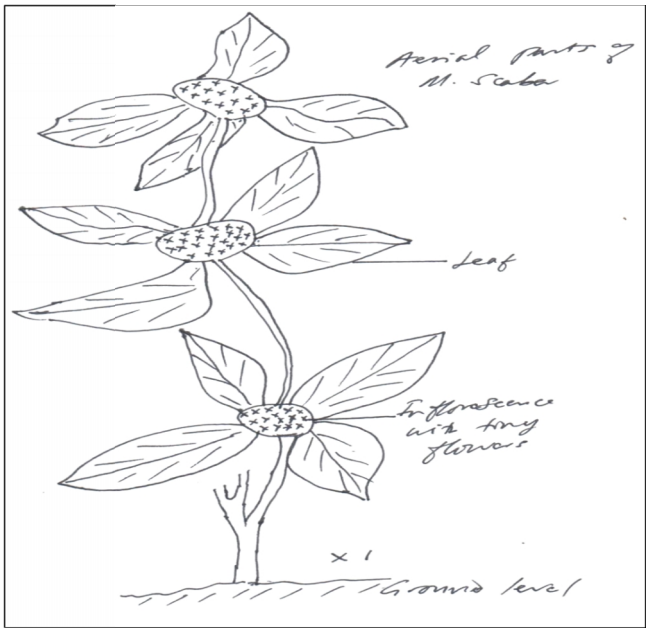



**Fig. 3. Sketches of the leaf and inflorescence of *Mitracarpus scaber***

*The leaves of M. scaber are lanceolate, 3-5 cm long, with scabrous upper surface. The inflorescence is a capitulum consisting of clusters of small white flowers that appear at different and mature at different times – such that sometimes only one flower may be seen on the circular head – the capitulum. The fruits are dehiscent capsules, up to 1 mm long.*

**Table 2. Descriptions of *A. paniculata*, *N. latifolia* and *M. scaber* and their and ACEs**

| Sample | Description/ pertinent comments |
|---|---|
| *Andrographis paniculata* (AP) as a crude herbal drug (CHD) is the dry and coarsely cut aerial parts. Family: Acanthaceae | *Andrographis paniculata* with voucher number NIPRD/H/3720 in the NIPRD's Herbarium, was grown in NIPRD's garden with seeds from India. The plant bears tiny inflorescences with minute white petals bearing purplish spots. The fruits have numerous tiny brown seeds (≤ 0.25 mm diameter) in linear-oblong capsules that taper at the ends. The leaves vary in shape and size, but predominately lanceolate. The herb is a perennial, usually growing erect to a meter tall. The stem is quadrangular or roughly round with longitudinal furrows. Both the leaves and branches are intensely bitter and odorless. |
| *Nauclea latifolia* (NL) as a CHD is the dry and coarsely cut root. Family: Rubiaceae | *Nauclea latifolia* with voucher number NIPRD/H/5579 in NIPRD's Herbarium, is a perennial small tree. It is widespread in African, hence is also called African peach. The samples here described were obtained from NIPRD's botanical garden. The plant bears an interesting flower - a large ball with long projecting stamens, for which reason, the plant also called pin-cushion tree. The fruit is brown or red, but unattractive. The stem bark of a mature tree is rough and heavily furrowed. The root system consists of many yellowish-brown branches. The parts are faintly aromatic have an exciting bitter taste. |
| Mitracarpus scaber (MS) as a CHD is the dry and coarsely cut aerial parts. Family: Rubiaceae | *Mitracarpus scaber* with voucher number NIPRD/H/4208 in NIPRD's Herbarium, grows well in most parts of Nigeria. The samples here described were collected from NIPRD's botanical garden. The plant grows erect up to 55 cm high and usually branched. The leaves are lanceolate, 2-4 cm long, with the upper surface bearing minute hairs. The plant manifests dense clusters of inflorescence, 6-14 mm across, with minute white flowers. The fruits are dehiscent capsules, about 0.5-1 mm long. Both the fresh plant and air-dried weed are practically odorless but taste slightly warm |
| Conavir is the active crude extract (ACE) of AP | Conavir is a granular water extract of the aerial parts of *Andrographis paniculata*. It is dark greenish brown, practically odorless, intensely bitter and is intended for formulation as an oral immunostimulant. |
| Niprd-AM1 is the ACE of NL | Niprd-AM1 is a granular water extract of the root of *Nauclea latifolia*. It is yellowish brown, odorless or faintly aromatic, with an exciting bitter taste and is intended for formulation as an oral antimalarial. |
| Niprifan is the ACE of MS | Niprifan is a waxy or sticky ethylacetate extract of the aerial parts of *itracarpus. scaber*. It is black or dark greenish brown, practically odorless and is intended for use as an external application. |

*The three plants above were identified by the Institute's ethnobotanist –Ibrahim Muazzam (associate of late Prof Nina Etkin), and botanists – Drs. Jemilat Ibrahim and Grace Ugbabe. The original identifying authorities of the plants are given in bracket after the plant as follows: Andrographis paniculata (Burm.f.) Wall. ex Nees; Nauclea latifolia (Smith); and Mitracarpus scaber (Zuccarini). The 3 plants are flowering plants and are all classified as dicots or eudicots.*

### 3.3 Preparation and Sampling of CHDs/ACEs are Conducted to Reflect GMP

"Standardization of herbal production" or "quality control of herbal production" consists of two types of controls: "inspection control" and "analytical control" [10]. Ideally, quality control of herbal production should commence with cultivation/ collection of the required parts, to assure greater uniformity of the parts collected, and to ease the laborious process of sampling. The inherent variability of biological materials and the opposing need for uniformity of materials for pharmaceutical production, require that collection of starting materials and the processes that materials undergo, be meticulously controlled. Thus, the preparative and sampling processes described in Section 2 are a recapitulation of actual industrial practices of "inspection control". Without such practices in collection, treatment and sampling, it would be impossible to apply GMP to herbal production. This would be because batch to batch variations would be unmanageably high. As would be seen in the results shown in Tables 3-5, without such handling and sampling, batch to batch variations (as SDs) would have been much higher, and hence unmanageable. Inspection control is as important as analytical control, especially in herbal production. Plate 1 has been included to give an idea as to how *N. latifolia* is merchandized and processed in Nigeria.

*A: Roots of Nauclea latifolia (called "tafashiya" or "Igiya" in Hausa, or "Oya" in Idoma, or "Egbesi" in Yoruba) being merchandized in Zaria, Kaduna State, Nigeria.23/7/08.*

*B: Parts of Nauclea latifolia roots being air-dried at a shade in NIPRD garden. 12/8/08.*

**Plate 1. Underground parts (root) of *Nauclea latifolia***

*Macroscopic/ organoleptic features – the roots of Nauclea latifolia consist of a thick, dark brown bark. The core has a yellow and woody texture, with a faint aromatic, and exciting bitter taste. N. latifolia is available in most ecological zones of Nigeria and most of West Africa. In June 2012 during a WAHO conference at Ouagadougou, it was observed that N. latifolia is similarly used in Burkina Faso as in the northern parts of Nigeria.*

## 3.4 Physicochemical Features of CHDs/ ACEs and Relevance to GMP/ GCP

While the preceding preparative and sampling approaches belong to "inspection control", the test results described under the present subhead belong to "analytical control". The two "controls" must go hand-in-hand to guide production and quality. The results in Tables 3-5 are shown to illustrate the purposes of the determinations and how the results can be used to guide GMP. This approach is in line with two of the three reasons/ aims for HDS, namely to: establish dosage; and to facilitate industrial production.

## 3.5 Establishing Dosage Based on Ethnomedical Data/Physicochemical Results

The following activities are required, using powdered *N. latifolia* (NL) as an example:

- If the CHD is not in a form that can be readily weighed or measured out with a spoon, the quantity prescribed by the herbalist is first converted to powder. The example (coarsely NL powder) can be weighed or measured in heaps with a spoon.
    - Let the average weight of a heap be 3.5 g.
- If the dose of NL prescribed by the herbalist were as follows:
    - Decoct 2 heaps with 1 "regular coke bottle" equivalent of boiling water (ie: 300 ml).
    - Allow the mixture to cool, and decant or filter the entire extract
    - Drink the entire extract in one draught
    - The above means that the dose of NL is: 7.0 g
- If Niprd-AM1, the ACE of NL, were to be given, the quantity of 1 dose is calculated from the water extractive value as follows:
    - Water extractive value of NL is: 16.56 % w/w (from Table 4)
    - From above 100 g NL is equivalent to: 16.56 g of Niprd-AM1
    - Hence 7 g of NL is equivalent to: 1.16 g of Niprd-AM1
- The foregoing calculations are based on air-dried samples, hence is necessary to know that air-dried sample of NL typically has an LOD value of 9.79 %w/w, while that of Niprd-AM1 is 10.81 %w/w.

## 3.6 Standardization Facilitates Industrial Production

Standardization of an herbal drug facilitates industrial production in the following ways:

- Establishing botanical identity enhances/ promotes confidence in planning for cultivation/ collection or procurement.
- Knowledge of expected values of LOD, TA, AIA, extractive (or solubility) and other parameters, including light absorption, pH and TLC profiles, allows for efficient arrangements for procurement by establishing practical acceptance or reject criteria (or specifications) for CHDs and ACEs.
- Choice of processing methods and types processing equipment depend on physicochemical properties of materials to be processed; hence prior knowledge of such properties is essential for planning schedules of processing and the type of equipment to procure.

**Table 3. Physicochemical features of AP and Conavir**

| Characteristic | CHD | ACE |
|---|---|---|
| Description of sample | The comminuted sample consists of dark green twigs and fragments of wrinkled leaves. Odor: faint or practically odorless. Taste: exceedingly bitter. | The freeze dried sample consists of dark greenish brown granules that are hygroscopic, odourless and very bitter. |
| Loss on Drying (LOD) %w/w | 10.64 ± 0.36 (7) | 10.54 ± 1.90 (7) |
| Total Ash (TA) %w/w | 14.10 ± 4.49 (7) | 27.50 ± 1.17 (7) |
| Acid Insoluble Ash (AIA)%w/w | 1.36 ± 0.27 (4) | Below detection |
| Water extractable matter (WEM) %w/w | 30.37 ± 2.63 (8) | - |
| pH of aqueous preparation (5%w/w) | Fresh extract: 6.65 ± 0. 25 | Solution: 8.45 ± 0.20 |
| Wavelengths: (λ200-300nm)<br>Solvent: water | Peak Trough<br>λ: 227.0 396.0<br>Abs: 2.126 -0.076 | Peak Trough<br>λ: 222.0 391.0<br>Abs: 1.558 -0.212 |
| Wavelength: λ225nm<br>Solvent: MeOH:$H_2O$<br>(52:48, v/v) x 150 dilution | Abs: 0.091 ± 0.015 (n=3) | Abs: 0.276 ± 0.031 (n=3) |

*The ACE was prepared by hot extraction with boiling water. The results represent Mean±SD. The number of determinations (n) is given in parenthesis. The TA of the ACE is about 2x that of the CHD. The few successful attempts to determine acid insoluble ash gave very low values (< 2 % w/w). The results suggested that the CHD is rich in bio-minerals, and that the TA results of both the CHD and the ACE consisted mostly of physiological ash. The conditions for the light absorption experiment were adapted from Suo et al. [21]*

**Table 4. Physicochemical features of the CHD and ACE of *Nauclea latifolia***

| **Characteristic** | **Raw material (CHD)** | | | | **Aqueous extract (ACE)** | | | |
|---|---|---|---|---|---|---|---|---|
| Description of sample | Yellow, coarse or finely cut material; practically odorless | | | | Yellow-brown granules; practically odorless or faint aroma | | | |
| | | | | | **1st Day** | | **14th Day** | |
| Loss on Drying (LOD) %w/w | 9.79 ± 2.00 (30) | | | | 6.50 ± 1.22 (8) | | 10.81 ± 0.44 (7) | |
| Total Ash (TA) %w/w | 3.09 ± 1.28 (57) | | | | 9.88 ± 2.60 (15) | | | |
| Acid insoluble ash (AIA) %w/w | Below detection | | | | Below detection | | | |
| Water extractable matter (WEM) %w/w | 16.56 ± 3.93 (29) | | | | - | | | |
| Wavelengths: (λ200-700nm) Solvent: | **Peak** | | **Trough** | | **Peak** | | **Trough** | |
| water. | λ | Abs | λ | Abs | λ | Abs | λ | Abs |
| Dilution: x 25 for herb mixture in water (1% | 688 | 0.055 | 697 | 0.053 | 688 | 0.075 | 697 | 0.072 |
| w/v); x 125 for solution of extract in water | 454 | 0.090 | 548 | 0.048 | 454 | 0.120 | 548 | 0.053 |
| (1%w/v). | 225 | 2.024 | 391 | -0.087 | 225 | 2.224 | 391 | -0.107 |
| A1%1cm at λ225 | 253 | | | | 278 | | | |

*Samples of the ACE were prepared by hot extraction with boiling water. The results represent Mean ± SD. The following should be noted: The ACE is highly hygroscopic but not deliquescent. The TA of the ACE is about 3x that of the CHD, but acid insoluble matter was below detection, suggesting that the CHD is rich in bio-minerals, and that the TA of the ACE mainly contained physiological ash. The spectra revealed 3 peaks and 3 troughs. The absorption in the visible region (400-600 nm) was consistent with the golden yellow color of both the CHD and the ACE.*

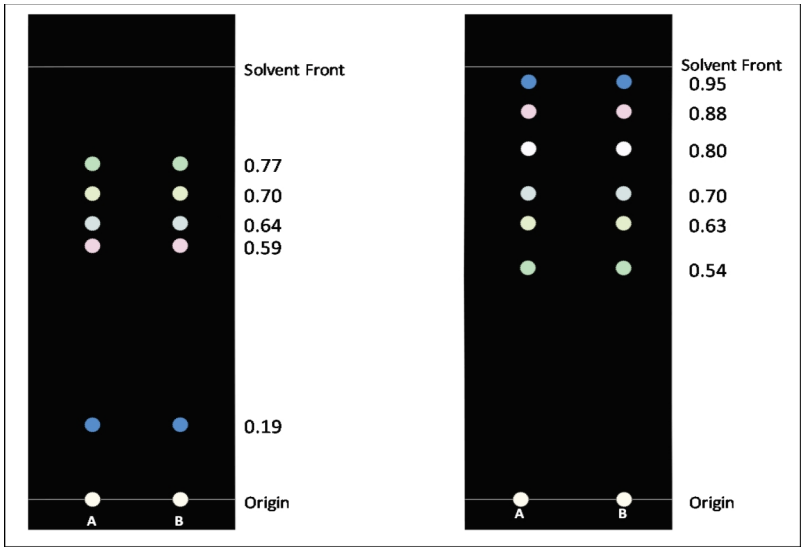
**Table 5. Physicochemical features of the CHD and ACE of *Mitracarpus scaber***

| Parameter (Mean ± SD) | Air-dried herb (CHD) | Et. acetate extract (ACE) |
|---|---|---|
| Loss on drying (LOD: % w/w ) | 10.29±1.81 (n=12) | 15.86±0.72 (n=12) |
| Total ash (TA: % w/w) | 12.44±2.95 (n=12) | 0.40±0.09 (n=11) |
| pH of 5 % w/v in water | 5.7±0.3 (n=5) | 6.9±0.3 (n=5) |
| Foaming Index (FI: as $ml^{-1}$) | Slight foam. FI ≤ 100 (n=5) | No foam. FI = 0 (n=5) |
| A 1%1cm at λ227 nm (MeOH/ $H_2O$: 80/20 v/v) | 325.8±15.6 (n=5) | 349.5±14.1 (n=5) |
| Extractability (% w/w) in: | Extractive value (n=8-12) | - |
| Solubility (ml/g) in: | - | Solubility (n=2-6) |
| Water | 28.37±1.77 | $>10^3$ |
| Methanol | 15.11±1.07 | 25.0± 0.0 |
| Ethylacetate | 14.02±1.89 | 25.0± 5.0 |
| Ethanol | 11.72±0.81 | 25.0± 0.0 |
| Acetone | 6.89±0.89 | 15.0±0.0 |
| Hexane | 4.11±0.47 | 55.0±5.0 |

*The high LOD results proved that the ACE is quite hygroscopic. The lower TA for the ACE probably suggests high presence of bio-minerals. The high water extractability of the CHD agrees with its high TA, and the fact that hexane (the least polar solvent) had the lowest extractive value. Under the conditions of the experiment, the solubility of the ACE did not change appreciably with the 4 organic solvents tested. The solutions of the ACE in the 4 organic solvents were clear and greenish-brown, but the solution with water was brown, and slightly cloudy, with no tinge of green.*

### 3.7 TLC and Phytochemical Tests Results and Their Relevance HDS and HDD

The result presented in Figs 4-5 and Table 6 respectively showed the TLCs of AP/ Conavir, NL/ Niprd-AM1 and MS/ Niprifan. Although the identities of the individual spots were not established, their number and positions were characteristic for each CHD or ACE; hence the TLCs are useful for the following purposes: i) identification of sample; ii) revelation of impurities/ adulterants / decomposition products and iii) monitoring effect of storage. The phytochemical tests were carried out mainly with the following aims: i) to obtain a general idea of the major types of phytochemicals present in the samples and ii) to determine whether or not potentially toxic entities like cyanogenic glycosides, anthraquinones and cardiac glycosides are detectable in the AP/ Conavir and NL/ Niprd-AM1 samples. In AP/ Conavir and NL/ Niprd-AM1 alkaloids, saponins, tannins and terpenoids were present, but none of the 3 potentially toxic entities were detected. NL/ Niprd-AM1 contained copious amounts of flavonoids, as might be expected from their yellow color. Tannins, saponins and anthraquinones were detected in MS/ Niprifan but the tests for alkaloids were negative.




**Normal Phase ($K_5$) viewed at 366nm** **Reverse Phase ($KC_{18}$) viewed at 366nm**

**Fig. 4. Phytochemical and diagrammatized TLC profiles of AP/ Conavir**

*Phytochemical tests indicated alkaloids, saponins, tannins and terpenoids in AP and Conavir, but cardiac and cyanogenic glycosides were not detected. Spot A is AP as ethanol extract; Spot B is Conavir dissolved in ethanol. Mobile phase for the Normal Phase was Hexane: Ethylacetate: Methanol (4:4:1). Mobile phase for the Reverse Phase was Methanol: Water (80:20).*

### 3.8 Effects of Storage on Quality Variables of AP/Conavir shown as Examples

The physicochemical results displayed in Tables 3-5 and Figs. 4 and 5 are “quality variables”. Therefore, the quality of samples can be monitored against lengths of storage under defined conditions of temperature and humidity. The results of the effects of storage at room temperature and humidity monitored at various intervals over a period of 39 months are shown in Tables 7-10 for AP/ Conavir samples as examples. Table 7 shows that the moisture content of the dried herb and extract did not change significantly during storage in glass bottles for up to 39 months. Table 8 shows that the water extractability of the herb (AP)

also did not change significantly, neither did the pH; but the greenish brown color of the extract (Conavir) faded slightly by the 21st month of dry storage of the herb. Table 9 also shows that the solubility of the dried extract (Conavir) in water decreased slightly but significantly by the 21st month storage, although the color and the pH did not. The emerging trend above is supported by Table 10, which shows that neither the extent of light absorption at λmax nor the chromatographic characteristics of the materials (ie: AP and Conavir) changed detectably up the 39th month of dry storage of herb (AP) and extract (Conavir). Unlike the trend in the bitterness results, the foaming indices of both the herb and the extract (also shown in Table 10) decreased slightly, but significantly by the 21st / 39th month of storage, as compared with the results at 0th month. On the whole the results show that most of the quality variables evaluated, including TLCs, remained largely unchanged up to the 39 month of storage, but a few began to decline as from the 21st month. This means both AP and Conavir may be stored up to 18 months.

**Table 6. Phytochemical and TLC Profiles of NL and Niprd-AM1 *latifolia***

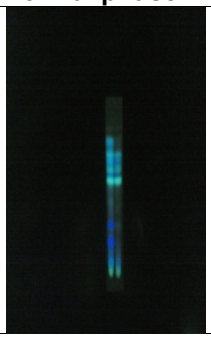
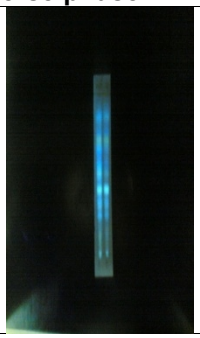
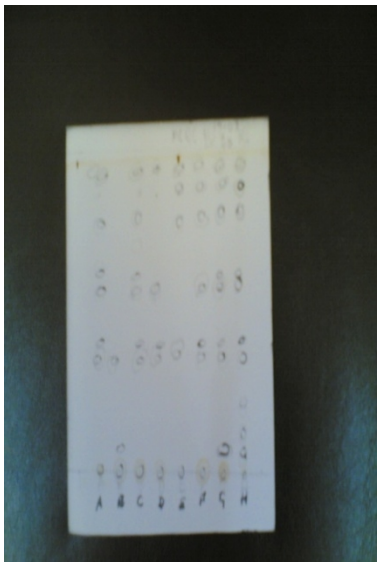
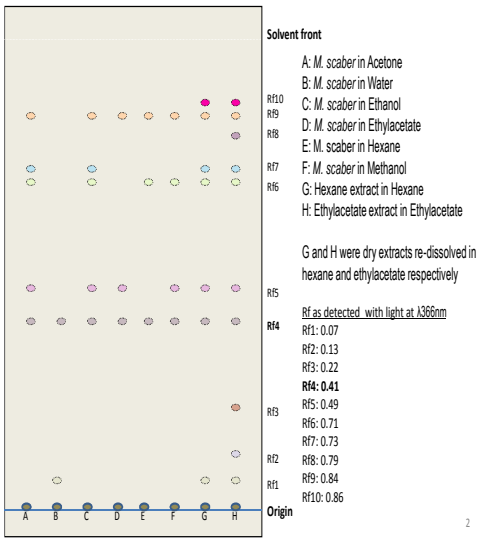
| Phytochemical constituent | Herb | Extract | Normal phase TLC | Reverse phase TLC |
|---|---|---|---|---|
| Saponins | + | + |  |  |
| Terpenoids | + | + | | |
| Alkaloids | + | + | | |
| Flavonoids | + | + | | |
| Cyanogenic glycoside | ? | ? | NL (L) and Niprd–AM1 (R) in ethanol developed with Hexane: Ethyl acetate (4:1) on K2 normal phase TLC, and viewed at 366 nm. NL yielded 9, while Niprd-AM1 yielded only 5 spots | NL (L) and Niprd–AM1 (R) in EtOH developed with Methanol: Water (4:1) on KC20 reverse phase TLC, and viewed at 366 nm. Both NL and the Niprd-AM1 yielded 9 spots. |
| Anthraquinones | ? | ? | | |
| Cardiac glycosides | ? | ? | | |

*Table 6. (+): Indicates present. (?): Indicates absent or below detection*

## 3.9 Results of Metallic Content Analysis/Limit Test for Heavy Metal

Plants as living organisms contain a wide range of metals that function in various ways especially as cofactors in many enzymic processes as in animals. For metals such sodium, potassium, calcium, magnesium, chromium, iron, copper, Nickel and so on, whose presence and functions in biological organisms are well established, their limits are not usually required to be determined in food and drugs. However, for the heavy metals typified by lead, mercury and cadmium, their limits are required to be established by law. Usually only one of the three – lead or cadmium – is selected to represent heavy metals. The results shown in Table 11 show that the level of lead in Conavir, being about 0.05 mg/ 100 g, is well below the limit of 10 ppm allowed for this metal in food and drugs. In Niprd-AM1, as shown in Table 12, the levels for both lead and cadmium, being less than 0.1 and 0.01 mg/ 100 g respectively are below the allowed limits, which for cadmium is 3 ppm (3 mg/ Kg). The levels for the other metals shown in Tables 13 and 14 were determined out of curiosity.






**Photograph of chromatogram** **Diagrammatized chromatogram**

**Fig. 5. Diagrammatized normal phase TLC of herb and extract of *M. scaber* Tannins, saponins and anthraquinones were detected. The tests for alkaloids were negative.**

*The TLC is a normal phase (K5 Silica with hexane: ethylacetate at 60:40 v/v as mobile phase) – diagrammatized amplify the following comments. 1). The herb in acetone (A) or ethanol (C) yielded 5 identical spots, while the herb in water (B) yielded only 2 spots – Rf1 and Rf4. 2). The herb in ethylacetate (D) or hexane (E) yielded 3 spots, while the herb (F) in methanol yielded 4. 3) The dry hexane extract (G) re-dissolved in hexane yielded 7 spots, while the ethylacetate extract (H) re-dissolved in ethylacetate yielded 10. 4). Notably, Rf4 was present in all the chromatograms, while Rf2 and Rf3 were present only in the H chromatogram. On the other hand Rf1 was present only in the B, G and H chromatograms. The results suggested that the effectiveness or spectrum of activity of MS extract depends upon the solvent used for extraction and that the ethylacetate followed by hexane might be the ideal solvent.*

**Table 7. Effect of storage on the moisture contents of AP and conavir**

| Months of storage in capped glass bottles at room temperature and humidity (RTH) | Loss on drying (mean ± SD) %w/w Herb (AP) | Extract (Conavir) |
|---|---|---|
| 0 | 9.84±0.66a (n = 7) | 10.07±1.90b (n = 7) |
| 3 | 8.64±0.59a (n = 5) | 10.84±1.03b (n = 5) |
| 9 | 9.14±0.66a (n = 7) | 12.14±2.33b (n = 5) |
| 21 | 10.77±0.86a (n = 5) | 9.84±2.06b (n = 5) |
| 39 | 8.96±0.46a (n = 5) | 11.44±2.03b (n = 5) |

*The results show that the moisture contents of both the herb and extract did not change significantly (P > 0.05) as denoted by (a) for herb and (b) for the extract, during the storage period of 0 to 39 months. The results imply that changes in moisture content as a contributory factor to moisture induced instability or hydrolytic spoilage would be minimal.*

**Table 8. Effect of storage on extractability/solubility/ appearance/pH on AP/Conavir**

| Months of storage in capped glass bottles at RTH | Herb (AP) | | | Extract (Conavir) | | |
|---|---|---|---|---|---|---|
| | Extractability % Mean ± SD | Appearance of extract | pH | Solubility (ml/g) | Appearance of solution | pH |
| 0 | 30.80±3.63[a] (n=7) | Clear, greenish brown. | 7.9 | 35±5 (n=7) | Clear, greenish brown. | 8.5 |
| 3 | 33.37±4.77[a] (n=5) | Clear, greenish brown. | 8.1 | 40±5 (n=5) | Clear, greenish brown. | 8.2 |
| 9 | 34.88±5.43[a] (n=5) | Clear, greenish brown. | 7.9 | 40±5 (n=5) | Clear, greenish brown. | 8.2 |
| 21 | 25.08±3.43[a] (n=7) | Clear, pale greenish brown. | 8.0 | 45±5[b] (n=7) | Clear, greenish brown. | 8.3 |
| 39 | 29.02±3.63[a] (n=5) | Clear, pale greenish brown. | 7.9 | 45±5[b] (n=5) | Clear, greenish brown. | 8.3 |

*Extractability and pH of the herb did not change significantly, but the greenish brown color faded slightly by the 21st month. Solubility of the dry extract decreased significantly by the 21st month, but the color and the pH did not. The values with ([a]) were statistically the same (that is: P > 0.05). The results for the 21st and 39th months marked with ([b]) differed significantly (P < 0.05) from those for 0 – 9 months.*

**Table 9. Effect of storage on light absorption and TLC characteristics on AP/ Conavir**

| Months of storage in capped glass bottles at RTH | Herb (AP) | | | Extract (Conavir) | | |
|---|---|---|---|---|---|---|
| | Absorbance at λ225 nm | TLC spots | | Light absorption | TLC spots | |
| | | NP | RP | | NP | RP |
| 0 | 0.083±0.015[a] (n=7) | 5 | 6 | 0.291±0.037[b] (n=7) | 5 | 6 |
| 3 | 0.107±0.025[a] (n=5) | 5 | 6 | 0.306±0.031[b] (n=5) | 5 | 6 |
| 9 | 0.115±0.025[a] (n=5) | 5 | 6 | 0.292±0.031[b] (n=5) | 5 | 6 |
| 21 | 0.078±0.015[a] (n=7) | 5 | 6 | 0.240±0.031[b] (n=7) | 5 | 6 |
| 39 | 0.091±0.015[a] (n=5) | 5 | 6 | 0.276±0.031[b] (n=5) | 5 | 6 |

*Solutions of herb and extract were made by thoroughly mixing 1 part of solute and with 100 parts of solvent (MeOH: $H_2O$ [52:48, v/v]), filtering, and diluting the filtrates by 150X with the same solvent. Absorbencies were measured at λ225 nm, using the solvent as the blank. Both ([a]) and ([b]) denote that any differences seen in the values were not significant (P >0.05). NP and RP mean normal phase and reverse phase respectively.*

**Table 10. Effect of storage on bitterness values and foaming indices of APs**

| Months of storage in capped glass bottles at RTH | Bitterness value | | Foaming index | |
|---|---|---|---|---|
| | Herb x $10^3$ | Extract x $10^3$ | Herb x $10^3$ | Extract x $10^3$ |
| 0 | 1.77±0.25$^a$ (7) | 18.65±3.09 (7) | 1.56±0.11$^a$ (7) | 8.72± 0.32 (7) |
| 3 | 1.53±0.16$^a$ (5) | 17.31±2.92 (5) | 1. 46±0.24$^a$ (5) | 9.31±0.63 (5) |
| 9 | 2.08±0.21$^a$ (5) | 17.87±3.11 (5) | 1.43±0.11$^a$ (5) | 8.68± 0.38 (5) |
| 21 | 1.31±0.18$^a$ (7) | 12.04±2.11$^b$ (7) | 1.38±0.12$^a$ (7) | 6.22±0.34$^b$ (7) |
| 39 | 1.43±0.19$^a$ (5) | 12.73±2.08$^b$ (5) | 1. 65±0.11$^a$ (5) | 6.15±0.34$^b$ (5) |

*The results show that both the bitterness value and the foaming index of the herb did not change significantly with storage during 0 – 39 months (P > 0.05), as indicated by lack of difference between the set of results marked ($^a$) in each case. By contrast, both the bitterness value and the foaming index of the extract decreased significantly with storage as from the 21$^{st}$/ 39$^{th}$ months of storage (P < 0.05), as indicated by ($^b$).*

**Table 11. Metallic content analysis/Limit test for lead in Conavirs**

| Metal/ No. replicates / Levels (mg/100g) | Na | K | Mg | Ca | Cr | Mn | Fe | Zn | Ni | Cu | Pb |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2.8 | 3.1 | 4.4 | 71 | 1.2 | 18 | 47 | 5.1 | 0.44 | 0.56 | 0.05 |
| 2 | 3.1 | 3.6 | 3.9 | 57 | 1.6 | 22 | 42 | 4.8 | 0.28 | 0.51 | 0.03 |
| 3 | 2.7 | 2.9 | 4.1 | 59 | 0.9 | 15 | 34 | 5.3 | 0.37 | 0.60 | 0.05 |
| **Mean** | 2.9 | 3.2 | 4.1 | 59 | 1.2 | 18 | 41 | 5.0 | 0.36 | 0.57 | 0.05 |

*WHO has established a "tolerable weekly intake" level for lead at 1.5 mg, corresponding to a daily lead intake of about 0.21 mg. The limit for lead in pharmaceuticals, according to the U.S. Pharmacopoeia and WHO [14] is 10 ppm (ie: 10 mg/ kg or 1 mg/ 100 g or 10 µg/ g). The same figure is cited by U.S. test labs as the acceptable limit for herbal products, dietary supplements, and drugs generally [24].*

**Table 12. Metallic content analysis/Limit test for lead and cadmium in Niprd-AM1**

| Metallic Element (mg/100g) | Na | K | Ca | Mg | Mn | Fe | Cu | Zn | Cd | Pb | Se | Cr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 6.08 | 2.32 | 54.3 | 297.3 | 0.81 | 0.08 | 1.24 | 0.70 | < 0.01 | < 0.1 | 2.35 | 0.53 |
| **2** | 6.22 | 2.11 | 55.5 | 229.9 | 0.72 | 0.12 | 1.08 | 0.60 | | | 2.22 | 0.41 |
| **3** | 6.12 | 2.38 | 51.9 | 251.3 | 0.85 | 0.10 | 1.22 | 0.65 | | | 2.48 | 0.47 |
| **Mean** | 6.14 | 2.27 | 53.9 | 259.5 | 0.79 | 0.10 | 1.18 | 0.65 | < 0.01 | < 0.1 | 2.35 | 0.47 |

*The limit for lead in drugs as per the USP and WHO [14] is 10 ppm. The same figure is cited by US test labs as the acceptable limit for herbs, dietary supplements and drugs generally [24]. The levels of Cd and Pb were the lowest - occurring below the allowed limits of 0.3mg/100g for Cd; and 1.0 mg/100g for Pb.*

## 3.10 Results of Application of GC-MS Spectroscopy in This Study

While some aspects of medicinal plant research (MPR) aim at fractionation, isolation and purification of phytochemical components of herbal preparations, such fractionated or purified entities are not covered by the definition of herbal medicine as per WHO [15,22] or as per NAFDAC [13] or the European Pharmacopoeia [23]. Fractionation, isolation and purification of herbal drug components may be considered if the aim is to identify suitable biomarkers for the material, but applications of hyphenated techniques such as the GC-MS, as we have done in this study, have the potential of facilitating such an aim even more efficiently without the arduous, usually unfruitful, task fractionation and purification. The results of our introduction of GC-MS to the study of Conavir, Niprd-AM1 and Niprifan are shown in Fig. 6./Table 13, Fig. 7./Table 14 and Fig. 8./Table 15.

### 3.10.1 Conavir

The molecules shown in Table 13 may be intermediates, constituents or fragments of natural constituents of *Andrographis paniculata* (AP). The C-H-O-containing entities may be associated with terpenoids like andrographolide, andrographon and stigmasterol, which are known constituents of AP. The C-H-O-N entities may be associated with alkaloids, which are also present in AP. The presence of an oxirane in AP may be significant, given the usually interesting biological activities of oxiranes. The results suggested that a more refined application of GC-MS will result in the selection of markers other than andrographolide.

### 3.10.2 Niprd-AM1

Some of the molecules in Table 14 may be intermediates, constituents or fragments of constituents of *Nauclea latifolia* (NL). The C-H-O-containing molecules may be associated with terpenoids like rotundic acid, stitosterol and quinovic acid, which are well known constituents of NL. The C-H-O-N-entities may be associated with alkaloids like strictosamide and angustoline that are also present in NL. The results suggested that a more refined application of GC-MS can result in the quantitation of markers other than rotundic acid and strictosamide.

### 3.10.3 Niprifan

The entities in Table 15 represent constituents of *Mitracarpus scaber* (MS) or their intermediates or fragments thereof. The abundance of long aliphatic chains as in myristic and arachidic acids; and of lipids like phenanthrone, androstadiene and squalene, is consistent with the hydrophobic nature of Niprifan. The benzene ring entities are evidently by-products of shikimic acid pathway via gallic acid - a known constituent of MS. Other C-H-O-entities are terpenoids like ursolic and oleanolic acids; and others like psoralen and tectoquinone, which are well known constituents of MS. The results suggested that the GC-MS technique can be applied to the quantitation of the foregoing constituents of *Mitracarpus scaber*.

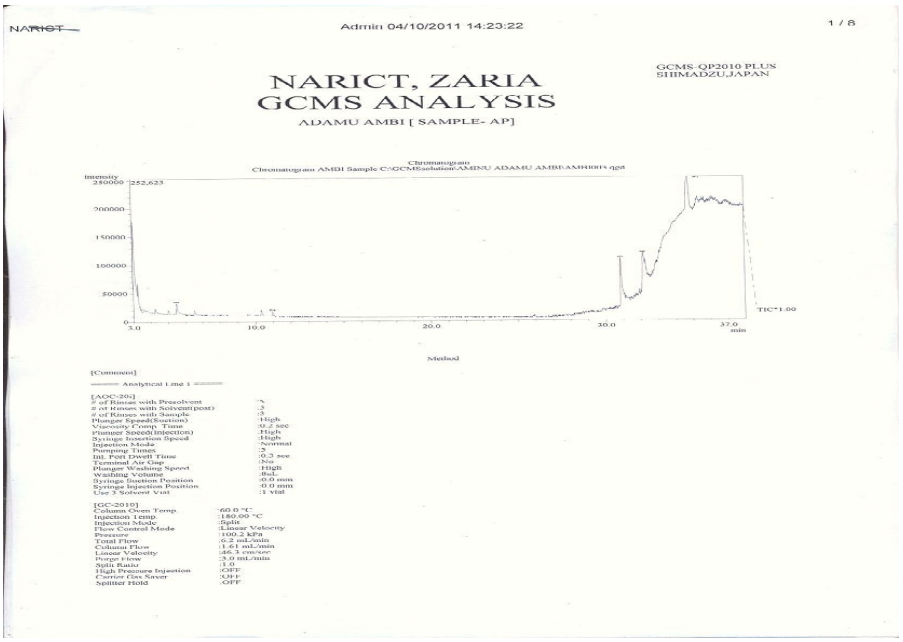
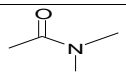
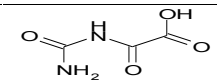
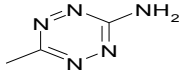
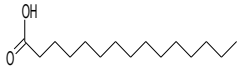
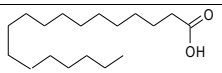
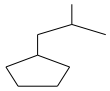
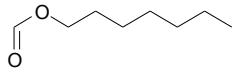
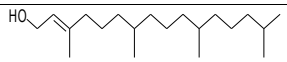
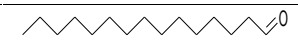
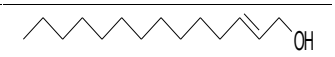
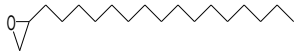



**Fig. 6. Gas chromatogram of aqueous extract of *A. paniculata* (AP)-Conavir**

*The chromatogram (abundance vs retention time) was generated with a Shimadzu GCMS-QP 2010 Plus. It shows 5 peaks corresponding Lines # 1 – 5 in the mass spectra. The sample was injected as 1µl of ethanolic solution containing 0.1µg of extract. Operational conditions were as indicated in the comment below the chromatogram.*

**Table 13. Summary of spectral data from GC-MS fingerprint of Conavir (AP)**

| Peak/ Line Number (#) as per GC/MS | RT (min) | Mass peaks | Base Peak | Some likely compds. based on comparison with NIST05 LIBRARY | Corresponding structures |
|---|---|---|---|---|---|
| Peak/ Line #1 | 5.5 | 6 | 44 | Acetamide<br>Mol Weight, 87 |  |
| | | | | Oxaluric acid<br>Mol Weight, 132 |  |
| Peak/ Line #2 | 11.0 | 5 | 42 | Terazin-3-amine<br>Mol Weight, 111 |  |
| | | | | Ethylenimine<br>Mol Weight, 43 |  |
| Peak/ Line #3 | 30.9 | 19 | 43 | Hexadecanoic acid<br>Mol Weight, 256 |  |
| | | | | Octadecanoic acid<br>Mol Weight, 284 |  |
| | | | | Cyclopentane (or Isobutylcyclopentane or Octyl formate) |  |

| | | | | | |
|---|---|---|---|---|---|
| Peak/ Line #4 | 32.1 | 23 | 83 | Mol Weight, 126 | |
| | | | | 1,9-Nonanediol<br>Mol Weight, 160 |  |
| | | | | Octyl formate<br>Mol Weight, 158 |  |
| Peak/ Line #5 | 34.7 | 30 | 57 | Phytol<br>Mol Weight, 296 |  |
| | | | | Pentadecanal<br>Mol Weight, 226 |  |
| | | | | Octadecen-1-ol |  |
| | | | | 2-<br>Heptadecycloxirane<br>Mol Weight, 282 |  |

*The molecules shown above may be intermediates, constituents or fragments of natural constituents of Andrographis paniculata (AP). The C-H-O-containing entities may be associated with terpenoids like andrograholide, andrographon and stigmasterol, which are known constituents of AP. The C-H-O-N entities may be associated with alkaloids, which are also present in AP. The presence of an oxirane in AP may be significant, given the usually interesting biological activities of oxiranes.*

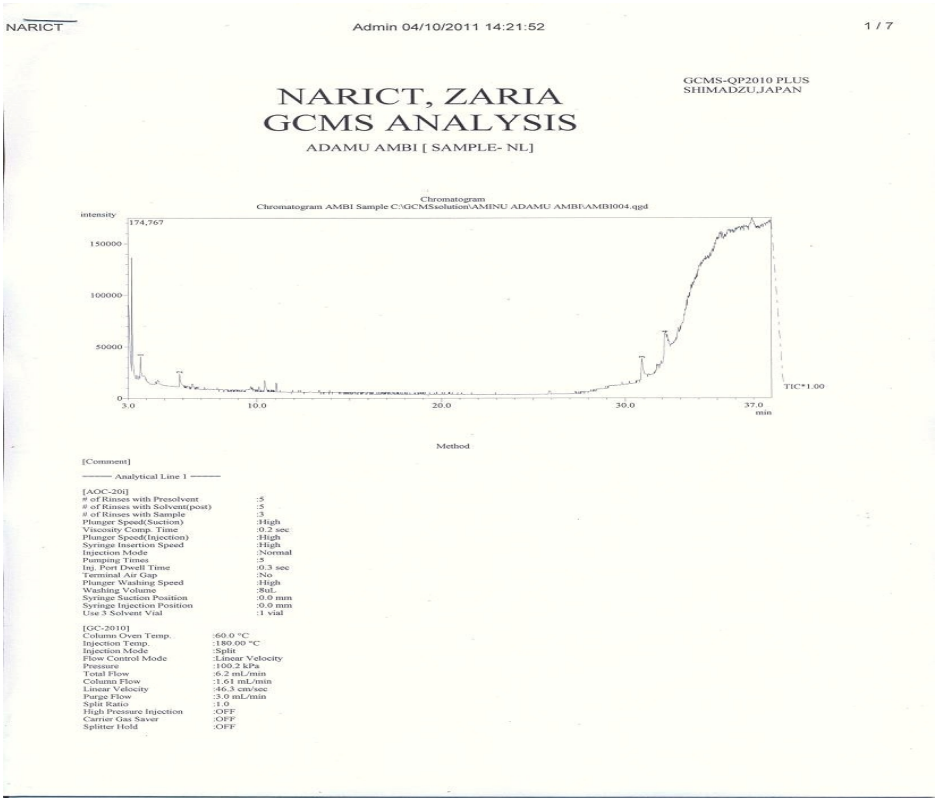
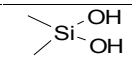
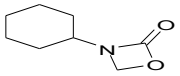
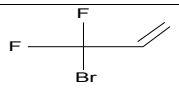
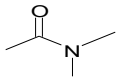
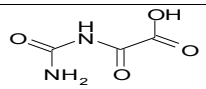
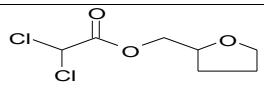
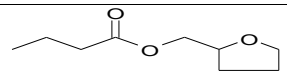
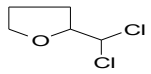
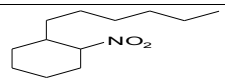
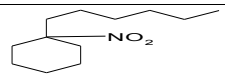
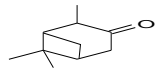
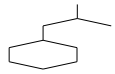



**Fig. 7. Gas chromatogram of aqueous extract of *Nauclea latifolia* (NL)-Niprd-AM1**

*The chromatogram (abundance vs retention time) was generated with a Shimadzu GCMS-QP 2010 Plus. It shows 4 peaks corresponding Lines # 1 – 4 in the mass spectra. The sample was injected as 1µl of ethanolic solution containing 0.1µg of extract. Operational conditions were as indicated in the comment below the chromatogram.*

**Table 14. Summary of spectral data from GC-MS fingerprint of Niprd-AM1 (NL)**

| Peak/ Line Number (#) as per GC/MS | RT (min) | Mass peaks | Base peak | Some likely compds. based on comparison with NIST05 LIBRARY | Corresponding structures |
|---|---|---|---|---|---|
| Peak/ Line #1 | 3.7 | 3 | 77 | Dimethylsilanediol Mol Weight, 92 |  |
| | | | | 3-Phenyl-1,3-oxazetidin-2-one Mol Weight, 149 |  |
| | | | | 3-Bromo-3,3-difluoro-1-propene, Mol Wt, 156 (likely colum break-down product) |  |
| Peak/ Line #2 | 5.8 | 7 | 44 | Acetamide Mol Weight, 87 |  |
| | | | | Silaethane (likely colum break-down product) Mol weight, 46 | $CH_3SiH_3$ |
| | | | | Oxaluric acid Mol Weight, 132 |  |
| Peak/ Line #3 | 30.9 | 8 | 83 | Tetrahydro-2-furanylmethyl dichloroacetate, Mol Wt, 212 |  |
| | | | | Tetrahydro-2-furanylmethyl butyrate, Mol Wt, 172 |  |
| | | | | Dichloromethyltetrahydrofuran Mol Weight, 154 |  |
| Peak/ Line #4 | 32.2 | 10 | 67 | 1-Hexyl-2-nitrocyclohexane Mol Weight, 213 |  |
| | | | | 1-Hexyl-1-nitrocyclohexane Mol Weight, 213, |  |
| | | | | Trimethyl[3,1,1]heptan-3-one Mol Weight, 152 |  |
| | | | | i-Butylcyclohexane Mol Weight, 140 |  |

*Some of the molecules above may be intermediates, constituents or fragments of constituents of Nauclea latifolia (NL). The C-H-O-containing molecules may be associated with terpenoids like rotundic acid, stitosterol and quinovic acid, which are well known constituents of NL. The C-H-O-N-entities may be associated with alkaloids like strictosamide and angustoline that are also present in NL. If the seemingly novel halogenated entities are not extraneous artifacts; then their presence might contribute to the popular anti-infective uses of NL, including its uses as an antimalarial and as a "chewing stick" for dental care and oral hygiene. It would seem however, that these halogenated and silicone-containing compounds are column break-down products.*

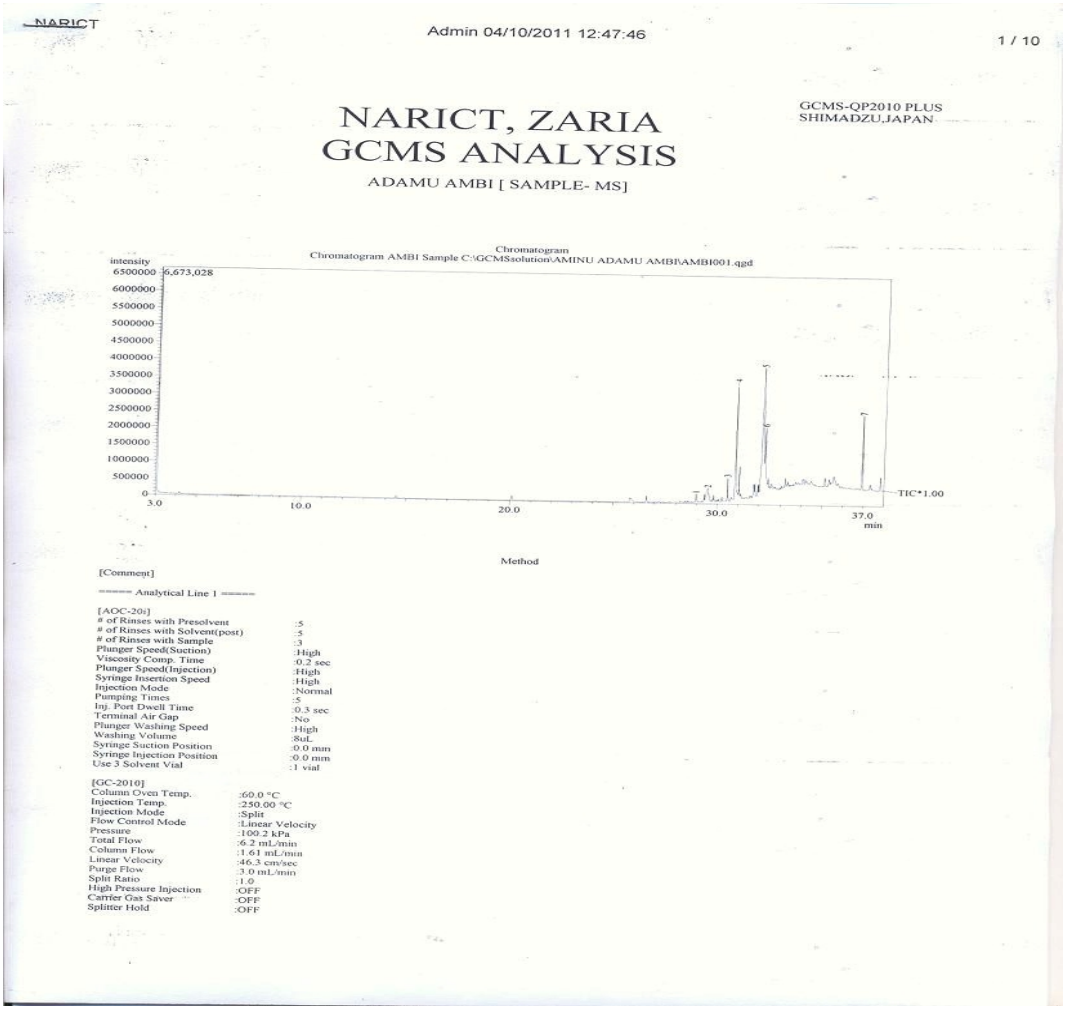
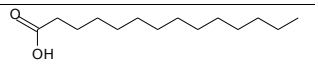
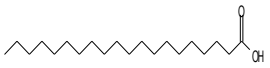
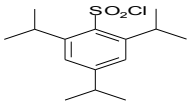
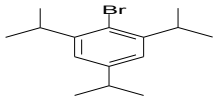
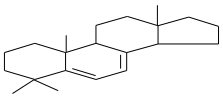
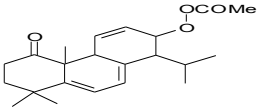
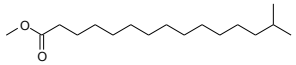
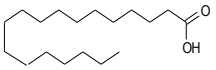
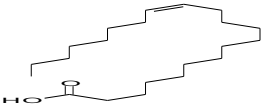
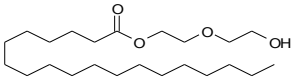
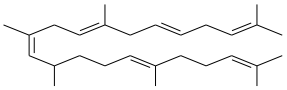



**Fig. 8. GCMS chromatogram of ethylacetate extract of *M. scaber* (MS)-Niprifan**

*The chromatogram (abundance vs retention time) was generated with a Shimadzu GCMS-QP 2010 Plus. It shows 7 peaks corresponding Lines # 1 – 7 in the mass spectra. The sample was injected as 1µl of ethanolic solution containing 0.1µg of extract. Operational conditions were as indicated in the comment below the chromatogram.*

**Table 15. Summary of spectral data from GC-MS chromatograph of Niprifan (MS)**

| Peak/ Line Number (#) as per GC/MS | RT (min) | Mass peaks | Base peak | Some likely compds. based on comparison with NIST05 LIBRARY | Corresponding structures |
|---|---|---|---|---|---|
| Peak/ Line #1 | 29.0 | 38 | 73 | Tetradecanoic acid (Myristic acid) Mol Weight, 228 |  |
| | | | | Eicosannoic acid (Arachidic acid) Mol Weight, 312 |  |
| Peak/ Line #2 | 29.5 | 56 | 187 | Benzenesulfonylchloride-2,4,6-isopropyl, Mol Weight, 302 |  |
| | | | | 1-Bromo-2,4,6-triisopropylbenzene Mol weight, 282 |  |
| | | | | 4.4-Dimethyl Androsta-5,7-diene Mol Weight, 284 |  |
| | | | | 14-Isopropyl-1-oxopodoc (Phenanthrone derivative), Mol Wt, 342 |  |
| Peak/ Line #3 | 30.5 | 55 | 74 | Methyl-14-methylpentadecanoate, Mol Wt, 270 |  |
| Peak/ Line #4 | 30.8 | 88 | 43 | Octadecanoic acid Mol Weight, 284 |  |
| Peak/ Line #5 | 32.1 | 95 | 55 | Erucic acid (Prifac 299) Mol Weight, 338, |  |
| Peak/ Line #6 | 32.2 | 60 | 43 | Clindrol SDG (Aqua Cera) Mol Weight, 372 |  |
| Peak/ Line #7 | 36.9 | 101 | 69 | Squalene (Spenacene, Supraene) Mol Weight, 410 |  |

*The molecular entities above are constituents of MS or their intermediates or fragments. The high abundance of long aliphatic chains as in myristic and arachidic acids; and of lipids like phenanthrone, androstadiene and squalene, is consistent with the general hydrophobic nature of Niprifan. The benzene ring entities are evidently by-products of shikimic acid pathway via gallic acid - a known constituent of MS. Other C-H-O-entities are terpenoids like ursolic and oleanolic acids; and others like psoralen and tectoquinone, which are well known constituents of MS.*

## 4. CONCLUSION

The data so far presented on *Andrographis paniculata*/ Conavir, *Nauclea latifolia*/ Niprd-AM1 and *Mitracarpus scaber*/ Niprifan by our laboratory and by studies elsewhere [25-28], as previously highlighted and discussed elsewhere [17], amply suffice for the registration of these drugs. But, as our experience with the study has shown, there is a need, for purposes of further research, for the following actions to be taken:

- The GC-MS studies should be refined and amplified for the purpose of selecting suitable biomarkers, which will pave the way for studies requiring bioassays.
- Depending upon the direction of further interest in Conavir, Niprd-AM1 and Niprifan, other techniques should be introduced and perfected for such purposes.

However, none of such further work is mandatory for the registration of these drugs by the NAFDAC or EMEA.

## CONSENT

Not applicable.

## ETHICAL APPROVAL

Not applicable.

## ACKNOWLEDGMENT

We would like to acknowledge Professor Charles Wambebe (DG, NIPRD: 1989-01) for approving, and Mr. John Atogwe (Botanist) for obtaining the seeds of *Andrographis paniculata* from India for planting in NIPRD; Dr. Uford Inyang (DG, NIPRD: 2001-09) for approving the research and for inviting the USA's NIH to NIPRD; and the NIH Library for free access to their magnificent facilities.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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