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PHARMACOGNOSTIC EVALUATION AND QUALITY CONTROL PARAMETERS OF ANDROGRAPHIS PANICULATA

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1. INTRODUCTION

Andrographis paniculata (Burm. f.) Nees (family Acanthaceae) is an annual herb native to India and Sri Lanka and now grown across South and Southeast Asia and parts of China. Known as kalmegh in Hindi and Ayurvedic texts and as chuan xin lian in Chinese medicine, it is used for fevers, sore throat, liver complaints and dysentery, and more recently as an ingredient of cold remedies sold in Scandinavia and elsewhere [1, 2, 3]. The bitter taste comes mainly from diterpenoid lactones, of which andrographolide is the best studied. Reviews of its pharmacology describe

Abstract

Andrographis paniculata (Burm. f.) Nees, known as kalmegh, is a widely used bitter herb in Ayurvedic, Chinese and Southeast Asian practice, yet commercial lots vary considerably in quality. This review, which reports no new data, brings together published work on the pharmacognostic evaluation of the leaf and aerial parts: botanical identity, macroscopic and microscopic features, physicochemical limits, phytochemical screening and chromatographic standardisation by andrographolide content. It also considers contaminants, adulteration, stability and the link between measured parameters and biological activity. The literature supports a workable set of identity, purity and assay tests, but reported ranges differ with origin, harvest stage and method, and few limits have been confirmed in collaborative studies.

anti-inflammatory, antimicrobial, hepatoprotective and immunomodulatory effects in experimental systems [1, 4]. Those findings matter to a patient or a manufacturer only if the material in the sack is the right

plant, in sound condition, with a reproducible amount of the active markers. Pharmacognostic evaluation is the discipline that checks exactly that.

Quality control of herbal raw material rests on a short list of questions. Is the plant correctly identified? Is it free from foreign matter, excess moisture and toxic contamination? Does it contain the marker compounds in an acceptable range? The World Health Organization (WHO) set out the basic methods for these tests in its manual on medicinal plant materials, and its monograph on *Herba Andrographidis* gives a description and general requirements [5, 6]. Practical laboratory handbooks add stepwise procedures for microscopy, ash values and extractive values [7, 8]. This review gathers what has been published on applying those methods to *A. paniculata*. It does not report new measurements. Instead it compares how different groups have defined identity, purity and assay, where their findings agree, and where the evidence is too thin to justify a fixed pharmacopoeial limit. The pharmacology is reviewed briefly, because it explains why particular markers were chosen.

The commercial setting adds pressure. Kalmegh is traded as whole dried herb, as cut and sifted leaf, and as powders and extracts blended into tablets, capsules and syrups. Each form hides different problems. Whole herb can be judged by eye, cut material by microscopy, and extracts only by chemistry. Reviews of herbal medicine regulation point out that quality depends on the whole chain from collection to finished product, since raw material often passes through collectors, village traders and processors before it reaches a manufacturer, and every handover is a chance for mixing, damp storage or substitution [9]. Bringing wild-collected species into managed cultivation tries to reduce these risks at source [10], but a manufacturer who relies only on supplier paperwork has little protection. A written specification, combined with in-house identity and assay tests on every incoming lot, is the minimum most quality systems expect, and it is the setting in which the parameters discussed below are applied.

2. BOTANICAL IDENTITY AND MICROSCOPY

The plant is an erect, much-branched herb that usually reaches between half a metre and a metre, with quadrangular stems that are slightly winged at the angles and swollen at the nodes. Leaves are opposite, lanceolate to ovate-lanceolate and glabrous, with an entire or slightly undulate margin and an acute apex. Flowers are small and white with pink or purple markings on the corolla, borne in lax panicles; the fruit is a linear-oblong capsule that splits to release small yellowish-brown seeds [3, 11]. Standard pharmacognosy texts agree on these points, and they form the macroscopic part of any identity test [12]. The drug as traded is usually the dried aerial part, and the leaf is the portion richest in bitter principles. Leaves are dark green above and paler beneath, and they turn brittle on drying. The taste is intensely and persistently bitter, which is itself a useful, if subjective, identity check.

In transverse section the leaf is dorsiventral. A single-layered upper epidermis covers a palisade layer and several layers of spongy mesophyll, and the midrib carries a small collateral vascular bundle. Cystoliths, which are calcium carbonate concretions, are conspicuous in the epidermal cells and are among the more distinctive features of the family; they are usually described as elongated bodies lying parallel to the leaf surface [3, 7]. Stomata are of the diacytic type, and trichomes are few, with glandular hairs reported on young leaves. Powder microscopy follows the same logic. Fragments of epidermis with cystoliths, diacytic stomata, spiral xylem vessels and mesophyll cells make up the usual picture. Clearing with chloral hydrate and staining lignified tissue with phloroglucinol and hydrochloric acid are described in the standard microtechnique texts [8, 13]. Micrographs published by different groups are broadly consistent, which makes these features dependable for identification.

Quantitative leaf microscopy puts numbers on the descriptive picture. Stomatal index, stomatal number, vein-islet number and palisade ratio vary little within a species when leaves of comparable age are compared, and

they help separate related Acanthaceae that are sometimes sold under the same vernacular name [7, 12]. The technique is cheap and needs only a microscope, a calibrated eyepiece and patience. Its weakness is that values depend on leaf position, maturity and growing conditions, so a single reported figure should not be read as a pharmacopoeial limit. Published pharmacognostic profiles of kalmegh generally give ranges from a small number of samples collected in one region [3, 7]. Until ranges are established on authenticated material from several agro-climatic zones, quantitative microscopy is best treated as supporting evidence rather than a pass-or-fail test. DNA-based methods are now discussed as a complement, and they are taken up in the final section of this review [14, 15].

Adulteration of kalmegh takes several forms, and the microscope is often the first tool to catch it. Dried herb may be bulked out with other leafy plants of similar colour, with stem and root material of the same species, or with exhausted marc left over from extract production. Powdered drug is the most exposed, because shape is lost and only cell fragments remain to be compared. A sound approach is to examine the powder against authenticated reference slides, to note the proportion of cystolith-bearing epidermis, and to run a TLC fingerprint on the same lot [7, 12, 16]. Marc is harder to detect by eye but tends to give itself away through a low extractive value and a weak bitter taste [17]. WHO recommends that suppliers keep reference samples and that purchasers verify identity at receipt, whatever certificates accompany the consignment [5], and cultivated lots, unlike wild collections, are easier to trace to a field and a harvest date [10]. The extra effort is usually repaid.

Harvest stage deserves a separate note, because it changes both the appearance and the chemistry of the drug. Growers in India usually cut the crop at about three to four months, around the onset of flowering, when the leaf mass is highest, and a second cut may follow. Studies of andrographolide accumulation during plant development report the highest leaf content close to this stage, with a decline as the plant matures and

the proportion of stem and fruit rises [18, 19]. The stem is poorer in bitter principles than the leaf, so a lot with a large stem fraction is of lower value even if it passes an identity check. That is why some specifications limit the stem or define a leaf-only grade, and why sellers charge more for such grades [17]. Drying method has also been compared, with shade or controlled low-temperature drying generally preferred to sun drying in order to limit losses of the lactones [18].

3. PHYSICOCHEMICAL PARAMETERS

Physicochemical constants are the working tools of raw-material control. Loss on drying measures moisture and volatile matter, and it matters because damp herb supports moulds and speeds the hydrolysis of glycosides. Total ash is the inorganic residue left after incineration, while acid-insoluble ash points to silica, sand and soil adhering to the drug. Water-soluble ash gives a further picture of mineral salts. These tests follow the procedures laid down in the WHO manual and in the practical texts [5, 7, 8]. For kalmegh, published values differ appreciably between laboratories, and the differences track how carefully the material was cleaned after harvest as much as the true composition of the plant [3, 17]. Foreign organic matter, mainly other plant parts and weeds, is determined by hand-sorting a weighed portion and is reported as a percentage. Because the tests are simple, they are also the first line of defence against dilution with sand, soil or stem.

Extractive values give a rough measure of how much drug dissolves in a chosen solvent, and so hint at the proportion of active or at least extractable constituents. Alcohol-soluble and water-soluble values are the usual pair, with both cold maceration and hot extraction described [7, 12]. In kalmegh the alcohol-soluble fraction is generally larger than the water-soluble one, which fits the moderate polarity of andrographolide and its congeners, although both fractions also carry flavonoids, tannins, sugars and chlorophyll [11, 18]. The tests are cheap, fast and sensitive to gross adulteration or exhausted material. Their limitation is lack of specificity. An extract can be heavy with inert polar matter and still contain little of the

diterpenoids. For that reason extractive values are used alongside chromatographic assay and not in place of it [17]. Some authors also report extract pH, swelling index and foaming index, but these add little diagnostic information for this drug.

Preliminary phytochemical screening appears in almost every pharmacognostic paper on the species. Extracts are tested for alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, glycosides and reducing sugars by classical colour and precipitation reactions [3, 8]. Reported results are fairly consistent: flavonoids, terpenoids, tannins and steroids are usually positive, while the alkaloid result varies with the reagent and the extract. A well-known weakness is that these tests are qualitative and prone to false positives, since chlorophyll and polyphenols interfere with several colour reactions. Reagents that react with the unsaturated lactone ring, such as the Kedde reagent, are used to visualise the diterpenoid lactones on plates [11, 16]. Screening is best seen as a descriptive fingerprint of the extract rather than evidence of quality. It tells the analyst which classes are present, not how much of anything is there.

Heavy metals, pesticide residues, aflatoxins and microbial load belong in any modern specification, and herbs grown in intensively farmed or industrially affected areas can fail on one or more of them. WHO guidance on contaminants and residues describes sampling, test methods and the reasoning behind limits for arsenic, cadmium, lead and mercury [20]. Published surveys of herbal materials show that metals and microbial counts can exceed accepted limits in a fraction of commercial lots, and there is no reason to expect kalmegh to be exempt [9, 20]. Microbial testing commonly covers total aerobic count, yeasts and moulds, and absence of *Escherichia coli* and *Salmonella*. The point to stress is that a lot can pass every identity test, carry a perfectly respectable andrographolide content, and still be unfit for use. Contaminant testing is therefore a separate and non-negotiable layer, not a by-product of the chemistry.

Storage conditions matter more than many specifications admit. Andrographolide is a

lactone with a conjugated exocyclic double bond and a free allylic hydroxyl group, so it is open to hydrolysis, isomerisation and dehydration, and its stability in solution depends strongly on pH [18]. Dry herb is less reactive than solutions, but warm, humid storage encourages fungal growth and a slow loss of marker content. Texts on solid dosage form stability describe the usual long-term and accelerated conditions for drug substances and products [21], and the same logic can be adapted to herbal raw material and extracts, even though such reviews were not written with crude plant drugs in mind. A sensible approach is to keep the dried herb in tightly closed containers, protected from light and moisture, and to retest marker content at set intervals instead of assigning an arbitrary shelf life.

4. CHROMATOGRAPHIC STANDARDISATION

The main bioactive constituents are *ent*-labdane diterpenoids. Andrographolide is the principal and most bitter lactone, accompanied by 14-deoxy-11,12-didehydroandrographolide, neoandrographolide and 14-deoxyandrographolide, with andrograpanin, isoandrographolide and several glucosides described in smaller amounts [1, 11, 19]. Flavonoids and other polyphenols are also present, mostly at lower levels than the diterpenoids in the aerial parts [11, 22]. Diterpenoid content is strongly influenced by plant part, season and geographical origin. Pholphana and colleagues, who studied three of the main diterpenoids in material from Thailand, reported marked variation between samples and plant parts, and similar variability appears in surveys of Indian accessions [19, 23]. That variability is the main reason why a plain minimum andrographolide limit has been argued to be insufficient on its own, and why multi-marker or fingerprint approaches have gained favour.

Thin-layer chromatography (TLC) remains the workhorse of pharmacopoeial identification. A common system spots a methanolic extract on silica gel and develops it with a mixture such as chloroform and methanol, or toluene, ethyl acetate and formic

acid, with andrographolide visualised under ultraviolet (UV) light at 254 nm, or by spraying with the Kedde reagent or anisaldehyde-sulphuric acid [16, 23]. The laboratory handbook edited by Stahl remains a standard reference for plate preparation and detection reagents [16]. High-performance TLC (HPTLC) with densitometry improves resolution and permits quantitation, and validated methods for andrographolide in raw material and formulations have been published [18, 23]. Compared with liquid chromatography the equipment is cheaper, many samples can be run side by side on one plate, and solvent consumption is lower. Plates are less suited to resolving closely related deoxy derivatives, however, and precision is usually poorer. For routine identity against a reference chromatogram, TLC is hard to beat.

Reversed-phase high-performance liquid chromatography (HPLC) with UV detection is the reference quantitative method. Sharma and co-workers described one of the earliest HPLC assays for andrographolide in kalmegh in the early 1990s, and later methods have used C18 columns with water or dilute acid and acetonitrile or methanol in isocratic or gradient mode, with detection near 223–230 nm where the conjugated lactone absorbs [18, 24]. Later work extended the approach to the other diterpenoids in a single run, giving profiles that can be compared between batches [19, 23]. Validation follows the ICH approach to analytical procedures, applied to botanical extracts: specificity, linearity, accuracy, precision, detection and quantitation limits, and robustness [25]. The usual hazards apply. Extraction efficiency depends on solvent, time and particle size; andrographolide can degrade during prolonged hot extraction; and co-eluting peaks in crude extracts can inflate results unless peak purity is checked. A reference standard of known purity is essential.

The main parameters reported for the herb, together with what each can and cannot show, are brought together in table 1. The entries fall into four groups, as the grouping in fig. 1 makes plain: tests of identity, tests of purity, tests of chemical content, and tests of stability. Identity tests are the most numerous

because they are the cheapest and have the longest history, whereas the content group is small yet decisive in practice, since a few chromatographic assays carry most of the weight at batch release [5, 18, 24]. A specification built only from identity and purity tests would pass leaf lots that had lost most of their diterpenoids during poor drying. Conversely, one built only from an andrographolide assay would pass a lot full of soil and stem. The table therefore argues for combining at least one test from each group, and for stating the method as well as the limit.

Isolation work supports all of this analysis, because a pure reference compound is needed before any assay can be calibrated. Rajani and colleagues described a rapid method for isolating andrographolide from the herb that relies on hot extraction followed by crystallisation, and it is widely cited as a practical route to a working standard [26]. In-house standards are acceptable for routine use only when their identity has been confirmed by spectroscopy and their purity has been assigned against a certified material. Sareer and co-workers compared extraction and isolation procedures for andrographolide and other constituents and noted that solvent choice, temperature and extraction time change yield considerably [18]. Laboratories that quote andrographolide percentages without describing these variables make comparison between papers almost impossible. A minimum reporting set would include the part of plant, drying method, particle size, solvent, extraction time and the source and purity of the standard.

5. PHARMACOLOGICAL RELEVANCE AND SAFETY

Why these markers? Because andrographolide and its relatives carry much of the reported biological activity. Andrographolide inhibits nuclear factor- κ B signalling in cell models, apparently through covalent modification of a reduced cysteine residue on the p50 subunit, which offers a mechanistic explanation for the anti-inflammatory activity seen in many experimental systems [27]. Effects on macrophage activation and antibody responses have been reported in mice [28], and hepatoprotective activity against

chemically induced liver injury has been shown in rodents, in part through antioxidant effects [29, 30]. Antimalarial activity of crude extracts has been observed against rodent malaria parasites, alone and combined with curcumin [31]. Enzyme inhibition studies, for instance against α -glucosidase and α -amylase, have raised interest in antidiabetic use [32]. None of this proves that a particular lot of herb is clinically useful. It does show that diterpenoid lactone content is a rational quality attribute.

Human data are more modest. A systematic review of trials in upper respiratory tract infection concluded that kalmegh preparations, mostly a standardised fixed combination, were more effective than placebo for symptom relief, with caveats about trial quality [33]. An early double-blind study of a standardised monodrug preparation in common cold reported fewer symptoms and better recovery [34], and a placebo-controlled study in rheumatoid arthritis reported reduced joint symptoms with a standardised extract [35]. Larger clinical evidence in other conditions is lacking. These trials share an important feature: the extract was standardised to a stated amount of herb or marker. That is exactly what raw-material pharmacognosy is meant to deliver. Without quality control upstream, trial results cannot be transferred to products on the market, and variation in marker content between brands may explain some of the inconsistency seen when different preparations are compared [1, 36].

Safety and pharmacokinetics also feed back into specification. Oral bioavailability of andrographolide is low, owing to extensive metabolism and efflux by P-glycoprotein, which means the amount in the dosage form is not the amount reaching the circulation [37]. Studies of a fixed combination in rats and in human volunteers documented rapid absorption and elimination [38]. A phase I study of andrographolide in healthy volunteers and in people infected with human immunodeficiency virus (HIV) found that the compound was generally tolerated at lower doses, with adverse effects limiting dose escalation [39]. Reports of hypersensitivity, mainly with injectable preparations used in

China, have been summarised in the pharmacological reviews [1, 36]. For oral herbal products the practical implication is simple. Dose-related claims should be tied to a measured marker content, and batches should be released against an assay and not against the weight of herb alone.

Table 1: Pharmacognostic and quality control parameters reported for *Andrographis paniculata* herb

Parameter	What it shows and its main limitation	Ref.
Macroscopy leaf, stem, flower	Quick identity from shape, colour and bitter taste; subjective and cannot detect powdered adulterants	[3, 12]
Leaf microscopy cystoliths, diacytic stomata	Distinctive epidermal features separate the species from related plants; needs a trained analyst	[7, 12]
Quantitative microscopy stomatal and vein-islet numbers	Supporting constants that shift with leaf age, position and origin	[7, 12]
TLC and HPTLC fingerprint	Compares band pattern with an andrographolide standard; fast but resolves deoxy derivatives poorly	[16, 23]
Foreign matter and acid-insoluble ash	Flags soil, sand and non-leaf parts; reported limits differ between laboratories	[5, 8]
Loss on drying	Controls moisture to limit mould growth and degradation of lactones	[5, 7]
Heavy metals and microbial load	Safety limits following WHO guidance; independent of marker content	[17, 20]
Extractive values	Cheap measure of extractable matter; non-specific for diterpenoids	[7, 12]
HPLC assay of andrographolide	Quantitative reference method; needs validated extraction and a reference standard of known purity	[18, 24, 25]
Multi-marker HPLC profile several diterpenoids	Allows batch comparison across more than one compound; reference chromatograms not yet agreed	[19, 23]
Stability retesting marker content on storage	Follows standard drug stability logic; published data on dried herb are sparse	[18, 21]

Finished products raise their own questions. Extracts used in clinical studies are normally prepared with defined solvents and standardised to a stated percentage of andrographolide or total andrographolides, and the WHO monograph lists traditional preparations as well as the crude herb [6]. A standardised extract is easier to dose than raw leaf, but the advantage depends on method transfer: the assay used in the factory must give the same answer as the assay in the control laboratory [25]. The fixed combination Kan Jang, studied in several of the trials cited above, is a case in point, since its pharmacokinetic and clinical work was carried out with material of declared marker content [34, 38]. For tablets and capsules made from powder, content uniformity and disintegration add further tests, although those belong to pharmaceutical technology rather than pharmacognosy. What the herb supplier controls is the starting point, and every later variation is multiplied on top of it.

Anticancer and immune-stimulating properties have attracted a good deal of laboratory attention. Kumar and co-workers examined compounds isolated from the plant and found cytotoxic activity against human cancer cell lines, along with stimulation of lymphocyte proliferation, for andrographolide and several related diterpenoids [22]. Structure-activity comparisons of this kind are useful for quality control because they show that the minor constituents are not always inert. A multi-marker assay would capture them, while a single-marker assay would not. At the same time, cell-line cytotoxicity is a long way from a therapeutic effect, and the low systemic exposure noted above makes extrapolation to patients uncertain [37]. The literature is therefore best read as a justification for chemical standardisation, not as evidence that the herb treats malignancy.

6. LIMITATIONS AND FUTURE DIRECTIONS

Several limits recur throughout the literature. Most pharmacognostic profiles were produced by single laboratories on a handful of samples, often bought from local markets with no voucher specimen. Without a voucher

deposited in a recognised herbarium, a later worker cannot confirm that the plant studied was really *A. paniculata*. Reported ash, extractive and moisture values therefore differ for reasons that cannot be separated into genuine biological variation and sampling or technique effects. Best-practice guidance for phytopharmacological research stresses authentication, voucher specimens, chemical characterisation of the material and full reporting of methods [40]. A good number of kalmegh papers meet some but not all of those expectations. For a regulator or manufacturer seeking numerical limits, the honest summary is that wide ranges have been reported and few have been tested between laboratories.

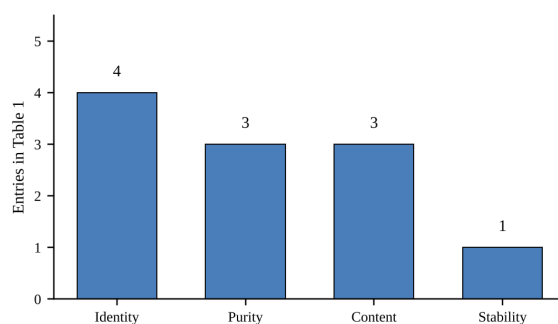


Fig. 1: Number of entries in table 1 by type of test

Regulatory expectations add another layer. Guidance on herbal specifications generally asks for a description, identification by at least two independent methods, tests for purity and contaminants, and an assay or equivalent chemical profile, with limits justified by data from a number of batches and methods validated along ICH lines [5, 20, 25]. Many published pharmacognostic papers on kalmegh supply the first half of that list but not the second. They lack batch-to-batch data, validation statistics and evidence that the proposed limits would reject genuinely poor material. A limit set at the mean of five samples will reject a fair share of acceptable lots. Ranges based on the observed spread, with allowance for natural variation and analytical uncertainty, are more defensible [6, 8]. Calling a paper a standardisation study is easy; delivering the batch statistics that justify the label is harder.

Molecular methods are an obvious addition. DNA barcoding with plastid and nuclear markers can identify species in whole or cut

material, and candidate markers have been tested on flowering plants generally and compared on medicinal plants specifically [14, 15]. The approach struggles with heavily processed material and extracts, where DNA is degraded or absent, and with mixtures, where a minor adulterant can be missed. It also says nothing about chemical content. Barcoding and chromatography therefore answer different questions and are better run together. For kalmegh, published barcode work appears to be thinner than for better-known commercial herbs, so reference sequences from authenticated vouchers would need to be deposited before the method becomes routine. Fingerprinting by liquid chromatography with mass spectrometry offers another route, giving a broader chemical picture than any single marker [19, 23].

Several practical steps would move the field forward. First, interlaboratory studies on authenticated, geographically diverse samples could turn the scattered ranges into defensible limits for ash, extractives, moisture and marker content. Second, assay methods should be harmonised, including extraction conditions, because the same lot can give different andrographolide results depending on sample preparation [18, 25]. Third, stability data on dried herb and extracts under controlled conditions would let manufacturers assign retest periods on evidence [21]. Fourth, an agreed reference chromatogram for the multi-marker profile would make fingerprint comparison objective instead of visual. Finally, work that links quality parameters to biological activity, not simply to chemistry, would test whether the chosen markers really predict effect. Bioassay-guided quality control is expensive, but even a modest anti-inflammatory cell assay run beside the HPLC profile would show whether chemically similar lots behave similarly [9, 27].

7. CONCLUSION

Pharmacognostic evaluation of *Andrographis paniculata* rests on a mature set of methods. Macroscopic and microscopic features, notably cystoliths and diacytic stomata, identify the leaf; ash, moisture and foreign-matter limits describe purity; and

TLC, HPTLC and HPLC establish diterpenoid content [3, 5, 7, 23]. Each test answers a different question, so a specification that omits any of the four groups in table 1 leaves a gap that the others cannot cover. Contaminant limits and stability retesting complete the picture and should not be treated as optional extras [20, 21]. Because andrographolide and its relatives carry much of the reported activity, content-based release is both chemically and biologically reasonable [27, 33].

The weaker side of the evidence is numerical. Reported ranges for ash, extractives and marker content come from small, single-laboratory studies, often without vouchers, and they cannot yet be turned into firm limits [8, 40]. Progress will depend on authenticated multi-site sampling, harmonised assay procedures, agreed reference chromatograms and complementary DNA-based identification [14, 15]. Until then, manufacturers are well advised to specify the method together with each limit, to test every lot for identity, purity and content, and to keep voucher samples of the material they accept. A specification of that kind costs little to run and protects the patient far better than a certificate alone.

8. ABBREVIATIONS

DNA-Deoxyribonucleic acid, HIV-Human immunodeficiency virus, HPLC-High-performance liquid chromatography, HPTLC-High-performance thin-layer chromatography, ICH-International Council for Harmonisation, TLC-Thin-layer chromatography, UV-Ultraviolet, WHO-World Health Organization

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10. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

11. CONFLICT OF INTERESTS

Declared none

12. REFERENCES

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