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Quality Standards for *Caturjata Carna* Evaluated with Official and Substitute Ingredients

Research Article

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Abstract

Background: In Ayurveda, single or multiple herbs mixed in a particular proportion are used for the treatment of different diseases. *Caturjata Curna* (CC) is a popular Ayurvedic medicine with therapeutic application in tastelessness, diseases due to vitiated *Kapha*, poisoning and discoloration. The present study is an attempt to standardize a polyherbal medicine CC. Methods: CC was prepared by mixing an equal proportion of ingredients including *Tvak* (*Cinnamomum verum*) - stem bark, *Ela* (*Elettaria cardamomum* (L.) Maton) - seed, *Tvakpatra* (*Cinnamomum tamala* (Buch.-Ham.) T.Nees & Eberm.) - leaf and *Nagakesara* (*Mesua ferrea* L.) - stamen in equal parts as per guidelines in Ayurvedic Formulary of India. Macro-microscopy, physico-chemical parameters, HPTLC fingerprinting, and spectroscopic parameters were determined according to standardised methodology available in Ayurvedic Pharmacopoeia of India. Results: *Caturjata curna* is brown coloured with a characteristic odour and aromatic taste. Powder microscopy showed the presence of diagnostic characters like horse shoe-shaped stone cells, perisperm cells with volatile oil droplets, paracytic stomata and endothecium layers of anthers indicating each ingredient of the formulation. HPTLC showed 13, 13 and 16 bands each under short UV, long UV and white light post derivatisation respectively in ethanolic extract of the formulation. Physico-chemical standards like loss on drying at 105º (10.39 %), total ash (4.1 %), acid-insoluble ash (0.90 %), ethanol- soluble extractive (10 %), water-soluble extractive (8.77 %) and pH of 10% aqueous solution (4.58) were recorded. The presence of cinnamaldehyde and 1-eicosene was confirmed by GC-MS and NMR studies. Conclusion: a monograph on quality standards for CC has been proposed which would serve as a document to control the quality of this polyherbal formulation.

Key Words: HPTLC, GC-MS, NMR, Polyherbal drugs, Standardisation, Substitute drugs, Tastelessness, Quality control.

Introduction

Herbal medicines are popular over synthetic drugs because of the efficacy, lesser side effects and affordability. According to EMEA (European Agency for the Evaluation of Medicinal products), the term "herbal drugs" denotes plants or plant parts that have been converted into phytopharmaceuticals by means of simple processes involving harvesting, drying, and storage (1). In India, herbal medicines are mainly based on the Ayurvedic system. The World Health Organization estimates that 80% of the word's inhabitants still rely mainly on traditional medicines for their health care (2). One of the major problems hindering the acceptance of herbal medicines is the lack of standard quality control profile documentation. The task of standardisation is really challenging due to variation in composition profile of single herbs and formulations made up of multiple herbs. Due to the complex nature and inherent variability of the constituents of plant-based drugs, it is difficult to establish quality control parameters for herbal drugs (3). The property of herbal medicine depends mainly upon the composition of chemical phyto-constituents in their extracted final product. Owing to the variability and complexity of chemical constituents present in herbal plant-based drugs there is a current need to develop a method for establishing quality control parameters for Ayurvedic formulations (4). Identification, isolation, purification and characterization of active ingredients in crude extracts from herbal plants is now possible relatively easily because of the development and implementation of high resolution separating analytical techniques like RP-HPLC (5).

To make the task still more challenging, in most of the cases, preparations are either with plants of mistaken identity or deliberate substitution due to the availability of cheaper substitutes. Thus standardization of the herbal formulations is an essential factor in order

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to assess the quality, purity, safety and efficacy of drugs (6).

Ancient Ayurvedic classical text *Sarangdhar Samhita* dating to 13th century A.E. explained the concept of polyherbal combinations to achieve greater therapeutic efficacy compared to single-drug therapy. Combination of the multiple herbs based on its Ayurvedic properties, better therapeutic effects are rendered with lesser toxicity (7). It is known that *Ayurvedic* herbals are prepared in a number of dosage forms, in which mostly all of them are polyherbal formulations (8). Another ancient Ayurvedic classical text *Charaka Samhita* (dates of composition are uncertain) described that *Ayurvedic* medicines have adverse effects when prepared or used inappropriately (9), which emphasizes the correct standardization of the formulations before administering to the patients.

Caturjata Curna (CC) is an important Ayurvedic polyherbal formulation used for the treatment of diseases like tastelessness, diseases due to vitiated *Kapha*, poison and discoloration mentioned by Acharya Sharngdhara during 13th Century A.D. (7,10). The ingredients of CC are *Cinnamomum verum* J.Presl stem bark, *Elettaria cardamomum* (L.) Maton seed, *Cinnamomum tamala* (Buch.-Ham.)T.Nees & Eberm. leaf and *Mesua ferrea* L. stamens at equal proportions. In the current study, CC prepared by standard method of preparation of *curṇa* detailed in Ayurvedic Formulary of India (10) was attempted for pharmacopoeial testing with reference to macro-microscopic, physico-chemical, high- performance thin layer chromatography (HPTLC)and spectroscopic parameters to obtain monographic data on its quality standards.

Material and Methods

Collection and identification of plant samples

Dry plant materials as per formula composition were collected from the raw drug section of SDM Ayurveda Pharmacy, Udupi. The plant materials were authenticated by macro-microscopy by Dr KN Sunil Kumar, Senior Research Officer, Department of Pharmacognosy, SDM Centre for Research in Ayurveda and Allied Sciences, Udupi, and voucher specimens of the raw drugs (No. SDM/UGC-MRP/CC/01-05) have been deposited in the crude drug museum (Figure 1).

Preparation of *Caturjata curna*

All the ingredients were washed properly to have no microbial load (11). The washed and dried raw drugs (Figure 1) of pharmacopoeial quality were finely powdered. The individual raw drug powders were passed separately through sieve number 44 followed by 85. Each ingredient was weighed separately and mixed together in the proportion specified (1 part each) and passed through sieve number 44 to obtain a homogenous blend and packed in an air-tight container (12). One kg of the formulation was prepared at the laboratory using standardized ingredients. Apart from using *Mesua ferrea* as the true ingredient, another set of the formulation was similarly prepared using *Mammea suriga* (Buch.-Ham. ex Roxb.) Kosterm, the substitute drug, for comparison.

Ethanol soluble extractive

Accurately 4 g of the sample was weighed in a glass flask with a stopper. 100 ml of ethanol (90%) was added. The flask was allowed to stand for 18 hours shaking occasionally every 6 hours. The content was filtered rapidly taking care not to lose any solvent. Twenty-five ml of the filtrate was pipetted out to a pre-weighed 100 ml beaker. It was evaporated to dryness on a water bath. It was kept in hot air oven at 105^{0}C for 6 hours, cooled in a desiccator for 30 minutes and weighed. The percentage of ethanol extractable matter of the sample was calculated. The experiment was repeated twice, and the average value was taken.

Figure 1. Macroscopy of ingredients/ substitutes of *Caturjata curna*

Water-soluble extractive

It was performed just like the procedure of ethanol soluble extractive but using distilled water as solvent.

Microscopy and physico-chemical standardisation

Powder microscopy of formulations and physico-chemical tests like total ash, water-soluble ash, acid insoluble ash, water and ethanol-soluble extract, loss on drying at 105°C and pH were performed following standard pharmacopoeial procedures (13).

Powder microscopy

A pinch of CC was mounted on a microscopic slide with a drop of glycerin-water. Characters were observed using Zeiss trinocular microscope attached with Zeiss AxioCam Erc5s digital camera under bright field light. Photomicrographs of diagnostic characters were captured and documented. Magnifications of the figures were indicated by the scale-bars.

Loss on drying at 105^{0}C

Ten grams of sample was placed in a tared evaporating dish. It was dried at 105˚C for 5 hours in a hot air oven and weighed. The drying was continued until the difference between two successive weights was not more than 0.01% after cooling in a desiccator. The percentage of moisture was calculated with reference to the weight of the sample.

Total Ash

Two grams of sample was incinerated in a tared platinum crucible at temperature not exceeding 450˚C until carbon free ash is obtained. Percentage of ash was calculated with reference to weight of the sample.

Acid insoluble Ash

To the crucible containing total ash, 25ml of dilute HCl was added and boiled. The insoluble matter was collected on ash-less filter paper and it was washed with hot water until the filtrate is neutral. The filter paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate, and ignited to constant weight. The residue was allowed to cool in a suitable desiccator for 30 mins and weighed without delay. The content of acid-insoluble ash with reference to the air-dried drug was calculated.

Determination of pH

One gram of sample was taken; 10 ml of distilled water was added, stirred well, and filtered. The filtrate was used for the experiment. The pH 4 solution was first introduced and the pH was adjusted by using the knob to 4.02 for room temperature 30°C. The pH 7 solution was introduced and the pH meter adjusted to 7 by using the knob. Introduced the pH 9.2 solution and checked the pH reading without adjusting the knob. Then the sample solution was introduced and reading was noted. Repeated the test four times and the average reading was taken as a result.

Qualitative HPTLC fingerprinting

Sample preparation

One gram of each of the ingredients was extracted with 10 ml of chloroform and filtered. The filtrates were concentrated separately and made up to 10 ml in a standard flask individually.

Caturjata curna with *Mesua ferrea* or *Mammea suriga* 5 g were extracted with 150 ml of chloroform using Soxhlet apparatus. The filtrate was concentrated and made up to 10 ml in a standard flask.

Mobile phase Toluene: ethyl acetate: formic acid (4.5: 0.5: 0.1) gave optimum separation. A comparative fingerprint of CC prepared using substituent *Mammea suriga* was developed using toluene: ethyl acetate (5: 1).

Application, development and densitometry:

Two concentrations such as 4 and 8 µl of extract of CC were applied on aluminium plate pre-coated with silica gel 60 F_{254} of 0.2 mm thickness (Merck, Germany) using LINOMAT 5 applicator (CAMAG). The plates were developed in a CAMAG glass twin trough chamber previously saturated with the mobile phase. The plate was dipped in vanillin-sulphuric acid (VS), and heated at 105 °C till the spots appeared (14, 15). The developed plates were visualized in CAMAG visualizing chamber and scanned in CAMAG SCanner 4 under 254 nm, 366 nm and at 620 nm post-derivatisation with vanillin-sulphuric acid spray reagent with the help of CAMAG Win CATS software. R_f values and densitograms were recorded.

NMR and GC-MS Analyses

Two grams of CC were soaked with 25 mL of dichloromethane for 24 hr. Then the mixture was filtered through Whatman filter paper no.1 and the filtrate was submitted for GC-MS. The filtrate was evaporated to dryness and it was employed for NMR analysis

GC-MS

GC-MS analysis of the extract of CC was performed using an Agilent 7890B GC comprising an auto-sampler coupled with an Agilent 7000G triple quadrupole GC MS system equipped with an Agilent HP-5MS (5% diphenyl / 95% dimethyl polysiloxane) fused a capillary column (30 m × 0.25 µm ID × 0.25 µm df). For GC separation, an injection volume of 2 µl was employed (split ratio of 20:1). The injector temperature was maintained at 280 °C, the oven temperature was programmed from 70 °C, with an increase of 15 °C/min to 150°C for 3 minutes, then increased by 20 °C/min to 270 °C, ending with a 15 min isothermal at 270 °C. For detection, an electron ionization system was operated in electron impact mode. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min and the. Mass spectra were taken at 70 eV; a scan interval of 0.5 s was used and fragments from 25 to 550 Da were identified. The solvent delay was 0 to 3 min and the run time was 29 min. Agilent Mass Hunter workstation software was used for data acquisition and analysis.

NMR analysis

The ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra for the dried extract of the formulation were recorded in $CDCl_3$ solution on BrukeAvance IV 500 spectrometer at 500.13 (^{1}H) and 125.35 (^{13}C) MHz respectively. Topspin 3.5 software was used for data acquisition and analysis.

Results

Microscopic features of powder of each herb

CC is a brown coloured fine powder with a characteristic odour and aromatic taste. Under the microscope it shows parenchyma with tannin and volatile oil, horse-shoe shaped stone cells, narrow lumened thick-walled tapering ended fibres; cells of testa with reddish-brown contents, irregularly walled perisperm cells with volatile oil droplets; unicellular conical covering trichomes with attached basal cells, epidermis in surface view with paracytic stomata; trichome with pointed and blunt tip, polygonal beaded epidermal cells of anther, endothecium layers of anthers. Incorporation of substitute drug *M. suriga* shows the presence of epidermis of petal with striated cuticle and pollen grains with warty exine (Figure 2).

Physico-chemical constants of each raw drugs of *Caturjata curna* and *Caturjata curna* containing *Mesua ferrea* or *Mammea suriga*

Loss on drying indicates the amount of water present in the sample; foreign matter is the presence of materials other than the part to be used; total ash is to find some of the physiological and non-physiological salts; to find out the silica or sand, acid insoluble ash is done; water-soluble ash is the water-soluble part of total ash indicating the inorganic content; to find out the percentage of active constituents, ethanol and water-soluble extractives are done. These physico-chemical values of ingredients (Table 1) indicate chemical standards of raw drugs used in the present study. In the formulation using *Mammea suriga* as *Nagakesara* minor difference in a few of physico-chemical constants such as total ash (+1.49), ethanol-soluble extractive (-2.85), and water-soluble extractive (+0.98) were observed (Table 2).

Table 1. Physico-chemical constants of raw materials used for the preparation of *Caturjata Curna*

Name of the ingredients	Results expressed as % w/w (n=3)				
	LOD	TA	AIA	ESE	WSE
Cinnamomum verum	9.18 ± 0	11.36 ± 0.018	0.69 ± 0.064	7.36 ± 0.85	7.48 ± 0.060
Elāttaria cardamomum	20.49 ± 0.091	6.89 ± 0.008	2.78 ± 0.168	3.99 ± 0.006	10.25 ± 0.99
Cinnamomum tamala	16.99 ± 0.687	3.38 ± 0.117	0.42 ± 0.2	8.95 ± 0.019	14.99 ± 0.098
Mesua ferrea	11.66 ± 0.039	4.22 ± 0.069	1.88 ± 0.088	16.25 ± 0.298	5.95 ± 0.054
Mammea suriga	12.66 ± 0.006	8.85 ± 0.088	2.89 ± 0.275	7.84 ± 0.171	11.99 ± 0.199

LOD – Loss on drying at 105º; TA - Total ash; AIA – Acid Insoluble ash; WSE – Water soluble extractive; ESE – Ethanol soluble extractive;
* Bark with outer cork was used for the study

Figure 2. Microscopic features of powder of each ingredient of *Caturjata Curna*

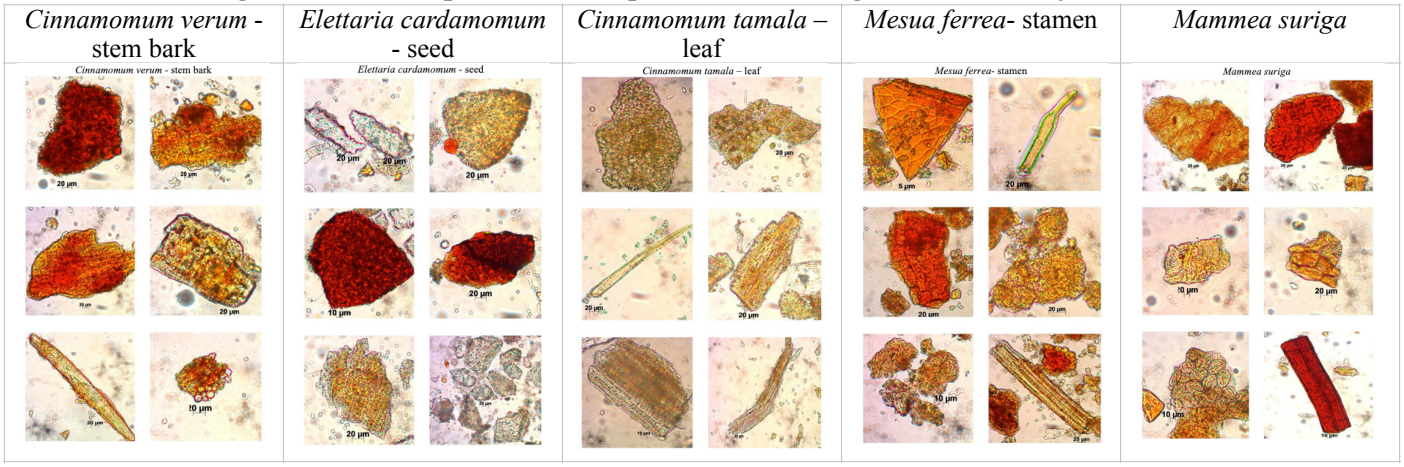


Table 2. Difference in physico chemical constants of *Caturjata cūrṇa* prepared using *Mesua ferrea* and *Mammea suriga*

Parameters (%w/w)	*Caturjata curna* with *M. ferrea*	*Caturjata curna* with *M. suriga*
LOD	10.39	10.60
TA	4.1	5.59
AIA	0.90	1.20
ESE	10.0	7.15
WSE	8.77	9.75
***p*H of 10% aqueous Solution**	4.58	4.42

LOD – Loss on drying at 105º; TA - Total ash; AIA – Acid Insoluble ash; WSE – Water soluble extractive; ESE Ethanol soluble extractive; * Bark with outer cork was used for the study

TLC photo of chloroform extract of *Caturjata Curna* and its ingredients

Under short UV, *Cinnamomum verum* - stem bark, *Elettaria cardamomum* - seed, *Cinnamomum tamala* - leaf, *Mesua ferrea* - stamen and CC showed 7, 2, 11, 12 and 13 bands (all green) respectively. Thirteen bands occurred in CC, of them 11 corresponded in R_f to bands from ingredients. Bands with R_f 0.08 was detected in *Mesua ferrea*; 0.14 in *Cinnamomum verum* and *Mesua ferrea*; 0.23 in *Cinnamomum verum* and *Mesua ferrea*; 0.29 was detected in all the ingredients except in *Elettaria cardamomum*; 0.37 and 0.46 were detected in *Cinnamomum verum* and *Mesua ferrea* respectively; 0.50 was observed in all the ingredients except in *Mesua ferrea*; 0.63 was found in all the ingredients; 0.70 and 0.77 were detected in *Cinnamomum verum*; and 0.97 occurred in all the ingredients except in *Elettaria cardamomum* (Figure 3.1).

Under long UV, *Cinnamomum verum*, *Elettaria cardamomum*, *Cinnamomum tamala*, *Mesua ferrea* and

CC showed 7, 3, 12, 7 and 13 bands (of different fluorescent colours) respectively. Out of 13 bands in CC, 11 corresponded to ingredients. Bands with R_f 0.06 was found in all the ingredients except in *Elettaria cardamomum*; 0.11 was from *Cinnamomum verum* and 0.28 was from *Cinnamomum tamala*; 0.36, 0.38. 0.49 and 0.53 were from *Cinnamomum verum*; 0.46 and 0.79 occurred commonly in all the ingredients; 0.60 was observed in all the ingredients except in *Elettaria cardamomum*; 0.98 was detected in *Cinnamomum tamala* and *Mesua ferrea* (Figure 3.2).

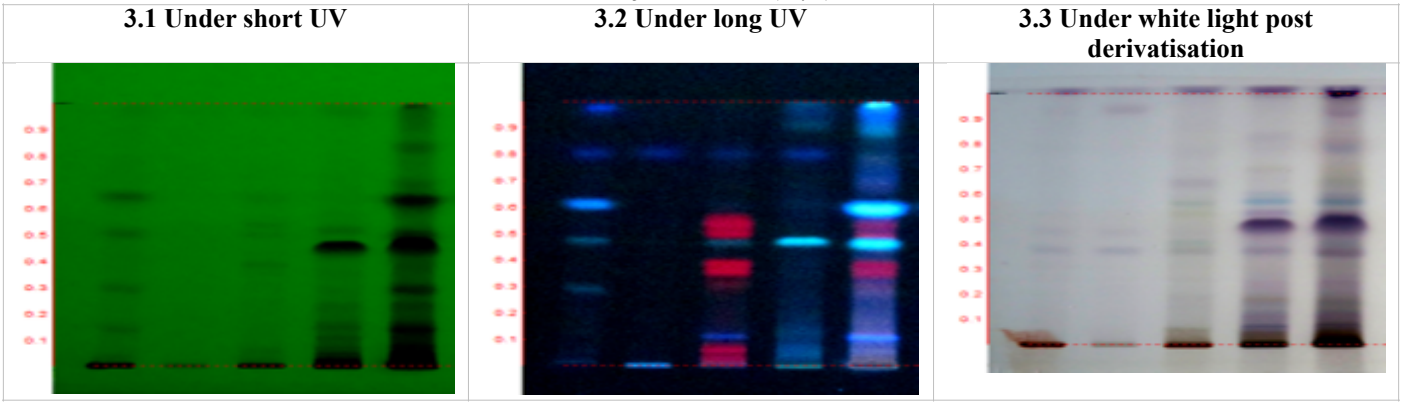
After derivatisation with VS, *Cinnamomum verum*, *Elettaria cardamomum*, *Cinnamomum verum*, *Mesua ferrea* and CC showed 5, 3, 12, 15 and 16 bands (of different colours) respectively. Except for band with R_f 0.92, all other 15 spots corresponded to ingredients. Spots with R_f 0.04, 0.11, 0.17, 0.26, 0.49, 0.60 and 0.83 were from in *Mesua ferrea*; 0.07, 0.24, 0.53, 0.57, 0.65 and 0.78 were from in *Cinnamomum verum* and *Mesua ferrea*; 0.37 (Purple) was observed common in all the ingredients; 0.41 was occurred in *Cinnamomum verum*. Most of the spots occurred from *Naga kesara* (*Mesua ferrea*) (Figure 3.3).

HPTLC fingerprinting was attempted for the detection of possible substitution of *M. ferrea* in CC with *Mammea suriga* with a qualitative fingerprint.

TLC photo documentation of *Caturjata Curna* formulation with *Mesua ferrea* and *Mammea suriga*

Under short UV, bands with R_f0.75 (dark green), 0.82 (green) and 0.88 (green) in CC corresponded with *M. suriga,* which is not found in CC with *M. ferrea.* In densitogram at 254 nm *M. ferrea* shows diagnostic peak at R_f0.40, while *M. suriga* shows twin peaks at R_f0.36 and 0.40. A unique peak was observed at R_f 0.85 (46.58 % area) in *M. suriga* which can be used as a diagnostic peak to tap authentic ingredient. The chromatogram can be an effective tool to identify the CC prepared using authentic and substitute drugs (Figure 3 & 4).

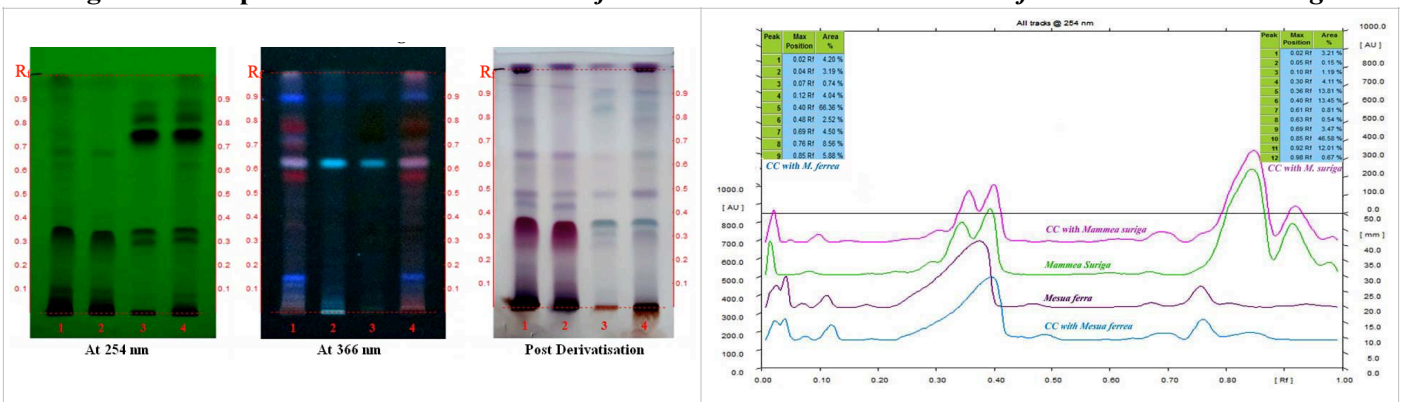
Figure 3. TLC photo documentation of chloroform extract of ingredients with *Caturjata curna* (4 µl)



Solvent system: Toluene: ethyl acetate: formic acid (4.5: 0.5: 0.1)

1 - *Tvak* (*Cinnamomum verum*); 2 - *Ela* (*Elettaria cardamomum*); 3 - *Tvakpatra* (*Cinnamomum tamala*); 4 - *Nagakesara* (*Mesua ferrea*); 5 – *Caturjata curna*.

Figure 4. TLC photo documentation of *Caturjata curna* formulation with *Mesua ferrea* and *Mammea suriga*



Track 1 – *Caturjata curna* with *Mesua ferrea* – 16 µl; 2 – *Mesua ferrea* – 4 µl; 3 – *Mammea suriga* – 4 µl; 4 – *Caturjata curna* with *Mammea suriga*– 16 µl

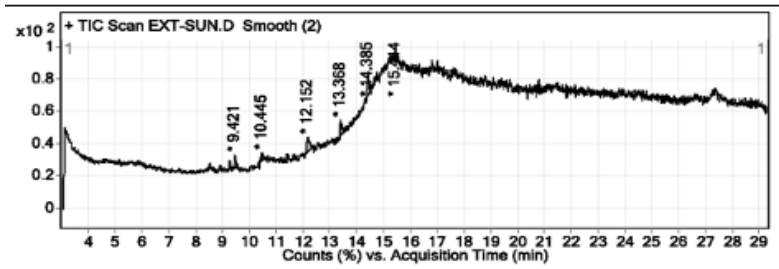
Solvent system: Toluene: ethyl acetate (5: 1)

Identification of marker compound by GC-MS and NMR Studies

GC-MS of chloroform extract yielded 7 peaks from CC with RT and area % details as mentioned in Figure 3-4. Two compounds 1-eicosene and *trans*-cinnamaldehyde have been identified NMR studies and confirmed by GC-MS analysis (Figure 5 & 6). In ^{1}H nmr spectrum (Figure 7b) of the CC extract showed

several signals. A doublet of doublet (J = 16 and 7 Hz) and doublet (J = 7 Hz) and 9.7 and 6.7 ppm respectively was due to the presence of *trans*-cinnamaldehyde. The ^{13}C nmr signals of *trans*-cinnamaldehyde were found at 131, 134 152 and 191 ppm (Figure 7a). The presence of 1-eicosene was identified by the ^{13}C nmr signals at 139 and 114 ppm which is due olefinic carbons. The ^{1}H nmr signal due to olefenic protons of 1-eicosene appeared as multiplets around 4.9 and 5.8 ppm. The signals due to aliphatic protons and carbons appeared in the region of 0.8-2.8 ppm and 11-35 ppm respectively. Further, cinnamaldihyde is the major compound of one of the ingredient *Cinnamomum verum* bark (16) and it is also present in *C. tamala* leaves (17). It is suggested that the identified marker is abundant in the formulation and hence can be used as analytical marker for quality control of CC.

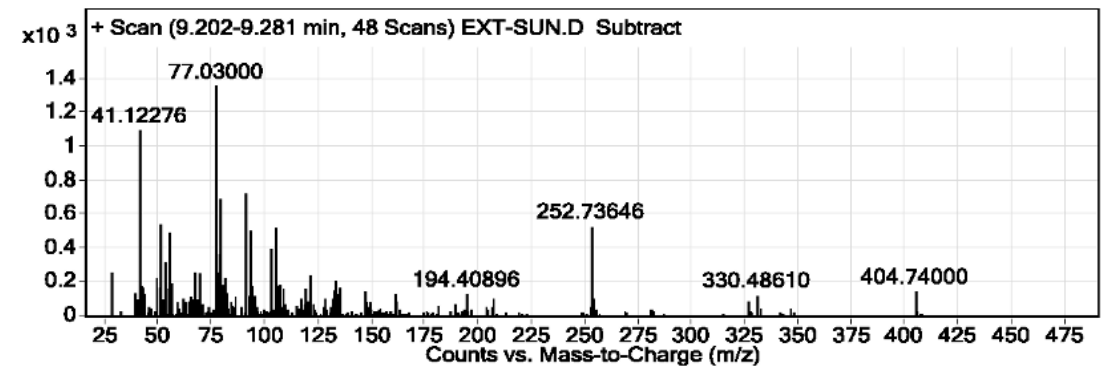
Figure 5. GLC of *Caturjata curna* and details of compounds eluted



Integration Peak List

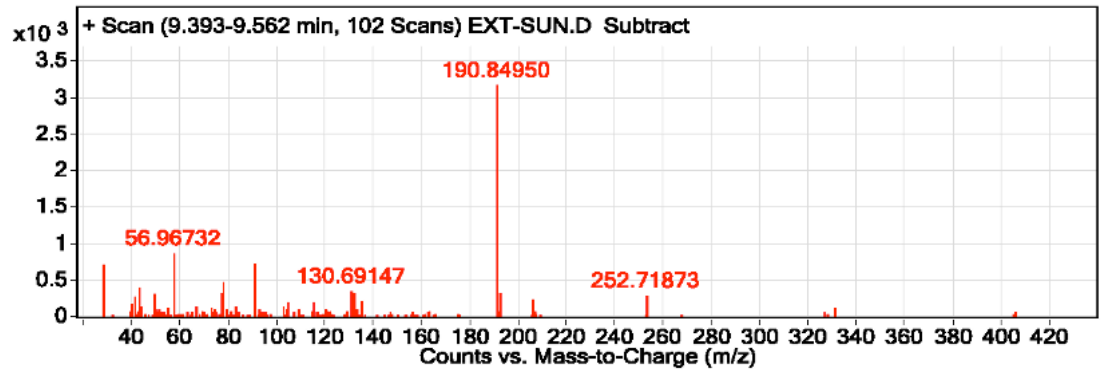
Start	RT	End	Height	Area	Area %
9.147	9.24	9.302	15813	37153	16.25
9.386	9.421	9.585	24971	116608	50.99
10.311	10.445	10.624	16503	93743	40.99
12.023	12.152	12.398	28506	228696	100
13.309	13.368	13.51	29807	147388	64.45
14.335	14.385	14.522	40011	175105	76.57
15.367	15.414	15.464	19005	45681	19.97

Figure 6. Mass spectra of compounds eluted by GLC of *Caturjata cūrṇa*



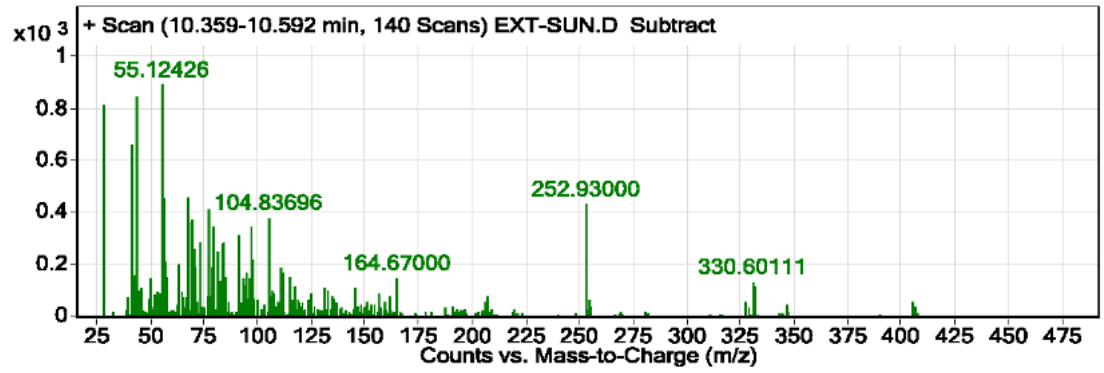
Peak List

m/z	Abund
41.12276	1099.88
50.93151	547.83
55.07	493.6
77.03	1359.85
78.92501	689.52
90.9	724.8
92.76	502.85
102.85	396.02
104.77044	518.33
252.73646	532.68



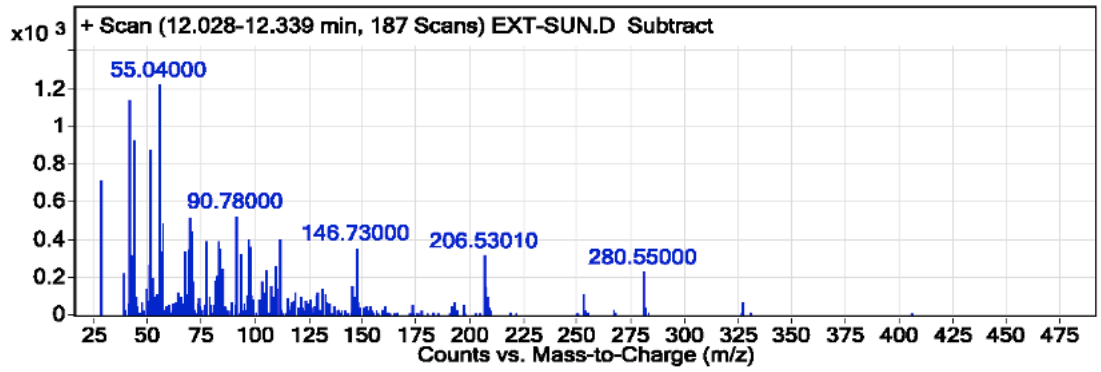
Peak List

m/z	Abund
28.11525	725.19
43.1118	415.07
56.96732	882.89
76.94	346.47
77.86	488.07
90.84	739.1
130.69147	363.27
190.8495	3185.11
191.01	687.94
191.87157	349.99



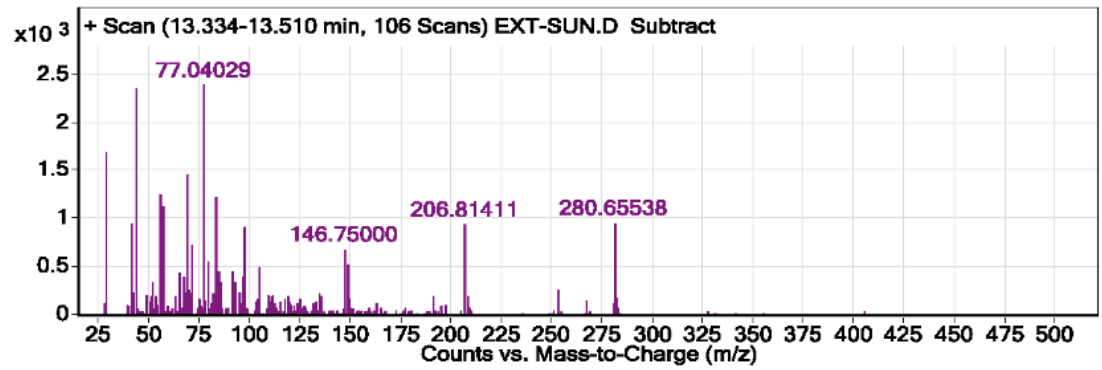
Peak List

m/z	Abund
28	811.54
41.14369	664.46
43.11118	846.35
55.12426	894.96
55.96	456.49
66.85	462.26
69.05	376.68
76.95	412.99
104.83696	382.42
252.93	434.78



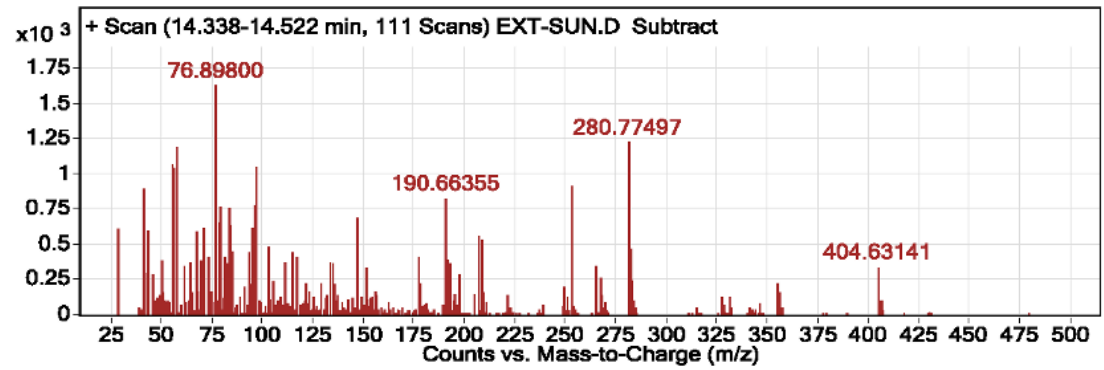
Peak List

m/z	Abund
28.2	721.42
41.21436	1142.35
43.14554	934.45
51.06204	882.65
55.04	1227.97
56.88	484.79
57.00673	493.23
69.03002	522.77
70.05771	450.74
90.78	530



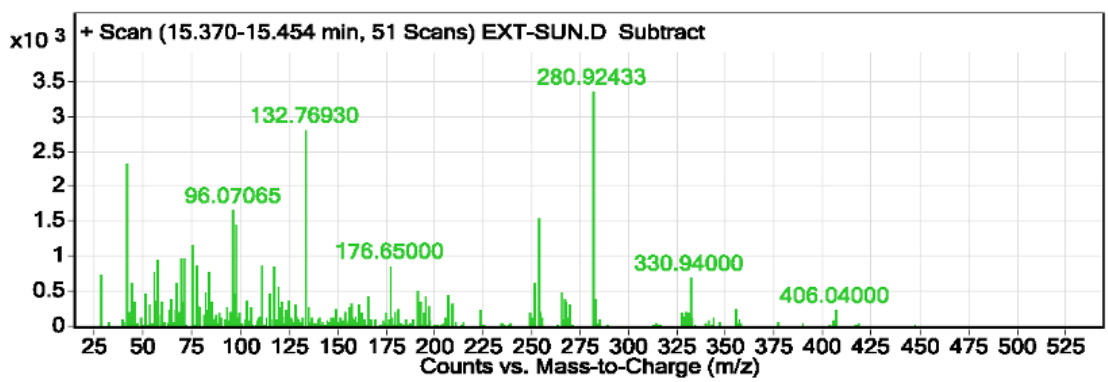
Peak List

m/z	Abund
28.2	1690.97
41.14185	957.26
43.23773	2360.62
55.04656	1264.1
57.04	1139.02
68.97	1469.98
77.04029	2398.34
83.03432	1240.54
206.81411	945.4
280.65538	963.59



Peak List

m/z	Abund
41.14578	903.46
55.13157	1080.66
56.05	1051.39
57.04374	1192.19
76.898	1637.21
96.13	784.29
96.94804	1056.62
190.66355	835.11
252.6	919.78
280.77497	1233.29



Peak List

m/z	Abund
41.22153	2338.29
57.11625	967.31
69.06147	991.54
70.89	985.82
74.95	1171.53
96.07065	1661.69
96.95	1473.38
132.7693	2811.02
252.85926	1545.76
280.92433	3359.34

Discussion

Adulteration/substitution is the major drawback in herbal products. In CC, *Mammea suriga* has been used as a substitute/adulterant. Adulteration is the practice of substituting the original crude drug partially or fully with other substances which is either free from or inferior in therapeutic and chemical properties or addition of low grade or spoiled or spurious drugs or entirely different drug similar to that of original drug substituted with an intention of enhancement of profits (18).

M. ferrea is morphologically and chemically completely different from *M. suriga*, the former being stamens it is coppery or golden brown colour; fragrant and possess astringent taste (19). Major chemical constituents of *Mesua ferrea* are mesuaferrol (20) and mesuanic acid (21, 22). *M. suriga* is a flower bud reddish-brown in colour, odour and taste faint and characteristic. Majorly it contains proanthocyanidins (23) and vitexin (24). The pharmacological actions of the two sources of *Mesua ferrea* would be different as they are chemically different.

There are very few attempts to identify marker compounds from extracts using MS or NMR. In some

studies, NMR has been used just as a fingerprinting tool. This study could identify a major compound of the formulation by employing GC-MS and NMR spectroscopy. Every AYUSH formulation must be studied to obtain these kinds of fingerprints along with conventional chromatography to raise the standards of the monograph on quality standards by identifying a compound present in it. Very few (25, 26) such monographic documents on standards of polyherbal formulations are published with high standards. There is a need for proposing reproducible data on standards of such formulations for worldwide acceptance of *Ayurvedic* polyherbal medicines.

Despite the establishment of the Drugs and Cosmetic Act to control the manufacture and quality control the regulation of Ayurvedic herbal preparation manufacturing is somewhat less stringent in India where most of the Ayurvedic polyherbal formulations are manufactured and exported. Since toxicity studies and clinical trials on herbal formulations are not mandatory for patent application and manufacturing license application (27), standardisation is not up to the mark. Standardisation of polyherbal formulations has been a challenging job for the regulators like Pharmacopoeial Commission of Indian Medicine and Homeopathy New Delhi as there are several formulations popular in *Ayurvedic* practice while very few have been standardised and Pharmacopoeial monographs published so far.

Figure 7 a.$^{13}C\{^1H\}$ NMR Spectra of *Caturjata cūrṇa* extract

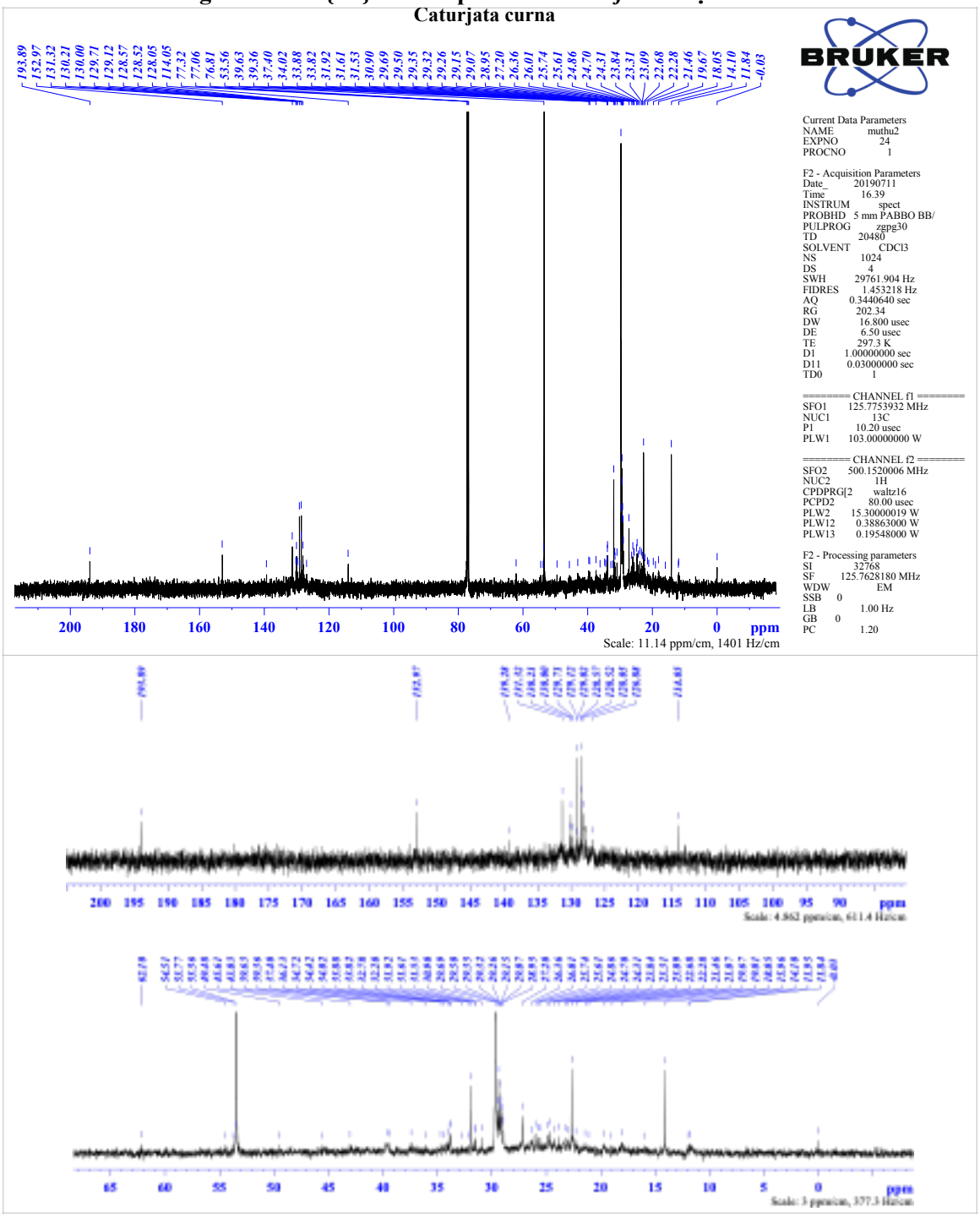
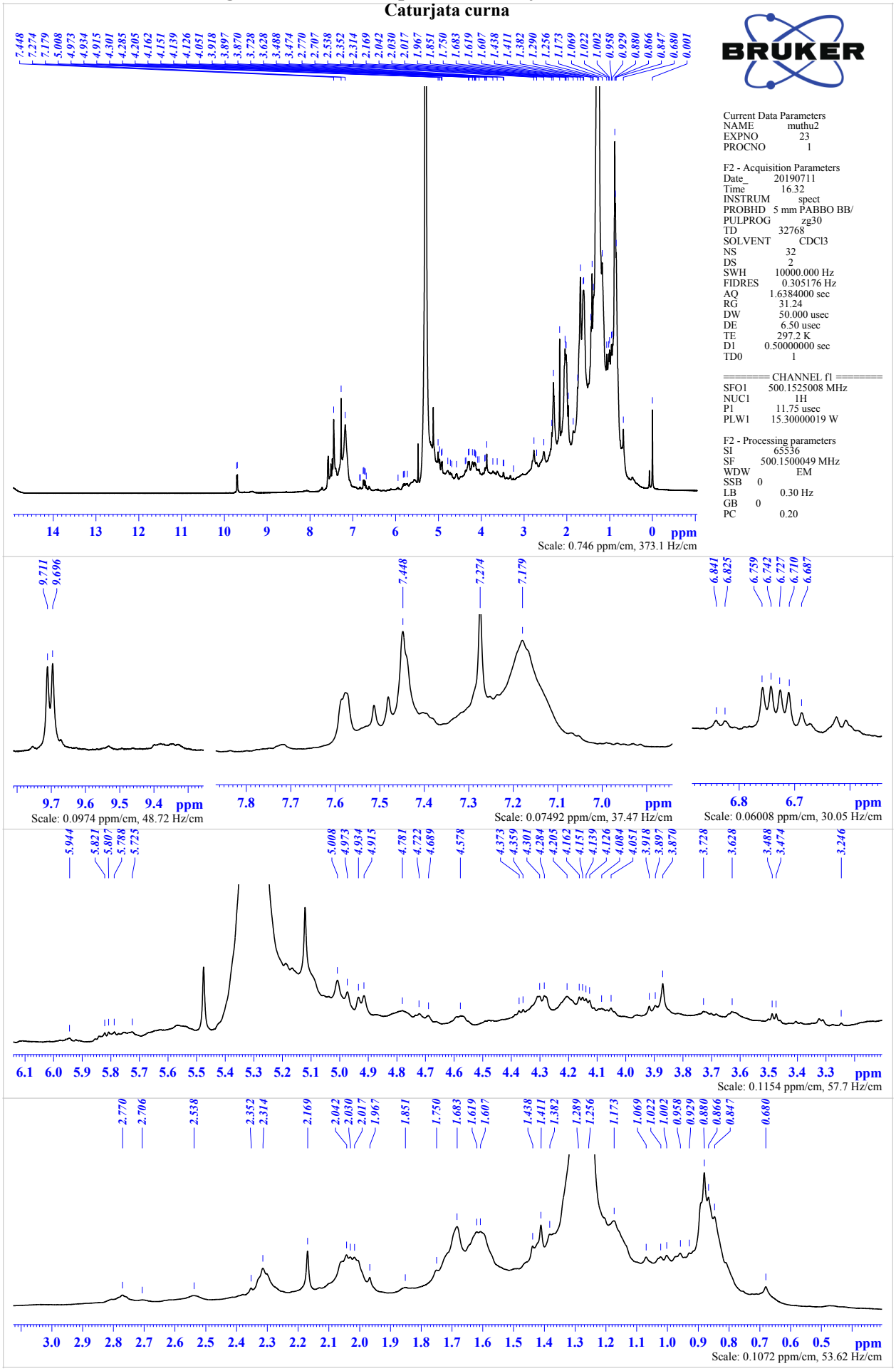


Figure 7 b.^{1}H NMR Spectra of *Caturjata curna* extract



Conclusion

From the result obtained the monographic data on quality standards for CC can be laid as follows:

Definition

Caturjata curna is a fine powder preparation made with the ingredients in the formulation composition given below.

Formulation composition

Cinnamomum zeylanicum	Stem Bark	1 part
Elettaria cardamomum	Seed	1 part
Cinnamomum tamala	Leaf	1 part
Mesua ferrea	Stamen	1 part

Methods of preparation

Take all ingredients of pharmacopoeial quality. Wash and dry all the ingredients. Powder all the ingredients and should completely pass through sieve number 44 and not less than 50 percent through sieve number 85. Weigh each ingredient separately and mix together. Pass the *cūrṇa* through sieve number 44 to obtain a homogenous blend and pack in an air-tight container.

Description

Caturjata curna is brown coloured fine powder with characteristic odour and aromatic taste.

Identification

Microscopy

Show sparenchyma with tannin and volatile oil, horse shoe shaped stone cells, narrow lumened thick-walled tapering ended fibres (*Cinnamomum verum*); cells of testa with reddish brown contents, irregularly walled perisperm cells with volatile oil droplets (*Ela*); unicellular conical covering trichomes with attached basal cells, epidermis in surface view with paracytic stomata (*Cinnamomum verum*); trichome with pointed and blunt tip, polygonal beaded epidermal cells of anther, endothecium layers of anthers (*Mesua ferrea*).

Thin Layer Chromatography

Under short UV 13 bands occurred in CC, of them 11 corresponded in R_f to bands from ingredients. Under long UV CC showed 13 bands out of which 11 corresponded to ingredients. After derivatisation with VS CC showed 16 bands. Except for band with R_f 0.92, all other 15 spots corresponded to ingredients. HPTLC fingerprinting was attempted for the detection of possible substitution of *M. ferrea* in CC with *Mammea suriga* with a qualitative fingerprint.

Physico-chemical standards

- ***Loss on drying at 105°*:** Not more than 10.39 per cent.
- ***Total ash*:** Not more than 4.1 per cent.
- ***Acid-insoluble ash*:** Not more than 0.90 per cent.
- ***Ethanol soluble extractive*:** Not less than 10 per cent.
- ***Water - soluble extractive*:** Not less than 8.77 per cent.
- ***pH of 10% aqueous solution*:** Not more than 4.58.

Spectroscopic studies

Presence of cinnamaldehyde and 1-eicosene were confirmed by GC-MS and NMR studies.

Other Requirements

***Microbial limits, aflatoxin, heavy metals, pesticide residue, and radioactive contaminants*:** Nil/Within limits of WHO.

Adulterants and substitutes

Mammea suriga is used as *Mesua ferrea*. Incorporation of substitute drug *M. suriga* shows presence of epidermis of petal with striated cuticle and pollen grains with warty exine. *M. suriga* renders high total ash (+1.49), low ethanol soluble extractive (-2.85) and high water soluble extractive (+0.98). In densitogram at 254 nm *M. suriga* shows twin peaks at R_f 0.36 and 0.40 and a unique peak at R_f 0.85 (46.58 % area).

Storage

Store in a cool, dry place in tightly closed containers, protect from light and moisture.

Therapeutic uses

Tastelessness; diseases due to visitation of *Kapha*; poisonous bites; discoloration of skin.

Dose: 2 to 4 g.

Anupana: Honey, ghee, water.

With the data obtained in the current investigation a monograph on quality standards for *Caturjata curna* of Ayurvedic Formulary of India has been proposed. This type of research is essential for deriving consistent standards with rapid authentication fingerprints as a contributing to herbal Pharmacopoeias.

Declaration of Interest

Authors declare that there are no conflicts of interest.

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