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RESEARCH ARTICLE

UFLC-MS Method Development of Vasicinone, Pellitorine, 6-Gingerol, Costunolide, Dehydrocostuslactone, Apigenin, and Validation of Piperine, Biflorin in Polyherbal Formulations

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Abstract

Standardisation of polyherbal formulations is necessary for the quality, safety, quantity, and efficacy of botanicals in marketed and newly established formulations. The Ministry of Ayush, Govt of India, recommended using polyherbal formulations to treat COVID-19, i.e., *Kabusura kudineer*, *Nilavembu kudineer*, etc., *Kabusura kudineer* is a *Siddha*-based formulation. It prevents and treats COVID-19 due to some botanicals in *Kabusura kudineer*, which have proven anti-inflammatory, anti-viral, and immunomodulatory effects. The current work focuses on establishing a standard protocol for the *Kabusura kudineer* marketed, a novel dosage form called *Hydaljss*08, and in plant species, mainly present in both formulations. Both formulations contain some similar crude drugs and their active constituents. They are *Zingiber officinale* rhizome, *Syzygium aromaticum* flower buds, *Adhatoda vasica* leaves, *Anacyclus pyrethrum* roots, *Saussurea lappa* roots, *Piper longum* fruits, *Clerodendrum serratum* roots, *Coleus amboinicus* roots, contain active phytopharmaceuticals are 6-gingerol, biflorin, vasicinone, pellitorine, costunolide, dehydrocostuslactone, piperine, and apigenin. Existing liquid chromatography methods were reported for individual above active compounds, but not in these formulations and combined dosage forms. Working UFLC methods have not been reported individually nor combined for the Biflorin. The current study aims to develop UFLC methods for 6-gingerol, biflorin, vasicinone, pellitorine, costunolide, dehydrocostuslactone, piperine, and apigenin in polyherbal formulations *Kabusura kudineer* marketed, *Hydaljss*08 and in isolated, fractions, extract of plant species present in both dosage forms. The preliminary identification of the phytopharmaceuticals in the polyherbal formulations, isolated fractions, and extract of plant species was done by TLC and IR spectrum. The developed liquid chromatography method was novel, simple, linear, and rapid for estimating 6-gingerol, biflorin, vasicinone, pellitorine, costunolide, dehydrocostuslactone, piperine, apigenin in a plant species, and Ayush-based formulations.

Keywords: *Hydaljss*08 Polyherbal Formulations, *Kabusura Kudineer Churna* Marketed, Marker Standards, Ultrafast Liquid Chromatography

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Abbreviations: BBE: Berberine; CET: Central European Time; Con: Concentration in(µg/ml); FT-IR: Fourier transform infrared Spectroscopy; DNA: Deoxyribonucleic acid, *Hydaljss*08: Developed Hydroalcohalic semi-solid fermented polyherbal formulation; HPLC: High-performance liquid chromatography; ICH: International Council for Harmonisation; KKR: Kabusura *kudineer* churna marketed; LOD: Limit of detection; LOQ: Limit of Quantification; mw: Molecular weight; RNA: Ribonucleic acid; RSD: Relative standard deviation; SARS-COV-2: Severe acute respiratory syndrome coronavirus 2; SD: Standard deviation; PDA: Photodiode Array Detector, TLC: Thin layer chromatography; UV: Ultra-violet Spectroscopy; UPLC/UFLC: Ultra-performance/ fast liquid chromatography; WHO: World Health Organization.

1. Introduction

Standardisation of polyherbal formulations is necessary for the quality, safety, quantity, and efficacy of botanicals present in the marketed and newly established polyherbal formulations. Standardisation of traditional medicine begins right from the collection of raw materials to extreme clinical applications[1]. World Health Organization (WHO) cooperates and supports health ministries in implementing systems for introducing classical phytomedicines into preliminary wellness programs, estimating immunity and potency, securing acceptable provender, and the graded control of primary and manufactured materials[2]. The Ministry of Ayush, Govt of India recommended using polyherbal formulations to cause COVID-19, i.e., *Kabusura kudineer churna*, *Nilavembu kudineer*, etc. *Kabusura kudineer churna* is a *Siddha*-based polyherbal formulation. It is used to prevent and treat COVID-19 due to some of the botanicals in *Kabusura kudineer churna*, which have proven anti-inflammatory, anti-viral, and immunomodulatory effects. The current work focuses on establishing a standard protocol for the *Kabusura kudineer* marketed and a developed novel polyherbal formulation called "*Hydaljss*08" in a plant species, mainly present in both formulations. Both formulations contain some similar crude drugs and their active constituents. They are *Z. officinale* rhizome dried (Zingiberaceae), *S. aromaticum* flower buds dried (Myrtaceae), *A. vasica* leaves dried (Acanthaceae), *A. pyrethrum* roots dried (Asteraceae), *S. lappa* roots dried (Asteraceae), *P. longum* fruits dried (Piperaceae), *C. serratum* roots dried (Lamiaceae) and *C. amboinicus* roots dried (Lamiaceae) contains active chemical compounds 6-gingerol, biflorin, vasicinone, pellitorine, costunolide, dehydrocostuslactone, piperine, and apigenin are with proved *in vitro, in vivo* effects of anti-inflammatory, anti-viral and immunomodulatory. Existing liquid chromatography methods were reported for assessing 6-gingerol, vasicinone, pellitorine, costunolide, dehydrocostuslactone, and apigenin individually, but not in these formulations and combined dosage forms. Working UFLC methods have not been reported individually, nor combined for the biflorin. The current study aims to develop liquid chromatography methods for 6-gingerol, biflorin, vasicinone, pellitorine, piperine, costunolide, dehydrocostuslactone, and apigenin in polyherbal formulations *Kabusura kudineer* marketed, *Hydaljss*08 and isolated, fractions, extract of *Z. officinale* rhizome (Zingiberaceae), *S. aromaticum* flower buds (Myrtaceae), *A. vasica* leaves (Acanthaceae), *A. pyrethrum* roots (Asteraceae), *P. longum* fruits (Piperaceae), *S. lappa* roots (Asteraceae), *C. serratum* roots (Lamiaceae) and *C. amboinicus* roots (Lamiaceae). The preliminary phytochemical screening was done for the phytopharmaceuticals in the polyherbal formulations, isolated, fractions, and extracts of plant species present in dosage forms by TLC and IR Spectrum. The developed liquid chromatography method was novel, simple, linear, and rapid for estimating 6-gingerol, biflorin, vasicinone, pellitorine, piperine, costunolide, dehydrocostuslactone, and apigenin in a plant species, and Ayush-based polyherbal formulations.

Zingiber officinale (Zingiberaceae) rhizomes are commonly called ″ginger". The plants of the Zingiberaceae family have different medicinal properties and particular aromas, and the ginger plant produces tuberous or non-tuberous rhizomes. The principal active constituents present in the rhizomes are zingiberene, zingiber-ole, gingerol, shogaol, zingerone, camphene, cineole, bisabolene, phellandrene, citral, borneol, citronellol, geraniol, 1-dehydrogingerdione, linalool, limonene, and camphene[3,4]. A new monoterpene, 10-O-b-D-glucopyranosyl-hydroxy cineole, and cyclic diarylheptanoid,1,5-epoxy-3-hydroxy-1-(3-methoxy-4,5-dihydroxy phenyl)-7-(4-hydroxyphenyl)-heptane, were isolated from the dried rhizomes of ginger[5]. zingiberene and 6-gingerol are potent immune-stimulating compounds that have been shown to improve humoral and cell-mediated immune

responses[6]. 6-Gingerol decreased Inducible Nitric Oxide Synthase (iNOS) and Tumour Necrosis Factor (TNF-α) expression by blocking protein kinase-C (PKC) and nuclear factor-κB (NF-κB) signalling pathways in lipopolysaccharide-stimulated mouse macrophages[7]. The *in vitro* anti-viral effects of 6-gingerol against Chikungunya virus (CHIK-V) infection using human Hepatocyte liver cancer cell line (HepG2)[8]. 6-Gingerol is a phytocompound from the *Z. officinale* rhizomes, a potential drug for COVID-19, and the anti-viral efficiency of 6-gingerol proved against the coronavirus disease by showing the interaction with multiple targets of COVID-19 including viral proteases, the highest binding affinity of Ribonucleic acid binding protein, and Spike proteins[9].

Syzygium aromaticum (Myrtaceae), clove flower buds with different vernacular names are *E. caryophyllus*, *C. aromaticus*, *C. silvestris, Jambosa caryophyllus,* and *Myrtus caryophyllus*. Clove buds are cultivated and indigenous to Indonesia's North Maluku Islands[10]. Active constituents of clove are eugenol, eugenin, kaempferol, rhamnetin, eugenitin, oleanolic acid, biflorin, and isobiflorin[11,12]. Biflorin promoted the production of immune stimulation in cytokines such as Interleukin-2, 6, 17, tumour necrosis factor-α, and interferons γ, inducing the immune profile toward a cytokines (Th1) response was able to stimulate mice splenocytes in albino, a laboratory-bred strain of the house mouse (Balb/c)[13]. The isolated phytocompounds eugenin, biflorin, and isobiflorin from the *clove* buds have proven anti-viral effects against the dengue viruses (DEN-V) by inhibiting the protease inhibitor[14].

Adhatoda vasica leaves (Acanthaceae) is a well-known plant drug in the classical system of medicine and a perennial shrub used to treat and manage respiratory illnesses such as asthma and bronchitis[15]. In the Indian indigenous system of medicine for over 2000 years, *vasaka* plants have been used[16]. The leaves of *vasaka* contain phytochemical compounds such as glycosides, saponins, alkaloids, tannins, flavonoids, and phenolics[17]. The plant comprises principle active are vasicol, vasicine, adhatodine, vasicinone, deoxyvasicinone, peganine, galactoside, and 9 - acetamido-3, 4 - dihydro pyrido-(3, 4-b)–indole, O–ethyl-A-D galactoside, vasakin, desmethoxyaniflorine, and 7- methoxy vasicinone respectively[18]. The leaves of the *vasaka* extract proved pepsin inhibitory enzymatic activity against the HIV-Protease[19]. *A. vasica* leaves showed potent immunostimulatory and antioxidant activity[20]. The principal active molecule responsible for the immunostimulatory effects of the *vasaka* extract has yet to be identified[21].

Anacyclus pyrethrum (Asteraceae), the Indian trade name *'akarkara'*, is a small hairy perennial herbaceous plant. The roots of the *akarkara* plant were aphrodisiac due to the presence of the bio-active compounds n-alkyl amides are deca-2E, 4E-dienoic acid iso-butyl amide (Pellitorine); deca-2E, 4E, 9-trienoic acid iso-butyl amide; deca-2E, 4E-dienoic acid 2-phenylethylamine, tetradeca-2E, 4E-dien8, 10-dioic acid iso-butyl amide (Anacycline), under-2E, 4E-dien-8, 10-dioic acid, pyracyclumines A–J, agrocybenine, 4, 6, 6-trimethyl-5,6-dihydro-2(1H)-pyridone, 3,5,5-trimethyl-1,5-dihydro-2H-pyrrole-2-one[22,23]. The essential compounds discovered in the roots of *akarkara* are inulin, pellitorine, anacyclin, phenylethylamine, polyacetylenic amides I-IV, and Sesamin[24]. *A. pyrethrum* roots hot water extracts were proven beneficial for mice *in vitro* and in *vivo* immune-stimulating effects[25]. Alkyl amides from various plants are proven to have potent immunomodulatory activity against the influenza virus and *SARS* coronavirus-2 by promoting the synthesis of tumour necrosis factor-α[26].

Saussurea lappa (Asteraceae) is indigenous to Pakistan, China[27]. *S. lappa* roots are a rich source of sesquiterpenoids, especially sesquiterpene lactones. The most critical phytochemical compounds isolated from the *S. lappa* roots, such as costunolide, dihydrocostunolide, isodihydrocostunolide, cynaropicrin, dehydrocostuslactone, lappadilactone, germacrenes, saussureal, cyclocostunolide, dihydro costunolide, saussuramines A-E, betulinic acid, betulinic acid methyl ester, mokko lactone, dihydrosantamarine, luteolin-7-O-β-D-glucoside, rutin, and apigenin-7-O-β-D-glucoside[28,29]. Costunolide and dehydrocostus lactone may have proven activity against the hepatitis B virus (HBV), and it's highly recommended that the anti-viral drugs be developed[30]. The safety and efficacy of decoction containing *Saussurea lappa* (qust), *Artemisia absinthium* (afsanteen), and *Unani* drugs were carried out in open, prospective single-arm clinical trial determined against the HBeAg-negative or positive chronic hepatitis B, hepatitis B virus (HBV), and HBsAg normalised alanine transaminase (ALT) significantly in study patients did not

show any side effects[31]. *S. lappa* roots isolated compounds dehydrocostus lactone was proven anti-allergic effects by inhibiting nuclear factor kappa B (NF-κB) to bind to the dimeric form of translationally controlled tumour protein (dTCTP)[32]. The isolated costunolide and eremanthin compounds from the *Costus speciosus* showed antioxidant properties, and the results proved the protective effects of costunolide and eremanthin from oxidative stress[33].

Coleus amboinicus roots (Lamiaceae), known as Indian borage, is a dicotyledonous plant widely used in Chinese folk medicine to treat cough, fever, sore throats, mumps, and mosquito bites. The plant is indigenous to Taiwan[34,35]. The principle active compounds present in *C. amboinicus* roots are quercetin, luteolin, eriodyctiol, caffeic acid, chrysoeriol, rosmarinic acid, gallic acid, tannic acid, apigenin, luteolin, salvigenin, quercetin, rutin, and rosmaric acid[36]. The *Batak* tribe women living in the islands of North Sumatra, Indonesia, consume the *Bangunbangun*'s leaves to increase breast milk production. The study was carried out on Apigenin in the leaves of *C. amboinicus* roots. The immunostimulatory activity was determined by measuring the levels of immunoglobulin G (IgG), immunoglobulin M (IgM), monocytes, and lysozyme in the *Bangunbangun*'s leaves[37]. The *invivo* Immunostimulatory activity was carried out for apigenin isolated from the *C. amboinicus* roots in untreated experimental autoimmune encephalomyelitis (E.A.E.) mice[38]. Apigenin therapy inhibited the synthesis of viral DNA, mRNA, and proteins without affecting the viral lifecycle, entry, and budding[39]. Apigenin is a polyphenolic compound with antioxidant effects. The study proved that apigenin therapy has antiviral activity against Enterovirus 71 (EV71) infection[40].

Clerodendrum serratum roots (Lamiaceae) are a deciduous shrub distributed in the forests of the Western ghats of India and are commonly known as *bharangi* (Sanskrit) and "*blue glory*" (English)[41]. *Bharangi* roots are bitter, acrid, anti-inflammatory, digestive, anthelmintic, antispasmodic, stimulant, and febrifuge and are helpful in inflammations, dyspepsia, colic, and flatulence[42]. The principle active components of *"blue glory"* are oleanolic acid, queretaroic acid, bauer-9-en-3-one, se-saponin A, lupeol, β-sitosterol, spinasterol, serratumin A, serratoside B, (þ)-catechin, apigenin-7-glucoside, luteoline 7–0-β-D-glucuronide, luteolin, scutellarein, apigenin, and 6-hydroxy luteolin[43]. Saponins isolated from the *Clerodendrum serratum* roots are isocahydropicenic acid[44]. Apigenin therapy inhibited the synthesis of viral DNA, mRNA, and proteins without affecting the viral lifecycle, entry, and budding[39]. Apigenin is a polyphenolic compound with antioxidant properties. Our study proved that apigenin therapy has anti-viral activity against Enterovirus 71 (EV71) infection[40]. Apigenin can inhibit (Foot mouth disease viruses) FMDV-mediated cytopathogenic effect and FMDV replication *in vitro*[45].

Piper longum dried fruits (Piperaceae), commonly known as long pepper, are widely distributed in the tropical, subtropical regions of the world and are native to South and Southeast Asia and the Indo-Malaya region[46,47]. The dried fruits of long pepper contain piperine, methyl piperine, piperidine, pellitorine, piperidine, tetrahydro piperine, sesamin, palmitic acid, and tetrahydropiperic acid[48]. Piperine showed proven inhibitory Hepatitis B virus (HBV) activity against the secretion of hepatitis B virus surface antigen (HBsAg), and HBeAg is the extractable part of the "core" antigen of the hepatitis B virus[49]. The administration of piperine and *P. longum* extract increased the bone marrow cellularity, α-esterase positive cells, and total WBC counts[50]. Piperine has shown proven *in vitro* and *in vivo* immuno-protective efficacy[51]. The piperine antitumor effect concerns the immune system's modulation to a cytotoxic helper type1[52].

2. Materials and Methods

2.1 Raw Materials

The authenticated raw materials *Zingiber officinale* rhizomes (Zingiberaceae), *Syzygium aromaticum* flower buds (Myrtaceae), *A. vasica* leaves (Acanthaceae), *Anacyclus pyrethrum* roots (*Asteraceae*), *Saussurea lappa* roots (Asteraceae), *Piper longum* dried fruits (Piperaceae), *Clerodendrum serratum* roots (Lamiaceae) and *C. amboinicus* roots (Lamiaceae) were purchased from the KRC crude drugs Chennai, Tamil Nadu, India (13.0827° N, 80.2707°E) and purchased materials dried in a hot air oven at 40°C or in room temperature until completely dry. Dried crude materials were pulverised using a grinder as a fine powder (Sieve No.10) and packed in a dry, airtight container until further use[53].

2.1.1 Extraction and Isolation of Vasaka Alkaloids (Vasicine and Vasicinone)

A weight of 250gm of *A. vasica* leaves powder was transferred in a conical flask of 2000 ml and macerated with 90% ethanol (1000 ml) for 48 hours. The extract is filtered, and the filtrate is concentrated up to the average value of 90% at 60°C. The filtrate is mixed with 25 ml of 5% acetic acid solution, warmed at 60ºC for 30 mins, and filtered off the coagulated mass. The acidic filtrate reduced its volume and was shaken with 3 × 25 ml of petroleum ether and 2 x 30 ml of chloroform to remove non-basic red colouring matter. The acidic aqueous extract is adjusted to pH 8.5 with dilute ammonia and extracted successively with 100 ml × 2 of chloroform till the aqueous layer gives a negative test with Dragendorff's reagent. The chloroform evaporated, and the alkaloidal mixture dried. Finally, it is dissolved in a minimum quantity of chloroform and extracted with acetic acid. The aqueous acidic extract is shaken with activated charcoal for 10 minutes and filtered. The filtrate is adjusted to pH 8.5 with dilute ammonia, and the alkaloids are extracted with chloroform. Chloroform is recovered, and the residue thus obtained is dried over calcium chloride and weighed.

2.1.2 Isolation of Vasicinone and Vasicine from Vasaka Alkaloids Mixture

Isolation of vasicinone and vasicine followed by the simple technique from the above mixture of vasaka alkaloids, i.e., TLC. The mobile phase is used as a chloroform: methanol (90:10). The above spot is vasicinone, and the below spot is vasicine. Collect each spot in a separate beaker and dissolve the scrapped silica gel mixture in an equal ratio of ethanol, methanol, and stir, warm for 10 min, filter the solution, and evaporate it[54].

2.1.3 Extraction, Fractionation, and Isolation of 6-Gingerol from Zingiber officinale Rhizomes

Weigh about 500 gm of ginger-dried powder placed in a 2000 ml conical flask, then pour a sufficient quantity of methanol and ethanol (1:1) kept aside for one week. Shaking is necessary occasionally. After one week, filter the extract and evaporate the solvent with a rota evaporator, obtaining a thick paste mass suspended in water. The residues of ginger resin were placed in water, which was removed by filtration, and the residue obtained was dried under a vacuum. The whole extract is washed with petroleum ether and evaporated. Acetone was added to the entire extract, separating the acetone soluble and insoluble portions and evaporating both. Evaporate the solvent from the acetone-soluble amount and dry it. Acetone soluble extract containing a mixture of gingerol was found at about 6.2 gm. The acetone insoluble portion was collected and dissolved in methanol and ethanol. The ethanol and methanol soluble portions were separated and organized. The ethanol and methanol insoluble portions were found to be about 0.197 mg. Gingerol was identified by TLC, and the mobile phase used as an n-hexane: ethyl acetate (7:3), and the R_f value was found to be 0.92[55,56].

2.1.4 Extraction, Fractionation, and Isolation of Biflorin from Syzygium aromaticum Flower Buds

Weigh about 500 gm of clove flower bud powder transferred in a conical flask (2000 ml), add ethanol 1.5 lit, keep aside for one week, and shake if necessary. The extract is filtered and collected the filtrate. Through the rota evaporator, evaporate the solvent. The extract was dried and weighed. About 49 gm of the dried extract was suspended in water and fractionated with n-hexane, ethyl acetate, butanol, and water. Butanol fraction was collected, and the solvent was evaporated. About 9.32 gm of butanol fraction was found; it contains a mixture of biflorin. The Biflorin was identified by TLC, and the mobile phase is used as methanol, water (4:6), hexane, and acetone (9:1). 20 gm of alcoholic crude dried extract dissolved in 90% ethanol was kept aside for nearly two months, and some crystals were present. The solution was filtered, and crystals were collected, and given for mass spectra Analysis[57,58].

2.1.5 Isolation of Biflorin from Butanol Fractions of Syzygium aromaticum Flower Buds

The butanol fraction of clove buds may be a biflorin mixture dissolved in an equal ratio of methanol and ethanol. TLC was prepared with silica gel-GF-254 and developed with mobile phases (methanol: water 4:6, hexane: acetone 9:1). Developed plates are dried and detected in an iodine chamber. Separated spots may be biflorin collected from the TLC plates with a scrapping technique and kept in a beaker. Gathered spots are

dissolved in an equal ratio of methanol and ethanol, and the mixture is warmed, filtered if necessary, and evaporated. Collect the separated compound and analyze it with the standard.

2.1.6 Extraction and Isolation of Pellitorine from Anacyclus pyrethrum Roots

About 100 gm of root powder was weighed and transferred into a 1000 ml conical flask and macerated with petroleum ether (60–80°C) for 72h. After ensuring complete extraction, the extract was collected, filtered, and dried under a vacuum using a rotary evaporator (Heidolph, Schwabach, Germany). The yield was found to be 1.3% w/w. The extract was stored in an air-tight vial till further use.

2.1.7 Isolation of Pellitorine from Pet-ether Extract of Anacyclus pyrethrum Roots

Pet ether extract of *A. pyrethrum* roots may be an alkyl amides mixture dissolved in an equal ratio of methanol, DMSO, ethanol, and filtered. TLC was prepared with silica gel-GF-254 and developed with mobile phases (Toluene: ethyl acetate 9.7:03). Developed plates are dried and detected in a UV chamber at 254, 366 nm. Separated spots may be alkyl amides collected from the TLC plates with the scrapping technique and kept in a beaker. Gathered spots are dissolved in an equal ratio of methanol and ethanol, and the mixture is warmed, filtered if necessary, and evaporated. Collect the separated compound and analyze it with the standard[59,60].

2.1.8 Extraction, Fractionation, and Isolation of Costunolide and Dehydrocostuslactone from Saussurea lappa Roots

Weigh about 500 gm of *S. lappa* roots powder transferred in a conical flask 2000 ml and macerated with hexane: propanol: methanol (2:1:1) for one week. Filter the extract and concentrate. The dried extract was suspended in water and fractionated with pet-ether, hexane, ethyl acetate, chloroform, toluene, butanol, and water[61].

2.1.9 Isolation of Costunolide and Dehydrocostuslactone

Hexane extract of *S. lappa* roots may be a dehydrocostus lactone and costunolide mixture dissolved in an equal ratio of methanol, ethanol, and DMSO and filtered. TLC was prepared with silica gel-GF-254 and developed with mobile phase (petroleum ether: acetone 9:1). Developed plates are dried and detected in an iodine chamber. The scraping technique was followed to separate the developed three spots from the TLC plates and keep them in a beaker. Collected spots are dissolved in an equal ratio of methanol and ethanol, and the mixture is warmed, filtered if necessary, and evaporated. Collect the separated compound and analyze it with the standard[62].

2.1.10 Extraction and Isolation of Apigenin from Clerodendrum serratum Roots

About 250 gm of *C. serratum* roots are transferred in 2000 ml of the conical flask and macerated with 95% ethanol for one week by a cold maceration method. The extract is filtered and concentrated with a rota evaporator. The dried extract was treated with lead acetate, resulting in a yellow residue that was then suspended in methanol, treated with hydrogen sulfide to remove lead, and filtered. After evaporating the filtrate, the residue was treated with boiling water and extracted with ether. The concentrated ether fraction was extracted with sodium hydrogen carbonate solution and acidified with HCL. Recrystallization from alcohol and water results in yellowish-brown amorphous solid or colourless crystals[63].

2.1.11 Extraction, Fractionation of Apigenin from Coleus amboinicus Roots

Weigh 500 gm of dried *C. amboinicus* roots powder transferred in a conical flask 3000 ml and macerated with ethanol for a week. Filter the extract, collect the filtrate and concentrate, and dry it. Dried extract suspended in water and fractionated with pet-ether, hexane, butanol, benzene, chloroform, diethyl ether, ethyl acetate, toluene, etc. Finally, collect, each fraction and evaporate the solvent. Butanol and chloroform fractions of *C. amboinicus* roots may be a mixture of Apigenin[64].

2.1.12 Extraction, Fractionation, and Isolation Piperine from Piper longum Dried Fruits

Weigh 350 gm of *P. longum* dried fruit powder placed for cold maceration with solvent methanol: ethanol (2:1) for up to one week. Filter, concentrate the extract,

and dry. The dried extract is packed and stored in a well-closed container until further use. The crude extracts half quantity taken for piperine isolation, and the remaining half portion partitioned for other compounds. The above-dried extract was suspended in water and partitioned with hexane, chloroform, ethyl acetate, butanol, and water. All fractions were collected and concentrated, and only the hexane fraction was found to be a yellow oily liquid, kept for one week in a cool place. Reddish yellow crystals were present; it was further washed with hexane and acetone. Finally, light-yellow crystals are present (200 mg)[65].

2.1.13 Isolation of Piperine

Crude extract of *P. longum* dried fruits is treated with 10 ml of 10% alcoholic KOH, leaving for 10-15 min to saponify resin. Filter the solution and collect the filtrate. Wash the residue with water. Filtrate is dissolved in ethanol and cooled (frozen) or leftover overnight. 2.2 gm of crude piperine was obtained. It was washed with acetone and methanol 2-6 times; finally, a pure colourless crystal of piperine (294.5 mg) was collected[66].

2.1.14 Chemical Marker and Chemicals

Vasicinone was purchased from Natural Remedies Pvt. Ltd., Bengaluru – 560100, Karnataka, India. Apigenin, piperine reference standard purchased from Yucca Enterprises, Mumbai India (19.0206° N, 72.8679° E). Pellitorine reference standards purchased from merck sigma-Aldrich, USA. 6-Gingerol, costunolide**,** and dehydrocostuslactone were purchased from Wuhan Chemfaces Biochemical Pvt. Ltd., Wuhan, China. Biflorin was purchased from the Biosynth-carbo synth, Pvt. Ltd., United Kingdom. Required HPLC grade solvents are called Acetonitrile, methanol, ethanol, acetone, chloroform, toluene, ethyl acetate, and pet-ether, lead acetate, and lead sulfide were purchased from the Central Drug House (CDH) Pvt. Ltd., Gujarat, India, and Collected from the Department of Pharmacognosy, and Pharmaceutical Chemistry, JSSAHER-JSS College of Pharmacy, Ooty, Tamil Nadu, India. (Longitude -76°42′25.56″E (76.7071), Latitude - 11°24′4.07″N (11.401127), and Chandra Labs, IDA-Prashanth Nagar, Hyderabad, Telangana, India (Longitude - 78.4271639, Latitude - 17.4760781[67].

2.1.15 UFLC System and Instruments

The liquid chromatography was carried out on a Shimadzu LC 2010 HPLC (UFLC) instrument composed of an ultrafast autosampler and a UV-VIS detector. The LC-2010 is also designed for ease of use by automating lab solutions software's analysis process. Inertsil C_{18} 3V (250*4.6mm and 5µm), RP-C_{18}-Hiber @ 250-4,6, Prosper R-STAR, Inertsil C_{18} 3V (250*4.6mm and 5µm), Inertsil C_8 (250*4.6mm and 5µm), Inertsil C_8 (250*4.6mm and 5µm), Inertsil ODS 3V (150*4.6mm and 5µm), and C_{18}-BDS, 130 A° column was used as the stationary phase. FT-IR Model-8400S (Shimadzu), KBR pressing, Software Shimadzu IR Solution, Shimadzu-LC-MS-8030[68].

2.1.16 Preparation of Standard and Sample Solutions

2.1.16.1 Standard Preparation

5 mg of each weighed vasicinone, biflorin, pellitorine, 6-gingerol, costunolide, dehydrocostuslactone, piperine, and apigenin taken and poured into an individual 50 ml volumetric flask, and dissolved in the solvent system, finally made up to the mark with the diluent. The Final concentration was 100µg/ml made with the mobile phase.

2.1.16.2 Sample Preparations

5 mg of each sample was taken and poured into a 50 ml volumetric flask; the sample was dissolved in the solvent system and finally made up to the mark with the diluent. The Final concentration was 100µg/ml made with the mobile phase.

2.1.16.3 Kabusura Kudineer (Marketed) Formulation Preparation

5 gm marketed *Kabusura kudineer churna* formulation was transferred in 250 ml of the beaker. A sufficient quantity of water and an equal amount of ethanol were added, stirring the mixture while applying heat until boiling the mixture. Filter the solution, collect the filtrate, and concentrate the solution. From the concentrate solution, 5ml was taken and poured into a 50 ml volumetric flask; the sample was dissolved in the mobile phase and made up to the mark with the diluent. The Final concentration was 100 µg/ml made with the mobile phase.

2.1.16.4. Hydaljss08 Formulation Preparations

Accurately, 5 ml of the liquid sample was transferred into a 50 ml volumetric flask, 30 ml of the solvent

system was sonicated for 10 min, and the final volume was mixed well with the solvent system. Centrifugation was done in this solution at 5000 rpm for 10 minutes for the dissolving purpose. The supernatant was further diluted 1ml to 10 ml with a solvent system, mixed well, and injected into the HPLC system. The prepared solution was stored under a refrigerator[69].

3. Results and Discussion

The preliminary identification of the 6-gingerol, biflorin, vasicinone, pellitorine, costunolide, dehydrocostuslactone, piperine, and apigenin was in the polyherbal formulations (marketed and new), and isolated, fractions, extract of *Z. officinale* rhizome (Zingiberaceae), *S. aromaticum* flower buds (Myrtaceae), *A. vasica* leaves (Acanthaceae), *A. pyrethrum* roots (Asteraceae), *P. longum* dried fruits (Piperaceae), *C. serratum* roots (Lamiaceae) and *C. amboinicus* roots (Lamiaceae) was done by using the TLC, a mobile phase used as an n-hexane: ethyl acetate (7:3), 6-gingerol, methanol: water (4:6), hexane: acetone (9:1) Biflorin, chloroform: methanol (90:10), vasaka alkaloids, toluene: ethyl acetate (9.7:03) Pellitorine, n-hexane: ethyl acetate: glacial acetic acid(12:4:0.4) Piperine, and hexane: ethyl acetate: acetic acid (6.2:2.8:1) Apigenin. The R_f value was found to be 0.92, 6-Gingerol., 0.75, 0.34, Biflorin, 0.56, vasicinone, 0.13-vasicine, 0.32 and 0.15, Pellitorine, 0.19, Piperine, 0.15, Apigenin, (*C. serratum* roots), 0.20, and Apigenin (*C. amboinicus* roots), data result chromatogram demonstrated in Figure 1. FT-IR spectrum of isolated fractions, extract of *Z. officinale* rhizomes (Zingiberaceae), *S. aromaticum* flower buds (Myrtaceae), *A. vasica* leaves (Acanthaceae), *A. pyrethrum* roots (Asteraceae), *S. lappa* roots (Asteraceae), *P. longum* dried fruits (Piperaceae), *C. serratum* roots (Lamiaceae), and *C. amboinicus* roots (Lamiaceae) was taken and data interpretation was done. Ultra-fast liquid chromatography method was developed for pellitorine in polyherbal formulations (*Kabusura kudineer* marketed and new *Hydaljss*08), and extract, isolated products of *A. pyrethrum* roots (Asteraceae). The column and mobile phase were used as an RP-C_{18}-Hiber @ 250-4,6, Purospher R-STAR, methanol: 0.1% formic acid in water (1:1). Standard 5 mg pellitorine purchased from Sigma Aldrich has developed two methods of liquid chromatography, in both methods standard drug developed three peaks. Identifying pellitorine peaks is difficult because the standard is mixed with other impurities or substandard products. For that condition developed liquid chromatography, three peaks' fractions are collected according to the retention time of chromatograms and given for mass spectra analysis. According to the molecular weight of pellitorine (mw:223.35), a peak was identified. The 3rd peak of the chromatogram matched the molecular weight of pellitorine (mw:223.35). The pellitorine retention time was 8.721 min and was detected at 260 nm in the PDA detector. The chromatogram and mass spectrum results data are summarized in Figure 2. Percentage purity calculation of pellitorine in the isolated extract of *A. pyrethrum* roots and polyherbal formulations by using ultra-performance liquid chromatography, data represented in Table 1.

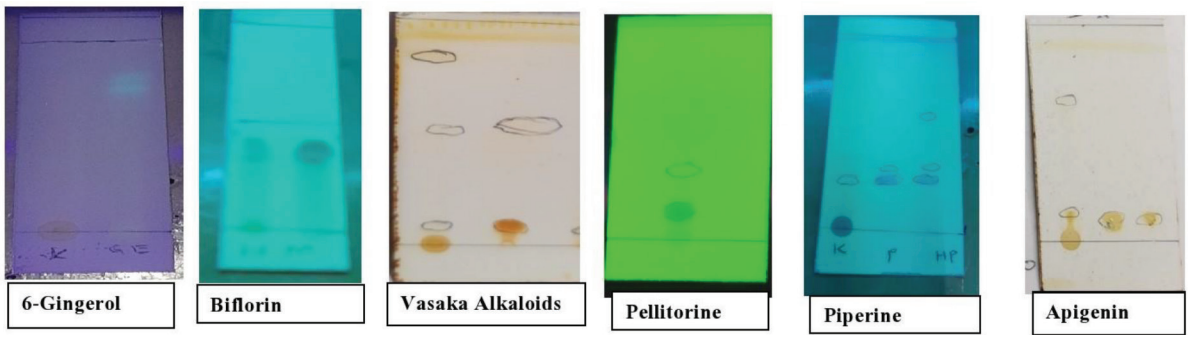


Figure 1. Typical Thin layer chromatograms of 6-Gingerol, Biflorin, Vasaka alkaloids, Pellitorine, Piperine, and Apigenin, in polyherbal formulations marketed "*Kabusura Kudineer*" and fractions, isolated phytopharmaceuticals from the selected plant species.

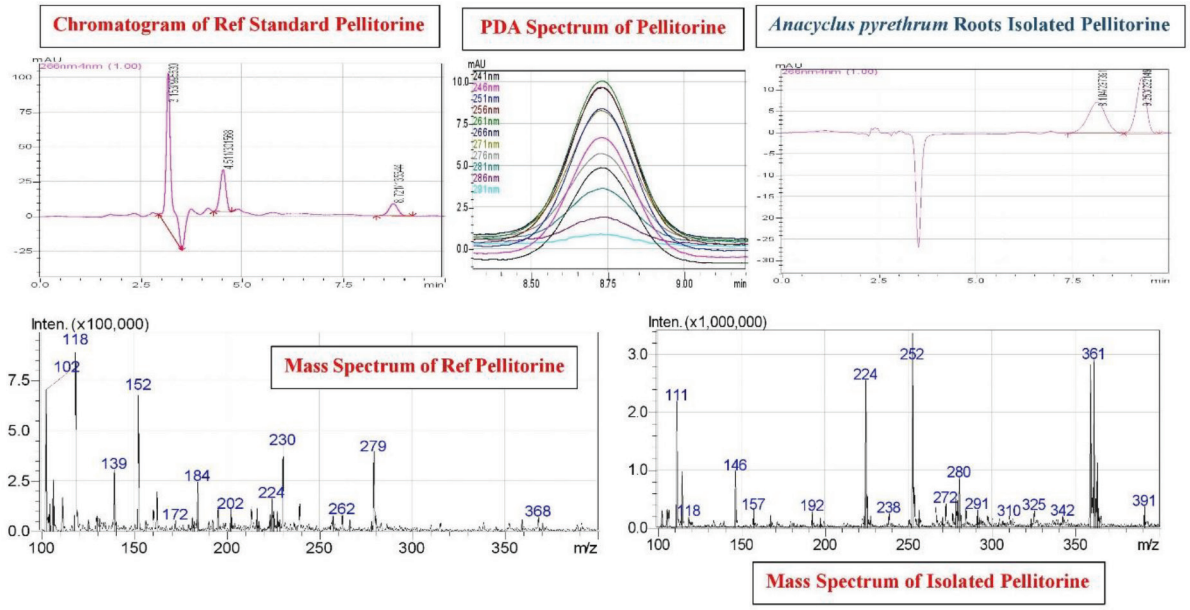


Figure 2. Typical UFLC chromatograms of Ref standard, isolated pellitorine from *Anacyclus pyrethrums* roots and Mass spectrum of pellitorine.

Table 1. Percentage purity calculation of pellitorine in Isolated extract of *Anacyclus pyrethrum* roots and polyherbal formulations by using liquid chromatography

S. No.	Sample	Peak area	Avg. peak area	% w/w	µg/ml
1	Standard pellitorine-from Sigma Aldrich	135344 136108	135726	97% of label claims	
2	Isolated pellitorine	237391	237391	174.9%	17.49
3	Pet-ether extract of *A. pyrethrum* root extract	72463	72463	53.3%	5.33
4	*Kabusura kudineer* marketed formulations	Pellitorine was not detected in *Kabusura kudineer*			
5	*Hydaljss08* formulation	Pellitorine was not seen in the "*Hydaljss08*" formulation			

UFLC method was developed for biflorin in polyherbal formulations *kabusura kudineer* (marketed and new-*Hydaljss*08), and isolated butanol fractions of *S. aromaticum* flower buds. The developed liquid chromatography method is novel, simple, linear, and rapid for estimating biflorin. The columns and mobile phase are used as an Rp-C_{18}-Hiber @ 250-4,6, Prosper "R" STAR, and methanol: water (1:1). Biflorin retention time was 4.806 min and detected at 340 nm in the PDA detector. The chromatogram data results are illustrated in Figure 3. Six concentrations were injected in liquid chromatography to analyze a standard's average peak area. Then, the regression equation: Y = 13888 + X = 16549 was obtained for biflorin, and the correlation coefficient (R2) was found 0.9996, respectively. Thus, outstanding linearity was exposed when the biflorin concentration ranged from 5-100 µg/ml; data results are summarized in Table 2 and Figure 4.

About 20 gm of alcoholic crude dried extract of clove flower buds dissolved in 90% ethanol and kept aside for nearly two months; some crystals are present. The solution was filtered and collected crystals were given for mass spectra analysis; the molecular weight of biflorin (354.31) matched with mass spectra data. Percentage purity calculation of biflorin in isolated, butanol fraction of *S. aromaticum* flower buds and polyherbal formulations by UFLC, data represented in Table 3.

UFLC method was developed for the 6-Gingerol, vasicinone, apigenin, costunolide, and dehydrocostuslactone, in polyherbal formulations *Kabusura kudineer* (marketed and new-*Hydaljss*08) and isolated, fraction, extract of *Z. officinale* rhizomes (Zingiberaceae), *A. vasica* leaves (*Acanthaceae*),

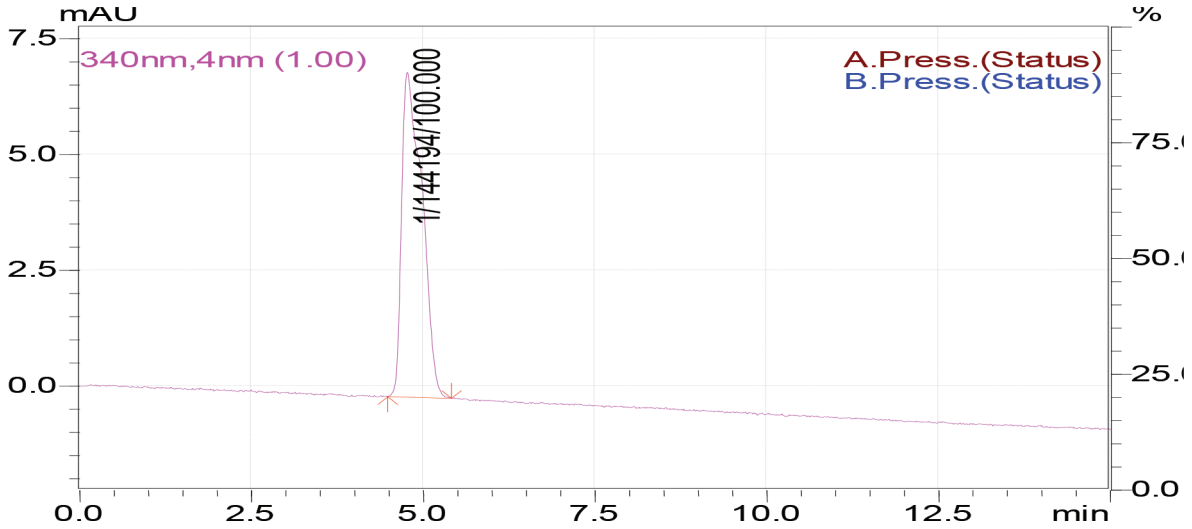


Figure 3. Typical UFLC chromatogram of Ref standard biflorin.

Table 2. Linearity of biflorin

Linearity and Range			Linearity data results of Biflorin		
S. No.	**Conc in µg/mL**	**Area**	**S. No.**	**Parameter**	**Biflorin**
1	5.0	78646	1	Correlation coefficient	0.9996
2	10.0	144194	2	Slope	13888
3	20.0	310181	3	Intercept	16549
4	25.0	369585			
5	100.0	1402179			
6	Avg.	12598.57648			

C. serratum roots (Lamiaceae), *C. amboinicus* roots (Lamiaceae), and *S. lappa* roots (Asteraceae). Columns, mobile phase used as an Inertsil C_{18} 3V (250*4.6mm and 5µm), Inertsil C_{18} 3V (250*4.6mm and 5µm), C_{18}-BDS,130 A°, Inertsil C_8 (250*4.6mm and 5µm), Inertsil C_8 (250*4.6mm and 5µm), and 0.05% Orthophosphoric acid: methanol (60:40)., 0.01% of triethylamine is mixed with phosphate buffer pH-7.0: acetonitrile (55:45), acetonitrile: water (1:1), phosphate buffer pH 3.5: acetonitrile (50:50), and combined phosphate buffer pH:7.0: acetonitrile (55:45). The retention time and detector used for the estimation of 6-gingerol, vasicinone, apigenin, costunolide, and dehydrocostuslactone was found to be 3.128 min, 2.182 min, 4.329 min, 3.425 min, 3.387 min, and detected at 280 nm (UV), 300 nm (UV), 340 nm (PDA), 225 nm (UV), 225nm (UV), in PDA, and UV detector, data results are illustrated in Figures 5-9. Percentage purity calculation of 6-gingerol, vasicinone, apigenin, costunolide, and dehydrocostuslactone in an isolated, fraction of plant species and polyherbal formulations by UFLC, data results were illustrated in Tables 4-8. The mass spectrum for pet ether soluble fraction of isolated ginger products was done to assess the 6-Gingerol (mw:294.15) identification and quantification, obtaining results demonstrated in Figure 10, and the results data matched with 6-Gingerol molecular weight.

UFLC development, documentation of piperine in polyherbal formulations, and Isolated hexane fraction of piperine from *P. longum* dried fruits were done by using columns, and the mobile phase was Inertsil ODS 3V (150*4.6mm and **5µm)**, pH 2.5 phosphate buffer: acetonitrile (60:40). The Piperine was detected in UV detector at a wavelength of 343 nm, and retention was found as 4.063 mins, data results represented as Figure 11. While determining the system suitability of piperine was injected six times in the concentration of 100µg/ml, to analyze accurate, precise results of the sample, and obtained chromatograms were recorded. A standard deviation of intraday (11433.78), interday (9253.9), and a % RSD of 0.2, 0.1, were found satisfactory. The plate count and tailing factor results were within limits, and the % RSD. was found to be 0.3%, so the system is suitable and gives precise results; data are summarized in Table 9.

A calibration curve and outstanding linearity were recorded for piperine by injecting 25-75µg/ml of marker solutions. The regression equation was found to be

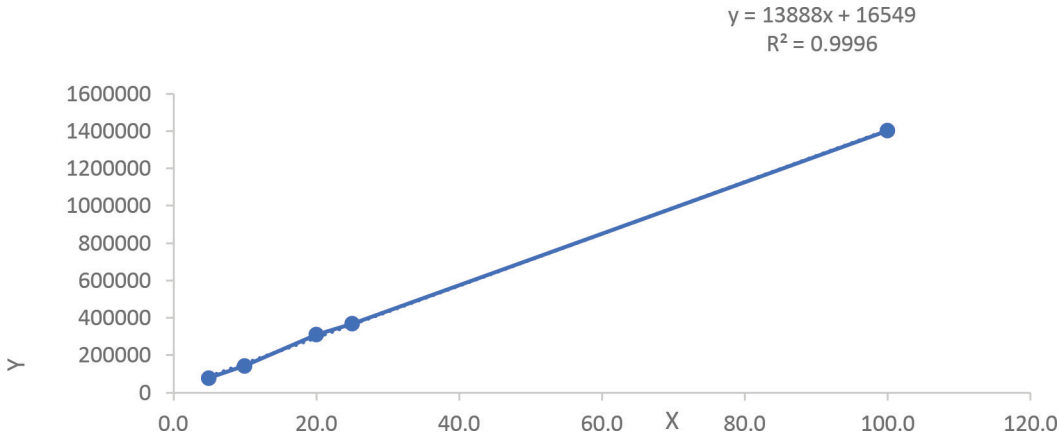


Figure 4. Typical linearity graph for Ref standard biflorin.

Table 3. Percentage purity calculation of biflorin in isolated butanol fraction of *Syzygium aromaticum* flower buds and polyherbal formulations by using UFLC

S. No.	Sample	Peak area	Avg. peak area	% w/w	µg/mL
1	Standard Biflorin is from Biosynth-Carbosynth, United Kingdom	144194 185977	330171	Label claims 98%	Label claims 98%
2	Isolated Biflorin	27810	27810	8.42 %	0.84
3	Butanol fraction of Clove buds	53161	53161	16.1 %	1.61
4	*Kabusura kudineer*-marketed	Biflorin not detected in *Kabusura kudineer* formulation-Marketed			
5	Hydaljss08 formulation	30606	30606	9.26%	0.926

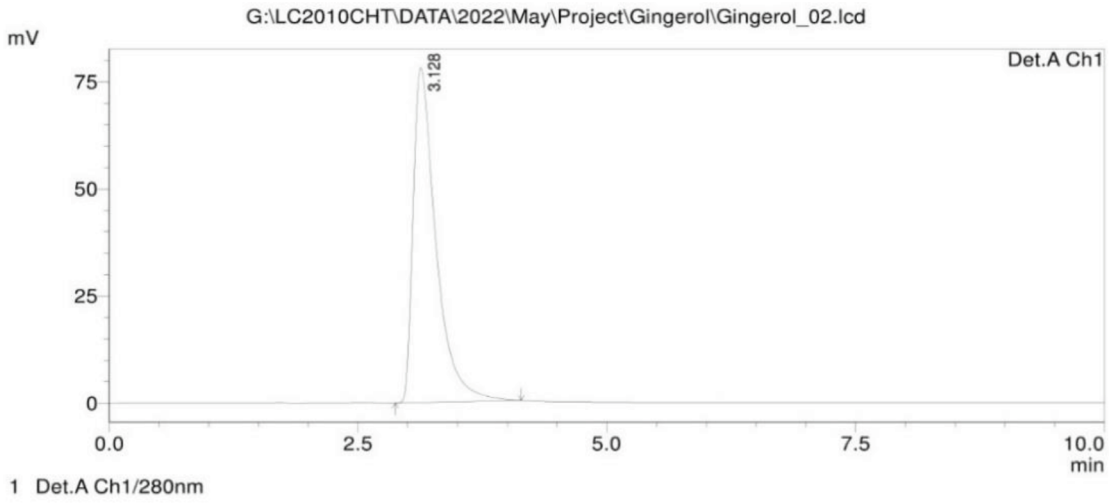


Figure 5. Typical UFLC Ref standard chromatograms of 6-gingerol.

Y = 165569 X -1830481, and the correlation coefficient (R2) of piperine was 0.9993, respectively, the results data summarized in Tables 9 and 10 and Figure 11. The LODS and LOQ of the piperine were found to be 1.96 µg/ml, and 5.93 µg/ml, with results data summarized in Table 10.

To study the recovery of the piperine, accuracy was performed three times to calculate the percentage of healing, and the percentage mean recovery of markers compounds was found to be between 98% and 101.2%. The results of the data were summarized in detail in Table 11.

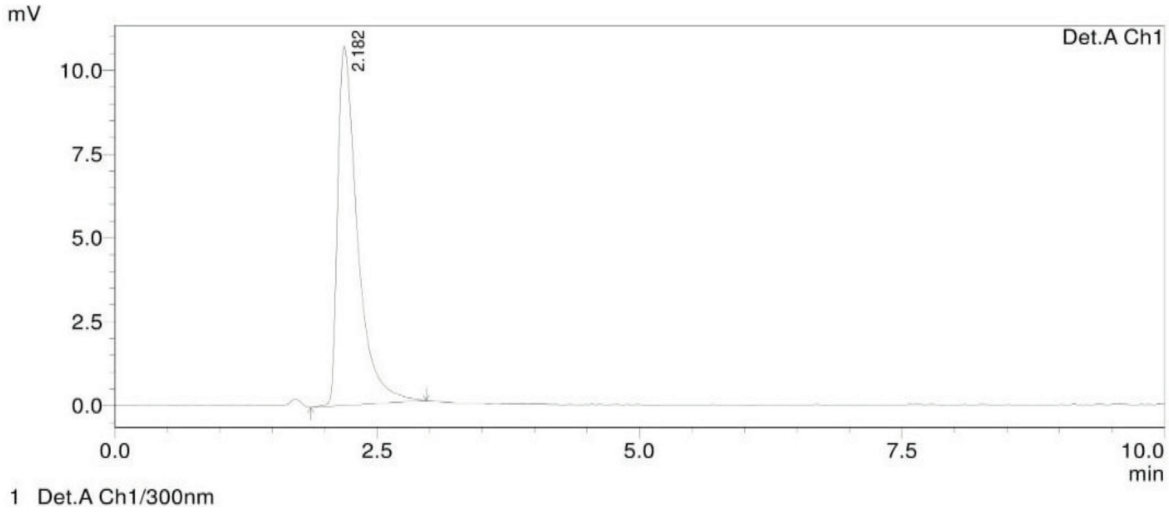


Figure 6. Typical UFLC Ref standard chromatograms of vasicinone.

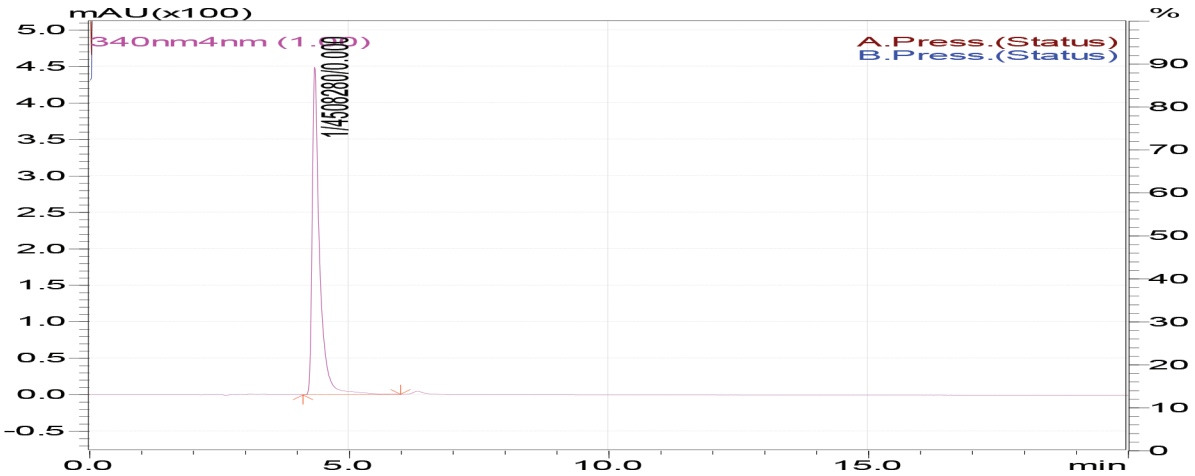


Figure 7. Typical UFLC chromatograms of Ref standard apigenin.

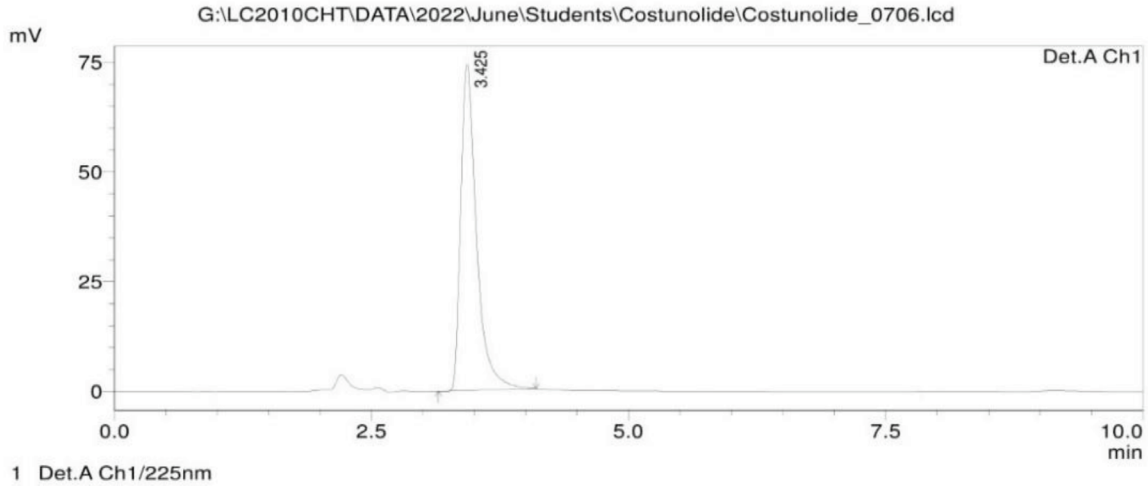


Figure 8. Typical UFLC chromatograms of Ref standard costunolide.

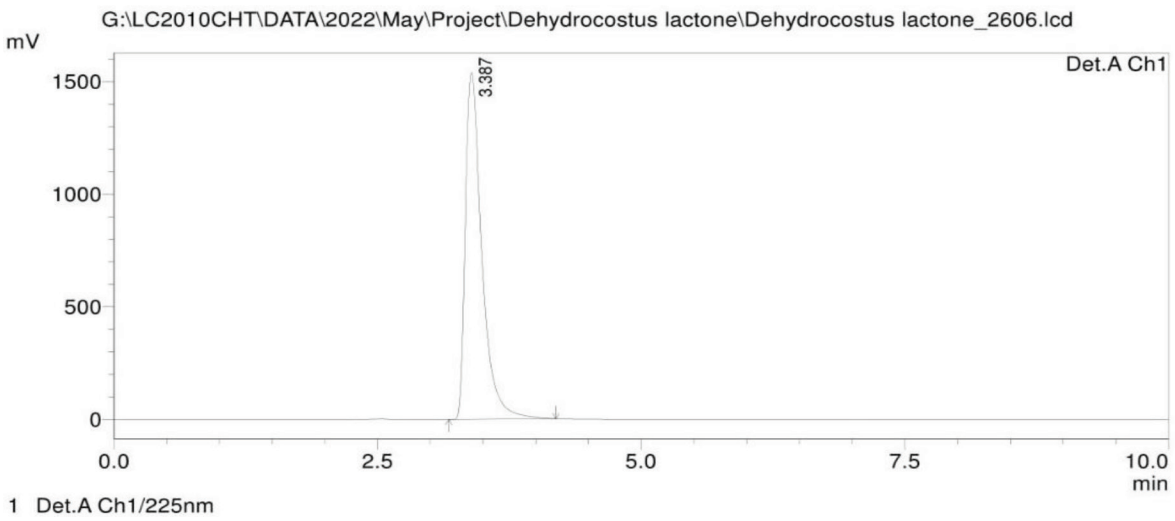


Figure 9. Typical UFLC chromatograms of Ref standard dehydrocostus lactone.

Table 4. Percentage purity calculation of 6-Gingerol in an isolated fraction of plant species and polyherbal formulations by using UFLC.

S. No.	Sample	Avg. peak area	% w/w	µg/mL	mg/mL
1	**6-Gingerol Standard**	1247633	Label claims 98%		
2	Ginger fractions are methanol-soluble	1098978	88.1	44.042	0.044043
3	Ginger pet-ether fraction isolated	3760207	301.4	150.69	0.150694
4	*Kabusura kudineer*-marketed	191976	15.4	7.693	0.007694
5	*Hydaljss*08 formulation	3117	0.2	0.124	0.000125

Table 5. Percentage purity calculation of Vasicinone in an isolated fraction of plant species and polyherbal formulations by using UFLC

S. No.	Sample	Area	%w/w	µg/mL	mg/mL
1	**Vasicinone Standard**	141095	Label Claims-95%		
2	*Hydaljss*08 formulations	192174	15.4	7.701544	0.007702
3	*Kabusura kudineer* -marketed.	20771	1.7	0.832416	0.000832
4	Vasaka Alkaloids Isolated.	98984	7.9	3.966872	0.003967
5	Vasicinone Isolated by TLC	37977	3.0	1.521962	0.001522

Table 6. Percentage purity calculation of Apigenin in an isolated fraction of plant species and polyherbal formulations by using UFLC

S. No.	Sample	Peak area	Avg. peak area	% w/w	µg/mL
1	Standard Apigenin	4508280 4007411	**4257486 /5=**851497.	**Label claims 98.05%**	
2	Butanol fraction of *C. amboinicus*	11476 11277	11377	1.3	0.036
3	Chloroform fraction of *C. amboinicus*	1982 4062	3022	0.035	0.0035
4	Isolated Apigenin from *C. serratum* roots	40489 30189	35339	4.15	0.4150
5	*Kabusura kudineer*-marketed	8702 5043	6873	0.80.7	0.0807
6	*Hydaljss*08 formulation	35806 12485	24146	2.83	0.2835

Table 7. Percentage purity calculation of Costunolide in an isolated fraction of plant species and polyherbal formulations by using UFLC

S. No.	Sample	Peak area	% w/w	µg/mL	mg/mL
1	Standard Costunolide	790310	Label Claims 98%		
2	SPTLC-III	3501082	443.0	221.50	0.22150
3	*Kabusura kudineer*-marketed	Costunolide was not detected in "*Kabusura kudineer*." Polyherbal formulation			
4	*Hydaljss*08 formulation	Costunolide was not detected in the "*Hydaljss*08" formulation			

Table 8. Percentage purity calculation of dehydrocostuslactone in an isolated fraction of plant species and polyherbal formulations by UFLC

S. No.	Sample Codes	Area	%w/w	µg/mL	mg/mL
1	Dehydrocostus lactone from Chem faces-China	16773087	Label claims 98%.		
2	Not Matched-Developed "*Hydaljss*08" formulation	Dehydrocostunolides peak not detected in "*Hydaljss*08"			
3	Not matched-*Kabusura kudineer*-marketed	Dehydrocostunolides peak not detected in "KKR"			
4	Ethyl acetate fraction of *S. lappa*	4920782	29.3	14.67	0.01467
5	Hexane fraction of *S. lappa*	23315106	139.0	69.50	0.06950
6	Petroleum ether fraction of *S. lappa*	7009489	41.8	20.90	0.02090
7	Toluene fraction of *S. lappa*	5789564	34.5	17.26	0.01726
8	Isolated Spot-II by TLC from the extract of *S. lappa*	1919349	11.4	5.72	0.00572

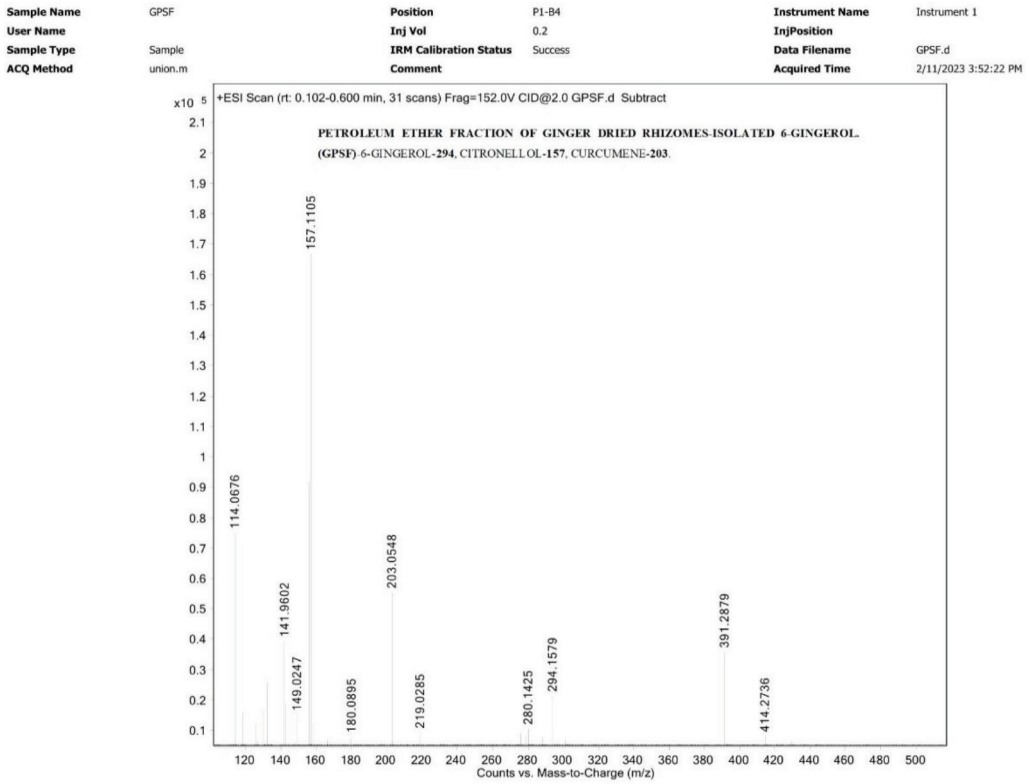


Figure 10. Mass Spectrum of 6-gingerol isolated (Pet-ether soluble) from ginger (mw: 294).

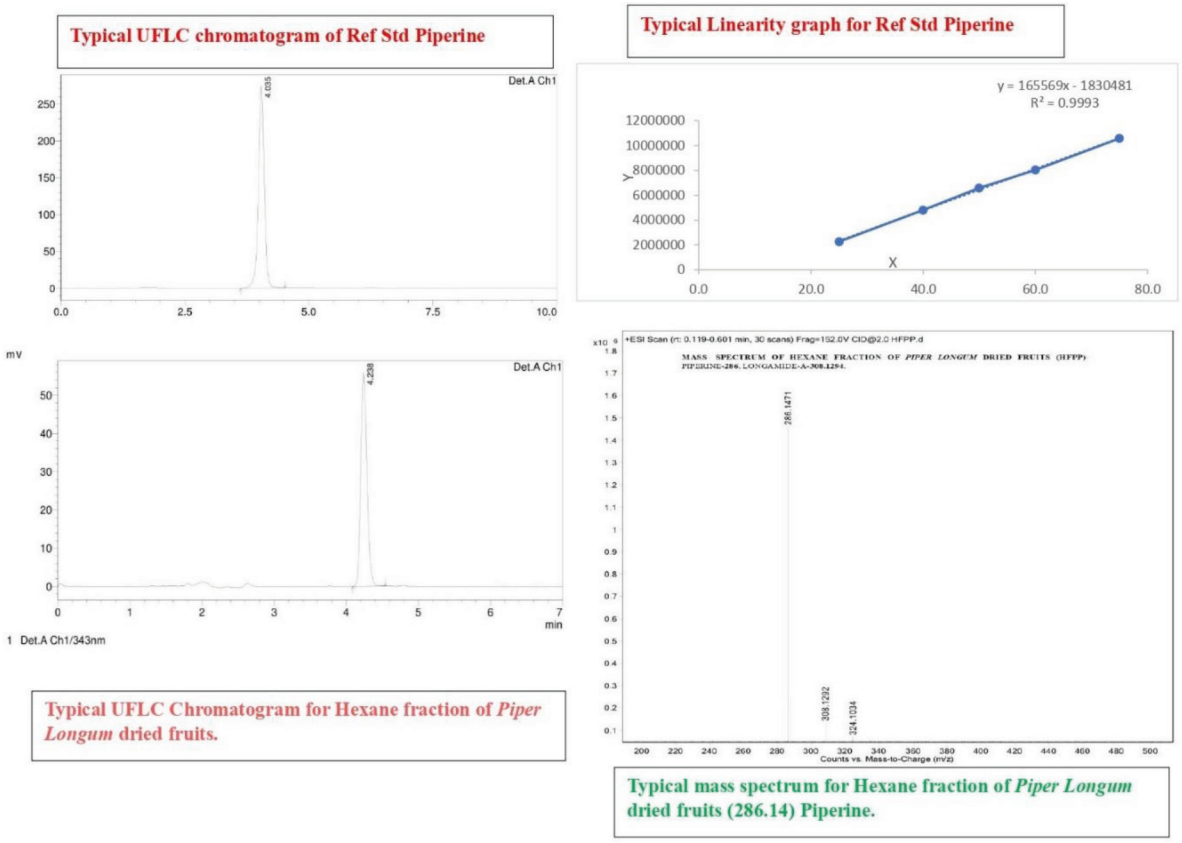


Figure 11. Typical UFLC chromatogram, linearity of Ref standard piperine and hexane fraction of piperine from *Piper longum* dried fruits chromatogram and Mass spectrum.

Table 9. Intra, inter-day results of method precision, results of linearity, and range of piperine

S. No.	Intra-day Precision (n=6)		Inter-day precision (n=6)		Linearity and Range	
	Con 100 µg /ml	Area	Con100 µg /ml	Area	Concentration (µg/mL)	Area
1	1	6621525	1	6601014	25.0	2253868
2	2	6599265	2	6606732	40.0	4808530
3	3	6621843	3	6601822	50.0	6584476
4	4	6597924	4	6599733	60.0	8021083
5	5	6610964	5	6617331	75.0	10571837
6	6	6622256	6	6589081	Avg.	1830481.0
7	Average	6612296	Average	6602619		
8	Std dev	11433.78	Std dev	9253.9		
9	% RSD	0.2	% RSD	0.1		

The percentage purity calculation of piperine in isolated hexane fraction of *P. longum* dried fruits, and polyherbal formulations by using UFLC data are represented in Table 12.

The developed ultra performance liquid chromatography method was a novel, simple, rapid, linear, and new for the estimation of vasicinone, biflorin, pellitorine, 6-gingerol, costunolide, dehydrocostuslactone, piperine, apigenin in *Ayurvedic, Siddha*-based polyherbal formulations and in related plant species[70,71]. Physico-chemical properities of 6-gingerol, Biflorin, Vasicine, Vasicinone, Pellitorine, Costunolide, Dehydrocostuslactone, Apigenin and Piperine was demonstarted in Table 13.

Table 10. System suitability and ruggedness results of piperine

S. No.	Concentration (µg/mL)	Area	Ruggedness Results of Piperine	
1	Linearity and Range	25-75 µg/ml	**Piperine**	**%Assay**
2	Regression equation	y = 165569 x -1830481	Analyst 01	98.1%
3	Correlation coefficient	0.9993	Analyst 02	99.0%
4	Theoretical plates	37584	%RSD	0.3%
5	Tailing Factor	0.962		
6	LOD	1.96		
7	LOQ	5.93		
8	Slope	165568.8		
9	Intercept	1830481		

Table 11. Results of recovery studies for piperine

S. No.	Concentration in (%)	Concentration in(µg/ml)	Area	% Recovered	% Recovery
1	50	50	3331573	98.8	99.3
		50	3337758	99.8	
2		50	3337390	99.4	
	100	100	6656606	98.3	97.9
		100	6645578	97.8	
		100	6658798	97.6	
3	150	150	10012082	100.5	100.9
		150	10034403	100.9	
		150	10049004	101.2	

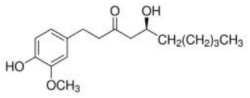
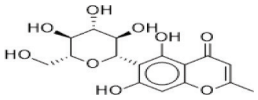
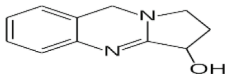
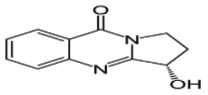
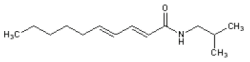
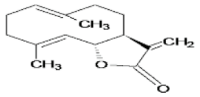
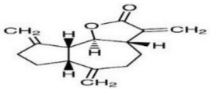
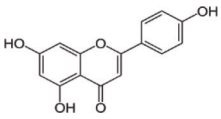
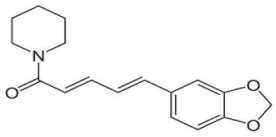
Table 12. Percentage purity calculation of piperine in isolated, hexane fraction of *P. longum* dried fruits and polyherbal formulations

S. No.	Sample	Area	%w/w Assay	in µg/ml	in mg/ml
1	**Piperine Reference Standard**	6621525	95.1% Label claims		
2	Hexane Fraction of Piperine from *P. longum* dried fruits	3307966	9.957 %	4.9957	0.0049957
3	Isolated Piperine in *P. longum* dried fruits	6553569	98.973 %	9.8973	0.0098973
4	Developed "*Hydaljss*08" polyherbal formulations	6794608	102.613 %	10.2613	0.0010263
5	*Kabusura kudineer* marketed	1658715	25.50 %	2.5050	0.0025050

4. Conclusion

The preliminary identification of the 6-gingerol, biflorin, vasicinone, pellitorine, piperine, costunolide, dehydrocostuslactone, and apigenin was in the polyherbal formulations, isolated fractions, extract of *Z. officinale* rhizome (Zingiberaceae), *S. aromaticum* flower buds (Myrtaceae), *A. vasica* leaf (Acanthaceae), *A. pyrethrum* roots (Asteraceae), P. *longum* dried fruits (Piperaceae), *S. lappa* roots (Asteraceae), *C. serratum* roots (Lamiaceae) and *C. amboinicus* roots (Lamiaceae) was done by TLC and FT-IR spectrum. The current developed, documented method is a simple, novel, linear, rapid, accurate, new, and reliable method that was designed to estimate for the 6-gingerol, biflorin, vasicinone, pellitorine, costunolide, dehydrocostunolides, piperine, and apigenin in polyherbal formulations *Kabusura kudineer* churna marketed, *Hydaljss*08 (new) and in isolated, fractions, extract of *Z. officinale* rhizome (Zingiberaceae), *S. aromaticum* flower buds (Myrtaceae), *A. vasica* leaf (Acanthaceae), *A. pyrethrum* roots (Asteraceae), *S. lappa*

Table 13. Physico-chemical properties and biological sources of 6-Gingerol, Biflorin, Vasicine, Vasicinone, Pellitorine, Costunolide, Dehydrocostuslactone, Apigenin and Piperine

S. No.	Name of the Compound	Molecular formula with mass	Chemical Structure	Biological Source	Nature of com pound	Pharmacological effects	Refer ences
1	*6-Gingerol*	$C_{17}H_{26}O_4$ 294.4		*Zingiber officinalis* rhizomes	Phenolic com pound	Anti-inflammatory, immunomodulatory, Antiviral effects.	3-9
2	Biflorin	$C_{16}H_{18}O_9$ 354.31		*Syzygium aromaticum* flower buds	*Phenolic gly cosides*.	Immunostimulatory Antiviral effects	10-14
3	Vasicine	$C_{11}H_{12}N_2O$, 188.23		*Adhatoda vasica* leaves	Quina zoline alkaloid	Antiviral effects, Immunomodulatory, Bronchodilator effects.	15-21
4	Vasicinone	$C_{11}H_{10}N_2O_2$, 202.213		*Adhatoda vasica* leaves	Quina zoline alkaloid	Antiviral Immunomodulatory Bronchodilator effects.	15-21
5	Pellitorine	$C_{14}H_{25}NO$ 223.35		*Anacyclus pyrethrum* roots	Amide alkaloid	Anti-cancer Immunomodulatory effects.	22-26
6	Costunolide	$C_{15}H_{20}O_2$ 232.32		*Saussurea lappa* roots	*Sesquiter pene lactone*	Anti-viral effects, Immunomodulatory effects.	27-33
7	Dehydrocostus lactone	$C_{15}H_{18}O_2$ 230.30		*Saussurea lappa roots*	*Sesquiter pene lactone*	Anti-viral effects Immunomodulatory effects	27-33
8	Apigenin	$C_{15}H_{10}O_5$ 270.0528		*Clerodendrum serratum* roots, *Coleus amboinicus* roots	Flavonoids	Anti-viral effects Immunomodulatory effects	34-45
9	Piperine	$C_{17}H_{19}NO_3$ 285.343		*Piper longum* fruits	Alkaloids	Anti-viral effects Immunomodulatory effects.	46-52

roots (Asteraceae), *P. longum* dried fruits (Piperaceae), *C. serratum* roots (Lamiaceae) and *C. amboinicus* (Lamiaceae) roots. Biflorin's existing ultra-performance liquid chromatography method has not been reported in individual and combined dosage forms. The obtained results revealed that the method showed good linearity for biflorin. Thus, it is acceptable to be used in the quantification of 6-gingerol, biflorin, vasicinone, pellitorine, costunolide, dehydrocostuslactone, piperine, and apigenin in *Ayurvedic*-based polyherbal formulations and Isolated, fractionated products in plant species.

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