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Research Article

Preparation, Quality evaluation and Gas Chromatography Mass Spectrometry (GCMS) Analysis of *Amalakyadi churna*

Agrawal Prajwal Ravindra[1], Veena KH[2*], Tejaswini Yarazarvimath[3]

1. PG Scholar, Department of Kaumarabhritya, KAHER's Shri BM Kankanawadi *Ayurveda* Mahavidyalaya, Deemed to be University, Belagavi, Karnataka, India.
2. Associate Professor, Department of Kaumarabhritya, KAHER's Shri BM Kankanawadi *Ayurveda* Mahavidyalaya, Deemed to be University, Belagavi, Karnataka, India.
3. Assistant Professor, Department of Kaumarabhritya, KAHER's Shri BM Kankanawadi *Ayurveda* Mahavidyalaya, Deemed to be University, Belagavi, Karnataka, India.

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Abstract

Background: *Amalakyadi churna* (AC) (drug powdered) is a familiar *Ayurveda* formulation mentioned in classical texts such as *Sharangdhara Samhita* (*Madhyamakhanda*, Chapter 6, *Shloka* 7*), Gadanigraha* (Part 1, *Churna adhikara*, Chapter 3, *Shloka* 372) and *Ayurvedic Formulary of India*. Systemic studies on AC was limited. In *Ayurveda*, it is chiefly used for treating fever, *ruchikara* (appetizer), *dipana* (digestive stimulant), *pachana* (carminative), *bhedi* (breaking) & *kapha* pacifying effect (phlegm). Aim: To prepare and evaluate quality of *Amalakyadi Churna.* Objective: To evaluate quality of AC by using GCMS profile. Materials and Methods: Evaluation tested quality, purity of AC using standard tests. Raw ingredients was authenticated and formulation was tested for colour, taste and odour. Physicochemical framework; LOD, acid insoluble ash, total ash and extractive values was determined. Microbial testing suggests safety, while calculate sodium, potassium. Phytochemical test recognizes bioactive compounds and GCMS analysis profiled quantified the chemical phytoconstituents. Results: AC showed proved organoleptic traits, acceptable physicochemical values, safe microbial limits and key minerals. GCMS finds 41 compounds. Phytochemicals (alkaloids, flavonoids, tannins, amino acids and monosaccharides) support its traditional use in *jwara*, *bhedi*, appetite improving, digestive aid and *kapha pacifier*. Conclusion: The bioactive compounds identified from GC MS profiling, which supports its *ayurvedic* use in stimulating appetite, digestion, *jwara*, *bhedi* & *kapha pacifier*.

Keywords: *Amalakyadi choorna*, Appetizer, Bioactive compounds, Flame photometry, Gas chromatography-mass spectrometry, Phytochemical profiling, Quality control

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Introduction

Acharya Sharangdhara explained formulation (AC) in *Sharangdhara Samhita* (*Madhyamakhanda*, Chapter 6, Shloka 7, *Churna Kalpana Adhyaya*) (1) and is also explained in the *Ayurvedic Formulary of India* and *Shodhal Gadanigraha* (2). As per WHO guidelines, quality evaluation of *ayurveda* medicines means measuring the quality of the raw plant materials through physical and chemical tests. It also contents choosing the right plant herbs and action them correctly. Most of the focus was often on measuring quality through tests like organoleptic characters, physicochemical parameters, phytochemical, microbial contamination, mineral levels using flame photometry and chemical profile through GCMS analysis.

In *Ayurveda*, as per classics, AC is indicated in all types of fever & acts as a *bhedi* (breaking), *ruchikara* (appetizer), *dipana* (digestive stimulant), *pachana* (digestive) and pacifier of *kapha* (reducing phlegm). AC was obtained by mixing 5 ingredients i.e. *Amalaki* (*Emblica officinalis gaertn;* fruit, laghu, ruksha, shita with madhurvipaka it is tridoshahar, rochak, dipana, anuloman*)* , *Haritaki (Terminalia chebula;* fruit, *laghu, ruksha* with *ushna, madhurvipaka* it is *dipana, pachana, krimighna, tridoshahar), Chitraka (Plumbago zeylinica Linn;* root, *lagh, ruksha, tikshna* with *ushna* and *katuvipaka* it act as *vatakaphahar, dipan, pachana, krimighna), Pippali (Piper longum Linn;* fruit, *laghu, tikshna* with *madhura vipaka* and *anushnashita* it act as *vata kaphahar, dipana, rasayan*) *and Saindhava* (*Rock salt laghu, sukshma* with *shita and Madhur vipaka* it act as *tridoshahar, ruchikara, dipana*) in same amounts.

*** Corresponding Author:**
Veena K H
Associate Professor, Department of Kaumarabhritya, KAHER's Shri BM Kankanawadi *Ayurveda* Mahavidyalaya, Deemed to be University, Belagavi, Karnataka, India.
Email Id: dratuldesai@rediffmail.com

This study offers a detailed standardization of *Amalakyadi Churna* with integrated GC-MS profiling in a single work. No prior studies have reported for this formulation. It demonstrates a link between phytoconstituents and classical *Ayurveda* actions like *Deepana*, *Pachana*, and *Jwara* management, also develops a validated analytical and chemical fingerprint for quality assessment. This study specifically merged API based standardization with advanced GC-MS analysis detect 41 compounds. It presents a holistic reference profile for quality control and validation, distinct from earlier limited reports.

Minor methodological modifications included refinement of extraction process utilizing acetone as solvent and calibration of GC-MS oven temperature settings and flow criteria to fit the *ayurveda* formulation. Also, formulation preparation and filtration steps was enhanced to confirm superior peak resolution and reliable identification of phytoconstituents.

Key Observations

This study revealed analytical correlation between detected phytoconstituents and classical *Ayurvedic* medicinal actions, it links with modern analytical proofs.

Unique Outcomes

A strong and validated multi aspect standardization model consolidating physicochemical, microbial, mineral, phytochemical and GC-MS profiling was formulated, determining a detailed reference fingerprint for *Amalakyadi Churna*.

In the current era of widespread adulteration, the validation of *ayurveda* formulation had become essential to ensure consistent quality. The aim is to prepare & evaluate the quality of AC and objective to identify, authenticate, to assess quality by using GCMS, which contain 41 compounds that enhance the above indications. As there was lack of quality evaluation, based on this AC selected for preparation & evaluation.

Materials and methods

Obtained all ingredients of AC from the KLE *Ayurveda* Pharmacy, Belagavi, Karnataka. Every ingredient was attentively checked for the presence of dust/germs particle. Total ingredients was reduced to a crushed mixture in a mortar and pestle. Every ingredient was then introduced through sieve 120.

Formulation composition

Preparation of AC

The raw ingredients "as shown in Figure 1" was taken from KLE *Ayurveda* Pharmacy and then pulverized into powdered cast. Total ingredients of pharmacopoeial quality was used in preparation. *Saindhava lavana* was cooked in metallic pan at low temperature till dust free. Total ingredients was thoroughly washed and soaked, initially dried in shade and further dried in hot air tray dryer to remove complete moisture "as shown in Figure 2".

Individual ingredient was crushed using a disintegrator and introduced through a sieve (mesh 120) to obtain a similar particle size. The crushed powdered was weighed individually and then mixed in same quantity to form a same blend. The final formulation "as shown in Figure 3" was kept in an airtight container and filled in a packed sealed container to keep safe it from light and moisture. The criteria and standard referenced in the study are derived from *Ayurvedic* Pharmacopoeia of India (API) monographs of the respective ingredients and formulations.

Table 1: Ingredients for *Amlakyadi Churna* (1,2)

Ingredients	Quantity
Amalaki	150 gm
Haritki	150 gm
Citraka	150 gm
Pippali	150 gm
Saindhav	150 gm
Total Quantity	**750 gm**

Figure 1: Raw ingredients: (a) *Amalaki*, (b) *Haritaki* (*Pathya*), (c) *Citraka*, (d) *Pippali*, (e) *Saindhava*, (f) Crushed Powder of individual ingredients

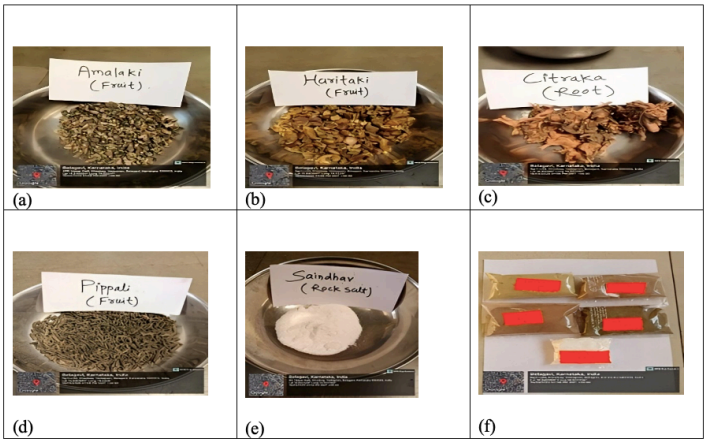
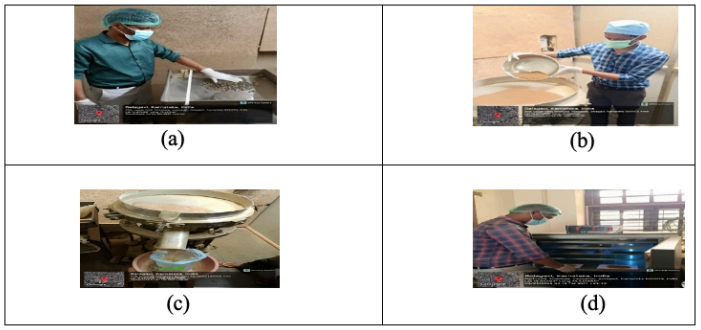


Figure 2: Stepwise Pharmaceutical Preparation of *Amalakyadi Choorna*



(a) Cleaning and sorting of raw ingredients, (b) Sieving and particle size standardization, (c) Pulverization and powder collection, (d) Drying of the prepared choorna under controlled conditions

Figure 3: Amalakyadi choorna

Organoleptic evaluation

Organoleptic evaluation of each ingredient and final product involves assessing the formulation based on its colour, odour, and taste. The organoleptic properties of given mixed *churna* was examined using the procedure outlined by using Siddiqui et al. (Table no.2,3,4) (5).

Physicochemical investigations

Physiochemical analysis of formulations was operated, the assessment of extractive values, total ash, acid insoluble ash, foreign matter, Ph and loss of drying. Standards followed the guidelines of the *Ayurvedic Pharmacopoeia of India* "Table 4." (3).

Determination of flame photometry

Na and K content in AC was determined using Systronics flame phototmeter 128 (μC, Sr. No. 8951, FPM compressor 126). Calibration was performed using Sigma Aldrich sodium chloride & potassium chloride standards over a 10 to 100 ppm range. Sample ash was prepared in muffle furnace, treated with HCL. The concentration applied for this acid treatment was 2 N hydrochloric acid (HCL) & filtered through Whatman ashless filter paper, resulting filtrate was used for flame photometric measurements "Table 5." (4).
Determination of Microbial load: "Table 5."
Determination of Phytochemicals: "Table 5."(5)

GC-MS analysis: as shown in Figure 4

1 g of the sample was elicited with 10 ml of acetone and 1 μL of the filtrate was examined using a Shimadzu GC-MS-QP2010SE system (Shimadzu Corporation, Kyoto, Japan) equipped with a capillary column (0.22 mm i.d., 40 m length) and helium as carrier gas. The oven T. program was: 80 °C (2 minutes), ramped @10 °C min^{-1} to 280 °C (10 minutes), then @20 °C min^{-1} to 330 °C (5 minutes). The injector was conducted in split mode (10:1) @ 280 °C, with a column flow of 1.50 mL min^{-1}, total flow of 19.5 mL min^{-1}, purge flow of 3.0 mL min^{-1}, linear velocity of 45.1 cm sec^{-1} & inlet pressure of 24.2 kPa. The ion source and interface temperatures was set at 200 °C & 300 °C, respectively, with a solvent cut time of 1.40 min. Data was acquired in scan mode from 2.00-3.00 min, with a detector gain of 0.95 kV, this method is validated, adopted from previously published and validated data with minor modifications (6), Central Instrumentation Facility of Nisargam Pvt. Limited Shivamogga- Karnataka, India. (Smith et al.2018; Zhang et al.2020. "Table 6." (5) and "as shown in Figure 4.

Analytical Results

Results

Pharmaceutical findings "Table 2, 3" and Analytic findings 'Table 4, 5, 6"

Table 2: Weight observation of raw drugs and their fine powder

Ingredient	Form	Weight (gm)	Weight loss
Amalaki	Raw	200 gm	50 gm
	Fine Powder	150 gm	
Haritki	Raw	200 gm	40 gm
	Fine Powder	160 gm	
Citraka	Raw	200 gm	30 gm
	Fine Powder	170 gm	
Pippali	Raw	200 gm	40 gm
	Fine Powder	160 gm	
Saindhava	Raw	200 gm	10 gm
	Fine Powder	190 gm	

Table 3: Organoleptic characters of raw drug and *Amalakyadi churna*

Drug	Part Used	Colour	Taste	Odour
Amalaki	Fruit	Grey to black	Sour and Astringent	-
Haritki	Fruit	Yellowish brown	Astringent	-
Citraka	Root	Reddish to Deep brown	Acrid	Disagreable
Pippali	Fruit	Black to Greenish black	Pungent	Aromatic
Saindhava	-	Pinkish white	Salty	Not specific
Amalakyadi Churna	-	Brown	Salty, Spicy	Pleasant

Table 4: Physicochemical analysis of raw drugs, Saindhava and *Amalakyadi churna* API Standards

Name of the drug	Foreign Matter	Ash readings	Acid insoluble Ash	Water soluble extractive	Alcohol soluble extractive	Ph readings	Test for Sodium	Test for Potassium	Loss on drying
Amalaki	Nil	3.073% (not more than 7 %) (7)	0.694% (not more than 2 %) (7)	52.641%	43.062%	3.09	-	-	-
Haritaki	Nil	2.684% (not more than 5 %) (8)	1.486% (not more than 5 %) (8)	75.084%	48.595%	3.81	-	-	-
Citraka	Nil	2.220% (not more than 3 %) (9)	0.789% (not more than 1 %) (9)	19.120%	17.453%	5.75	-	-	-
Pippali	Nil	6.995% (not more than 7 %) (10)	0.443% (not more than 0.5 %) (10)	35.160%	11.318%	3.10	-	-	-
Saindhav (Rock salt)	-	-	-	-	-	6.98	Present	Present	-
Amalakyadi Churna	-	22.693% (not more than 27 %) (11)	0.493% (not more than 0.6 %) (11)	70.061%	35.517%	3.21	-	-	5.114%

Table 5: Microbial Limit Test, Microbial Load Test as shown in Figure 5. A], Flame Photometry, Phytochemicals and Glycosides of *Amalakyadi churna* (Qualitative & Quantitative)

Micro-organisms	**Results in *Amalakyadi churna***
Escherichia coli	Not present
Staphylococcus aureus	Not present
Pseudomonas aeruginosa	Not present
Salmonella abony	Not present
Name of drug	***Amalakyadi churna***
Total Bacterial Count	No growth
Total Fungal Count	No growth
Name of drug	***Amalakyadi churna***
Total Sodium content	5540 ppm
Total Potassium content	431.1 ppm
Phytochemicals in *Amalakyadi Churna*	**Test result**
Carbohydrates	-ve
Reducing sugar	-ve
Monosaccharides	+ ve
Pentose Sugar	-ve
Non reducing sugar	-ve
Hexose Sugar	-ve
Proteins	-ve
Amino acids	+ ve
Steroids	-ve
Flavanoids	+ ve
Alkaloids	+ ve
Tannins	+ ve
(Positive= +ve, Negative= -ve)	

Table 6: GCMS profile details of *Amalakyadi Churna* with the possible medical role of individual molecule

Peak	**R time**	**Area**	**Area%**	**Height**	**CAS#**	**Name**	**Medical Role**
1	13.166	214196589	41.64	4656767	87-66-1	1,2,3-Benzenetriol	Antimicrobial, antioxidant (12), neuroprotective, anti-inflammatory
2	20.501	65981917	12.83	8804577	18836-52-7	2-4-Decadienamide, N-isobutyl-, (E,E)-	Appetiser, antioxidant, antimicrobial (13), anti-inflammatory, immune-modulatory, neuroprotective
3	9.792	51435076	10.00	2292253	67-47-0	5-Hydroxy-methylfurfural	Antioxidant (14), anti-inflammatory, neuroprotective
4	23.351	47124529	9.16	3015547	18634-87-2	Pipataline	Antimicrobial, Anti-inflammatory, antiparasitic, appetiser, antiparasitic, carminative, gut microbial balance, saliva & enzymes stimulant.
5	11.852	15970932	3.10	3050240	501-52-0	Hydrocinnamic-acid	Not known

6	8.706	14570307	2.83	1863416	28564-83-2	1)4H-Pyran-4-one, 2) 2,3-Dihydroxy-5,6-dihydroxy catechol	1)Antimicrobial, antioxidant (15), anti-inflammatory, neuroprotective. 2)Antioxidant, anti-inflammatory, antimicrobial, neuroprotective.
7	15.571	10092009	1.96	2723388	2595-97-3	D-Allose	Antioxidant (16), neuroprotective, immune-modulatory, antimicrobial
8	5.974	9520358	1.85	651621	56-81-5	Glycerin	Laxative (17), moisturizers
9	7.747	6947809	1.35	1286702	20690-71-5	2-Hexanone, 3-methyl-4-methylene	Not known
10	3.395	5588787	1.09	1297147	98-1-1	Furfural	Soluble in water, an Antioxidant, antimicrobial (18) and anti-inflammatory
11	6.506	5273471	1.03	587364	5926-95-4	2H-Pyran-2,6(3H)-dione	Antioxidant, antimicrobial (19), anti-inflammatory
12	14.440	4437265	0.86	1486365	23261-20-3	1,2:5,6-Dianhydro-galactitol	Not known
13	25.281	3896253	0.76	886079	30511-80-9	(E)-9-(1,3-Benzodioxol-5-yl)non- 8-enoic acid	Anti-inflammatory, antimicrobial, antioxidant, digestive aid, (inhibits-Enzymes-COX, lipoxygenase), neuroprotection
14	5.564	3824099	0.74	1217780	2170-3-8	2,5-Furandione, dihydro-3-methylene	Not known
15	11.211	3317976	0.64	1494113	589-82-2	3-Heptanol	Not known
16	6.236	2933915	0.57	1329482	10230-62-3	2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-on	Antimicrobial, antioxidant, anti-inflammatory, neuroprotective, cytoprotective.
17	7.893	2930128	0.57	774151	611-13-2	Methyl 2-furoate	Antioxidant, anti-inflamatory, antimicrobial.
18	24.023	2856615	0.56	1092128	23512-53-0	E-5-Benzo[d][1,3]dioxol-5-yl-N-isobutyl	Anti-inflammatory, antimicrobial, digestive stimulant, antioxidant, immune-modulatory, neuroprotective.
19	18.584	2826847	0.55	671496	5736-91-4	1) Benzaldehyde, 2) 4Pentyloxy-Hexadecenoic- acid	Antimicrobial, antioxidant, anti-inflammatory, CNS effects.
20	17.628	2526974	0.49	674356	481-42-5	Plumbagin	Antimicrobial, anti-inflammatory (20), antioxidant, neuroprotective, antiparasitic, digestive problems, psychiatric, appetiser.

21	14.540	2457427	0.48	674132	118-65-0	Bicyclo 7.2.0-undec-4-ene-4, 11, 11 trimethyl-	Not known
22	16.108	2470513	0.48	1316256	111-82-0	Dodecanoic acid, methyl ester	Antioxidant, Immune-modulatory, antibacterial (21), anti-inflammatory, immunosuppressi-ve, antioxidant, antispasmodic.
23	11.508	2298248	0.45	587301	141-91-3	1) Morpholine, 2)2,6-dimethyl	Antimicrobial, antidepressants, antioxidant, anti-inflammatory (22).
24	14.340	2300395	0.45	974167	140-10-3	trans-Cinnamic-acid	Antibacterial (23), neuroprotective, antioxidant, anti-inflammatory
25	17.098	2247437	0.44	1210063	7425-74-3	1,6-Anhydro-beta-D-glucofuranose	Antioxidant, antimicrobial.
26	20.604	2288771	0.44	795361	57-10-3	n-Hexadecanoic-acid	Antioxidant, antimicrobial, anti-inflammatory (24).
27	27.934	2275157	0.44	970024	188426-70-2	Piperdardine	Anti-inflammatory, antioxidant antimicrobial, digestive, (may stimulate saliva, digestive enzymes & intestinal motility, antiparasitic.
28	7.341	2051732	0.40	306968	123-76-2	Pentanoic acid, 4-oxo-	Not known
29	20.326	1953014	0.38	856401	112-39-0	Hexadecanoic-acid, methyl ester	Antioxidant, antimicrobial, anti-inflammatory
30	8.131	1905348	0.37	613074	4956-6-3	aS-triazine-3,5-[H,4H]-dione, 6-{dimethylamino}	Not known
31	8.578	1841933	0.36	689372	123-76-2	Pentanoicacid, 4-oxo-	Not known
32	21.880	1841198	0.36	936901	85896-31-7	13-Tetradecenal	Not known
33	21.989	1695379	0.33	454741	24738-51-0	(2E,4E)-N-Isobutyl-dodeca-2,4-dienamide	Not known
34	6.285	1509392	0.29	811790	17793-95-2	cis-1,2-Dihydro-catechol	Antioxidant (25), anti-inflammatory, neuroprotective and detoxification
35	18.258	1462951	0.28	495403	2156-96-9	Decylacrylate	Not known
36	21.323	1389308	0.27	418752	35431-4-0	Naphthalene, decahydro-1,1-dimethyl	Not known
37	5.253	1343614	0.26	454741	108-94-1	Cyclo-hexanone	Antimicrobial (26).
38	21.286	1349917	0.26	811790	477-43-0	Dehydrocostus-lactone	Antioxidant, antimicrobial, anti-inflammatory (27), immune-modulatory, hepatoprotective, neuroprotective.
39	7.165	1205402	0.23	495403	25465-18-3	1,4-Dioxin, 2,3-dihydro-5,6-dimethyl-	Not known

40	10.714	1182449	0.23	418752	3128-7-2	Heptanoic-acid, 6-oxo	Antimicrobial, neurological, antioxidant (28).
41	9.454	1125668	0.22	334168	120-80-9	Catechol	Antioxidant, neuroprotective (29)-inflammatory, detoxification.

Instrument: Shimadzu GCMS-QP 2010SE; Helium carrier gas; Capillary column (0.22 mm ID × 40 m).
Injection: 1 µL, split ratio 10:1, injector T-280°C, interface T-300 °C, ion source T-200 °C.
Oven program: eighty °C (2 minutes)
→ 280 °C @10 °C/minute (10 min stop)
→ 330 °C @20 °C/min (5 min stop).
Flow: Column 1.5 ml/min, total 19.5 millilitre/minute, velocity 45.1 centimetre/second, purge flow 3.0 millitre/minute.
MS conditions: Scan mode, m/z 35–500, scan velocity 1666 u/sec, solvent cut 2 min.

Figure 4: Chromatogram analysis of *Amalakyadi churna*

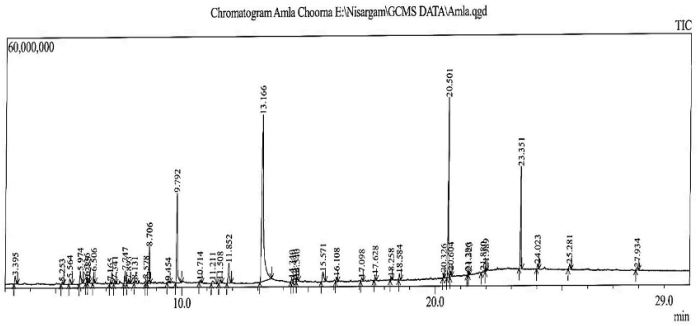
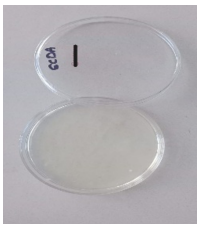
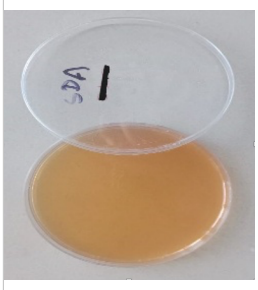
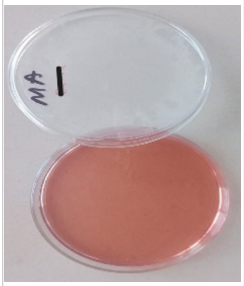
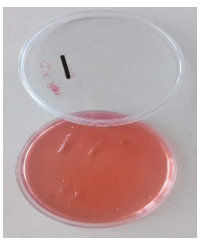
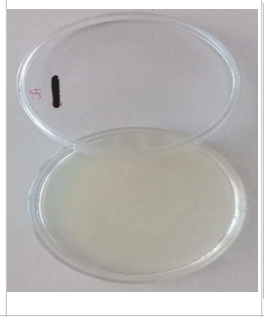
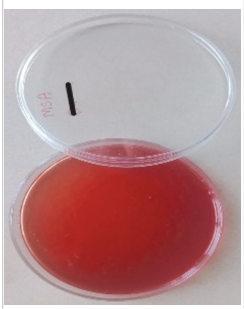


Figure 5. Petri plates of Microbial Load Test

1: Soybean Casein Digest Agar (SCDA) Plate	2: Sabouraud Dextrose Agar (SDA) Plate	3: MacConkey Agar Plate
		
4: Xylose Lysine Deoxycholate (XLD) agar.	**5: Cetrimide agar.**	**6: Mannitol Salt Agar (MSA).**
		

Discussion

Organoleptic profiling is well established in *Ayurveda* pharmaceuticals as a reliable preliminary quality control tool. Hence, applying this approach to AC is scientifically justified for ensuring authenticity and quality evaluation. AC, a classical *Ayurvedic* polyherbal formulation, has been comprehensively tested through organoleptic, physicochemical, microbial and GCMS analysis, which collectively substantiate its therapeutic potential.

Organoleptic profiling revealed a characteristic brown colour, salty spicy taste and pleasant odour, consistent with its ingredients drugs *Amalaki, Haritaki, Chitraka, Pippali & Saidhava- lavana*. Physicochemical evaluation showed a moderate total ash value (22.693%), high water soluble extractive content (70.061%), low acid insoluble ash (0.493%) and minimal LOD (5.114%), suggest stability, purity and predominance of water soluble phytoconstituents.

Phytochemical testing says the presence of flavonoids, which enhance digestion, exert anti-inflammatory actions and modulate gut harmones (ghrelin & leptin) (30); alkaloids that stimulate gastric secretion & increased nutrient assimilation: tannins with antimicrobial & gastroprotective properties (31); amino acids suggesting to *rasayana* (rejuvenative) (32) activity and essential mineral such as sodium & potassium, which regulate digestive secretions, gastrointestinal motility & cardiovascular functions (33).

GCMS analysis further finds bioactive compounds including pipataline (9.16%), 2e, 4e. n. isobutyldodeca 2,4 Dienamide (12.83%) as potent appetite stimulants & gut motility stimulator; glycerin (1.85%) & fatty acid esters like Dodecanoicacid ester methyl, Hexadecenoic acid ester methyl as ATP sources & palatability stimulator; & plumbagin (0.49%) with digestive stimulant, antimicrobial & antipyretic activity. Abundant phenolic antioxidants, notably 1,2,3-benzenetriol (41.64 %), catechol and cis-1,2-dihydrocatechol, confirmed strong free radical scavenging activity, supporting gut health and mitigating fever induced oxidative stress. Additional compounds include 4H-pyran-4-one derivatives enhancing flavor and compliance, 5-hydroxymethylfurfural (10.00%) with antioxidant and appetite improving potential, dehydrocostus lactone with anti-inflammatory and hepatoprotective effects, and rare sugars eg.D-allose supporting gut mobility as shown in Figure 5. B]

Other identified phytoconstituents, including trans-cinnamic acid, hydrocinnamic acid, n-hexadecanoic acid, cyclohexanone and piperdine, further invest digestive, expectorant, laxative, mucolytic, and antimicrobial properties, validating its *kapha* pacifying and *bhedi* (mild laxative) claims. Microbial analysis says the absence of germs like *Escherichia coli, Salmonella abony*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* demonstrating compliance with *Ayurvedic* Pharmacopoeia safety standards. Such findings highlight that the diverse phytoconstituents of *amalakyadi churna* act synergistically to increase digestion, improving appetite, regulate bowel function,

alleviate fever, gave antioxidant defense and support systemic rejuvenation.

After GCMS analysis, compounds was identified from library - NIST20. Organoleptic evaluation: confirms authenticity & consistency, Physicochemical analysis: checks purity, moisture, flame photometry: Assesses mineral(Na,K) content, Microbial testing: ensures safety from harmful microoganisms, Phytochemical screening: Identifies bioactive compounds supporting efficacy, GCMS profiling: Provides chemical fingerprint for composition and batch consistency.

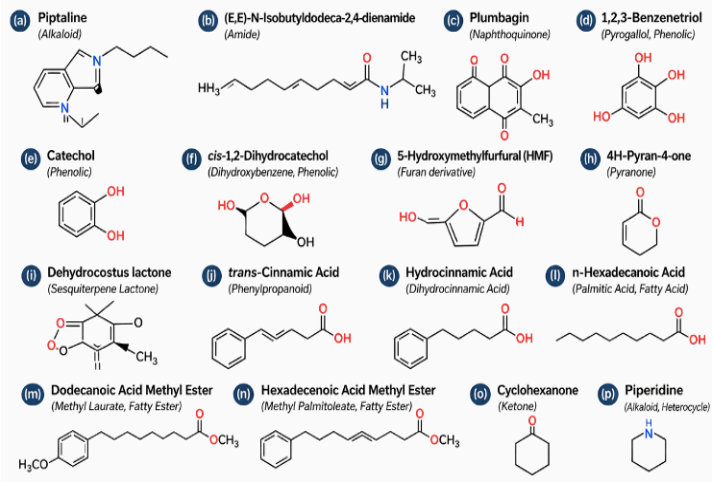
GC–MS analysis of *Amalakyadi Churna* showed clear molecular ion peaks (M^+) and characteristic fragmentation patterns consistent with the detected phytoconstituents, clarify their structural reliability. Phenolic compounds eg.1,2,3-benzenetriol (pyrogallol) (M^+ m/z 126), catechol (M^+ m/z 110) and cis-1,2-dihydrocatechol (M^+ m/z 114) exhibited fragmentation via loss of hydroxyl groups (–OH, 17 Da) and dehydration, creating stable aromatic ions at m/z 109, 93 and 81. 5-Hydroxymethylfurfural (M^+ m/z 126) and 4H-pyran-4-one derivatives (M^+ m/z 114) mentioned cleavage of aldehyde and furan rings with formation of fragment ions at m/z 97, 81 and 69 due to loss of CHO and CO units. Fatty acid derivatives such as n-hexadecanoic acid (M^+ m/z 256), dodecanoic acid methyl ester (M^+ m/z 214) and hexadecenoic acid methyl ester (M^+ m/z 268) showed typical ester and acid fragmentation involving McLafferty rearrangement, producing significant ions at m/z 74 (base peak for methyl esters), along with step by step losses of CH_3 and C_2H_5 groups. Alkaloidal and amide compounds including piperidine (M^+ m/z 85) and (E,E)-N-isobutyldodeca-2,4-dienamide (M^+ m/z 225) manifested nitrogen-centered fragmentation giving iminium ions at m/z 70 and 86 due to α-cleavage close nitrogen atom. Plumbagin (M^+ m/z 188) showed characteristic quinone fragmentation with peaks at m/z 173 and 159 due to loss of CH_3 (15 Da) and CO (28 Da). Likewise, trans-cinnamic acid (M^+ m/z 148) and hydrocinnamic acid (M^+ m/z 150) suffered decarboxylation (loss of CO_2, 44 Da) forming fragments at m/z 104 and 106, followed by aromatic ring cleavage. Dehydrocostus lactone (M^+ m/z 232) exhibited sesquiterpene lactone fragmentation with loss of CO and CO_2 units, generating ions at m/z 214 and 189. Cyclohexanone (M^+ m/z 98) displayed α-cleavage producing m/z 55 as a major fragment. Overall these molecular ion peaks and diagnostic fragment ions align with NIST library spectral matches, Hence strongly validating the GC–MS detection of bioactive constituents in the *Amalakyadi churna*.

GC–MS data analysis has been throughly reassessed by correlating the finding chemical constituents with their corresponding mass spectral fragmentation patterns, molecular ion peaks (m/z values), and characteristic diagnostic ions, instead of depending primarily on database matching. Each compound was critically assessed established on its chemical structure, expected fragmentation behavior and consistency with reported literature data and known phytochemical profiles of the ingredients of *amalakyadi churna*. If feasible, plausible fragmentation pathways (such as loss of CH_3, CO, CO_2, and cleavage of ester, amide and aromatic bonds) were examined to confirm the identified m/z values.

Ash values identified in the present study were observed to be under permissible limits as per *Ayurvedic* Pharmacopoeia of India (API) for *ayurvedic* drug standardization, indicating good purity and minimal inorganic contamination. Acid insoluble ash values also verified the absence of excessive siliceous matter and adulteration. The relatively higher ash value of *Amalakyadi Churna* may be due to the presence of *Saindhava* and mixed mineral constituents in the formulation (API) as shown in Table 4.

Figure 5.B] (A) Chemical structures of Bioactive Compounds identified in *Amalakyadi choorna.*

(a) Piptaline, (b) (E,E)-N-Isobutyldodeca-2,4-dienamide, (c) Plumbagin, (d) 1,2,3-Benzenetriol (Pyrogallol), (e) Catechol, (f) cis-1,2-Dihydrocatechol, (g) 5-Hydroxymethylfurfural (HMF), (h) 4H-Pyran-4-one, (i) Dehydrocostus lactone, (j) trans-Cinnamic acid, (k) Hydrocinnamic acid, (l) n-Hexadecanoic acid (Palmitic acid), (m) Dodecanoic acid methyl ester (Methyl laurate), (n) Hexadecenoic acid methyl ester (Methyl palmitoleate), (o) Cyclohexanone, (p) Piperidine.



Conclusion

The standard procedures used in this study based on *Ayurvedic* Pharmacopoeia of India (API) guidelines for the preparation rather than WHO or ICH guidelines. The prepared AC evaluate for its quality confirms the standard mentioned in API. The GCMS profiling of AC shows that the phytoconstituents present in AC having the property like improving appetite, digestion, to decrease fever also supports in ayurveda indications like bhedan (breaking) and kapha pacifier (reduce phlegm).

Abbreviations

AC: *Amalakyadi choorna* ; **GCMS**: Gas Chromatography Mass Spectrometry; **API**: *Ayurvedic* Pharmacopoeia of India, KAHER: KAHER's Shri BM Kankanawadi *Ayurveda* Mahavidyalaya; LOD: Loss of Drying; Ph: pH (Potential of Hydrogen); Na: Sodium; K: Potassium; R time: Retention time; CAS#: Chemical Abstracts Service Registry Number; +ve: Positive; -ve: Negative; MS: Mass Spectrometry; ppm: parts per million, eg. Example.

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