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RESEARCH ARTICLE

Quality control assessment of *Amlak kshudbodhak*: The traditional recipe of Ayurveda

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Abstract

Amlak kshudbodhak (AK) is a promising appetizer, offering a sweet and sour flavour. It is usually taken half an hour before a meal because it helps in digestion and nutrient absorption. In the present study, pharmacognostic standardization, physicochemical properties, antioxidant and quality control evaluation of AK was conducted. According to standard procedures, the AK recipe was analyzed for phytochemical identification, total flavonoids, total phenolic content, *in vitro* radical scavenging assays, high performance thin layer chromatography (HPTLC) analysis, microscopical characteristics, physicochemical properties and the presence of aflatoxin, heavy metals, essential minerals and pesticides. HPTLC profiling confirmed that the AK extract contains a range of phytochemicals. *In-vitro* radical scavenging assays showed significant, dose-dependent inhibition by AK aqueous (AQ), alcoholic (AL) and hydroalcoholic (HA) extracts. Powder microscopy of AK revealed polygonal, thin and straight walled epicarp cells, fibers with pits, broken fragments of tracheids, lignified spiral thickenings, stone cells and elongated, irregular mesocarpic cells of *Phyllanthus emblica* L. Furthermore, aflatoxins, heavy metals, minerals and pesticides were assessed according to standard limits, along with total ash, acid-insoluble ash, water-soluble extractive value and alcohol-soluble extractive value. The results of the study showed that AK exhibited the highest antioxidant activity and was free from aflatoxin, heavy metals and pesticide residues. However, essential minerals were detected and microscopical studies confirmed the presence of medicinal plant material. This recipe may be beneficial for individual with malabsorption syndrome, as it is rich in vitamin C, helps prevents communicable and non-communicable diseases and may reduce the risk of hepatitis.

Keywords: aflatoxin; antioxidant; Ayurveda; *Phyllanthus emblica*; pharmacognostical study; traditional recipe

Introduction

Food is given par importance in Ayurveda, a traditional Indian system of medicine, as it nurtures human beings. Proper selection of food, it's processing, quantity and dietary schedule is described in detail in Ayurveda, helping to maintain holistic health and happiness. Diet plays a vital role in maintaining and improving immune response because it provides the nutrients that support it. Ayurvedic literature discusses the undeniable advantages of traditional Indian recipes for boosting immunity. Hence, it is essential to identify such a traditional wealth of knowledge, document traditional food recipes and validate their immune-enhancing potential through modern techniques.

Amalaki, also known as amla and botanically identified as *Phyllanthus emblica* L., is highly valued in Ayurveda for its therapeutic and health benefits. This fruit is reported as the best *Vayastapana* (anti-aging) and one of the best Rasayana Dravyas (rejuvenating drug). Amla is also classified as Nitya Sevaniya Dravya (to be consumed daily) (1). In addition to its immunomodulatory effects, it has multiple therapeutic benefits, such as antioxidants, anti-inflammatory properties and anti-aging properties (2, 3). Fresh amla fruit has a shelf life of 5 to 6 days and is susceptible to early spoilage (4). However, with advances in food technology, amla is now processed into various products such as juice, squash, candy, pickle, murabba, jam, jelly, mouth freshener, ice cream and sauce (5).

Considering the health benefits of amla, it is essential to explore traditional recipes from ancient Ayurvedic texts. In this study, a recipe called as AK in Kshemkutuhalam was selected. To ensure the quality of ingredients and to document the morphological changes occurring during the processing, microscopy of the final recipe was performed, along with tests for aflatoxins, pesticides and heavy metals. A preliminary physicochemical analysis of AK was also conducted.

Materials and Methods

Kshemkutuhalam

This Sanskrit text provides Ayurvedic dietetics recipes and detailed information on a variety of food elements. A recipe containing amla as the main ingredient, reported with therapeutic uses equivalent to immunomodulating activity, was selected from this book (6).

Procurement of food recipe

AK was prepared according to the recipe described in the Kshudbodhak yoga section (6). After decoding the Sanskrit verse, its raw ingredients and method of preparation were finalized by Ayurveda experts. All functional raw materials were obtained from an Ayurvedic store and authenticated by the Institute Botanist Dr. Arun Gurav, Research officer (Botany), Regional Ayurveda Research Institute, Pune, Maharashtra. Samples are stored in the institute museum with voucher specimen numbers: such as *Phyllanthus emblica* (amla) (14842), *Curcuma longa* (turmeric) (15348), *Piper nigrum* (black pepper) (15721) and *Zingiber officinale* (ginger) (4132).

Preparation of the recipe

In Ayurveda, fruit of amla is considered as Rasayana (Rejuvenator) and consists of five Rasas (tastes) except salt. Thus, it is blend of six tastes if mix with salt and become complete food. As its name suggest, AK consist mainly appetizer like asafoetida, ginger, turmeric, black pepper and long pepper, which work synergistically to improve digestion, enhance taste perception and help for metabolism. Due to the Yogavahi (carrier effect) property of pippali, results into enhancing the effectiveness of other ingredients. Rich source of vitamin C and combination of appetizer make this recipe blend of immunomodulator and improve gut health. Recent research suggests amla improves digestion, malabsorption and immune modulatory and improves gut health (6). Thus, this recipe can help to modify weak digestive system, enhance metabolism and modulate immunity also.

Excessive stress, poor diet and modern lifestyles are known to contribute to Agni Mandya (weakened digestive fire) and Aruchi (loss of appetite). In addition to providing symptomatic relief, amlak-based appetite stimulants improve agni and correct dosha imbalances. Its uniqueness can be adapted into multiple palatable forms, such as churna, syrup and candy, thereby enhancing patient compliance, especially in paediatric and geriatric settings. Recent research suggests amla improves digestion, malabsorption, immune-modulatory and gut health (7).

Fig. 1, the recipe AK was prepared by grating amla fruit finely, placing in a bowl, greased with ghee and then adding fine powder of asafoetida, turmeric, black pepper, long pepper, dry ginger, rock salt and edible sesame oil and mixing well.

High performance thin layer chromatography (HPTLC)

A precise weight of 2.74 g of AK lyophilized powder was measured in 10 mL of methanol. Sonicated for 15 min at 25 °C, centrifuged at 10,000 rpm for 10 min and supernatant was collected in a glass vial for HPTLC analysis. Mobile phase contained, toluene: ethyl acetate: methanol: formic acid: glacial acetic acid (6: 8: 3: 1: 1 v/v/v/v/v). Utilizing a pre-coated

Fig. 1. Final product of *Amlak kshudbodhak.*

silica gel 60F254 thin layer chromatography (TLC) plate (Merck) with a consistent 0.2 mm thickness, apply 10 µL of AK on different tracks. The plate develops for up to 80 mm in the specified mobile phase. After the plate had dried, observed at 254 nm and 366 nm wavelengths. In the stage of derivatization, heat the plate at 105 °C until the colour of the bands or spots appears after dipping it in the natural product reagent (NP) and the anisaldehyde sulphuric acid reagent (ASR) (8).

Preparation of extract

In Ayurveda, mainly it has been advised to consume formulations either in the fresh form like juice, paste or dried form like powder, decoction and processed form like Asava and Arishta (fermented form and self-generated alcohol). Thus, use of aqueous, alcoholic and hydroalcoholic extracts of this recipe falls in accordance with scientific principles related to phytochemistry, solubility and bioavailability (9). These extraction methods enable bioactive compounds to be isolated and concentrated according to their chemical nature, ensuring maximum therapeutic efficacy and bioavailability. Aqueous solvents extract polar compounds, such as glycosides, tannins, polysaccharides, flavonoids and vitamins. Alcoholic solvents extract non-polar or moderately polar compounds, such as alkaloids, essential oils, resins and flavonoids. A hydroalcoholic solvents is a broad-spectrum extraction method that combines the advantages of both solvents. It is useful when a plant contains both polar and non-polar active constituents. Hydroalcoholic extracts often exhibit superior pharmacological activity *in vitro* and *in vivo* studies.

Coarse powder of 50 g of AK was soaked into 250 mL of solvent and kept for shaking for 48 hr at room temperature. After 48 hr, the solution was filtered by Whatman filter paper no.1. Hydro alcoholic and alcoholic filtered solutions were processed for rotary vacuum evaporator for removal of alcohol. After that aqueous, alcoholic and hydroalcoholic solutions were further processed for lyophilisation (Model-Free Zone 4.5 Liter Benchtop Freeze Dryers, Labconco, USA) to make concentrated powder of prepared extract.

Determination of flavonoid content

The aluminium chloride colorimetric method had been employed to ascertain total flavonoid contents (10). Sodium nitrite ($NaNO_2$) (0.3 mL of 5 %) was combined with 1 mL of aqueous extract in a flask holding 4 mL of distilled water using a pipette. Aluminum chloride ($AlCl_3$) (0.3 mL of 10 %) had been added after 5 min. After 6 min, this mixture was combined with 2 mL of 1 M sodium hydroxide (NaOH). Following that, distilled water had been added to make the mixture's volume to 10 mL. The reaction mixture's absorbance at 510 nm was measured at a multimode reader, Synergy HTX, Biotek, USA.

Determination of phenolic content

The Folin–Ciocalteu method had been used to calculate the total phenolic content for each extract (11). 1 mL of AK aqueous, alcoholic and hydroacloholic extracts and 1 mL of Folin-Ciocalteu reagent were combined. Following a 5 min incubation period, 4.0 mL of sodium carbonate (Na_2CO_3) was added to the mixture and distilled water was added to bring the volume to 10 mL. The solution was centrifuged for five minutes at 2000 rpm after being allowed to stand at room temperature in the dark for two hours to measure the absorbance at 760 nm was measured in multimode reader, Synergy HTX, Biotek, USA.

DPPH assays

The aqueous, alcoholic and hydro-alcoholic extract's capacity to scavenge DPPH radicals was assessed (12). 100 µL of tris-buffer was added to a DPPH solution (0.1 mM DPPH in methanol) along with 25 µL of AK and standard ascorbic acid. After that, 125 µL of DPPH solution was thoroughly added as well as allowed to stand for 30 min at room temperature and absorbance at 517 nm was measured in multimode reader, Synergy HTX, Biotek, USA.

FRAP assays

FRAP assay was used to calculate the reduction of AK extracts (13). 1.5 mL of potassium ferric cyanide (1 %), 1.5 mL of phosphate buffer (0.2 M, pH = 6.6), along with 0.5 mL of AK and standard BHT had been mixed. The mixtures incubate for 20 min at 50 °C. After adding 1.5 mL of 10 % trichloro acetic acid (TCA) to the mixture, the mixture had been centrifuged for 10 min at 6000 rpm. 1.5 mL of the solution's upper layer, 1.5 mL of distilled water and 0.3 mL of ferric chloride ($FeCl_3$) (0.1 %) were mixed. In the presence of AK and BHT to study the Fe^{3+}/Fe^{2+} transformation and absorbance values were measured at 700 nm in a multimode reader, Synergy HTX, Biotek, USA.

$ABTS^{\cdot+}$ assays

The decolourization assay is predicated on Wolfenden and Wilson's extracts with antioxidant method of reducing the $ABTS^{\cdot+}$ radical (14). A volume of 10 µL of AK and standard ascorbic acid had been added to 990 µL of $ABTS^{\cdot+}$ free radical solution, as well as the mixture had been left in the dark at room temperature for 60 min and absorbance was measured at 734 nm in a multimode reader, Synergy HTX, Biotek, USA.

Macroscopic and organoleptic analysis

The macroscopic as well as organoleptic features of AK had been observed and documented (15).

Powder microscopy

One mg of lyophilized powder of AK of the sample was stained on a different slide using 0.1 % w/v phloroglucinol and a 10 % diluted hydrochloric acid (HCl) solution, washed with water and mounted in 50 % glycerine. Furthermore, safranin (1 % safranin solution in 50 % ethyl alcohol) was added to stain. The microscopical characteristics of the prepared slides had been assessed with an Olympus BX 43 and LC 30 camera (16, 17).

Physicochemical analysis

The standard pharmacopeial guidelines were followed in the physicochemical investigations, which included loss on drying, total ash value, acid insoluble ash value, alcohol soluble extractive, water soluble extractive and pH (18, 19).

Aflatoxin analysis

The test for aflatoxins (B1, G1, B2 and G2) had been determined using a standard method according to standard pharmacopeial guidelines (18-20).

Heavy metal analysis

The heavy metal analysis for lead (Pb), arsenic (As), mercury (Hg) and cadmium (Cd) was determined by using standard pharmacopeial methods (18-20).

Essential minerals analysis

Pharmacopeial methods were used to study essential minerals like iron (Fe), calcium (Ca), sodium (Na), potassium (K), magnesium (Mg), copper (Cu), manganese (Mn), phosphorous (P), chromium (Cr) and zinc (Zn) was determined using standard pharmacopeial methods (18-20).

Pesticides residues analysis

The pesticide residues had been determined using standard pharmacopeial methods of different pesticides (18-20).

Statistical analysis

Graphpad Prism version-5 (California, United States) for Windows was used to conduct statistical analysis. For each group, the values have been presented as mean ± SD for 3 replicates, with significant level of $p < 0.05$.

Results

HPTLC analysis

The phytochemical screening studies have been carried out by quantitative HPTLC analysis. Table 1 shows that HPTLC analysis of AK with standards quercetin, ellagic acid, 6-gingerol and ascorbic acid. Fig. 2-4 were obtained by scanning at UV 254 nm and 366 nm, respectively. The Rf values of the methanolic extract of AK have been screened for phytochemical identification. HPTLC quantitative testing revealed that the methanolic extract of AK contained presence of quercetin and their Rf value was 0.62, ellagic acid and Rf value was 0.46, 6-gingerol Rf value was detected as 0.68 and ascorbic acid Rf value was detected as 0.12.

The Rf values of peak area, peak height and unknown substance of peak area depicted in Table 2. The Rf values calculated for the phytoconstituents present in the AK extract would be helpful in identifying the unknown compounds by comparing them with the reference standards and from the values of peak area, the concentration of the compounds can

Table 1. HPTLC analysis of *Amlak kshudbodhak* in comparison with standards quercetin, ellagic acid, 6-gingerol and ascorbic acid

S. No.	Track No.	Name of standards			
		Quercetin	**Ellagic acid**	**6-gingerol**	**Ascorbic acid**
		Rf- 0.62	Rf- 0.45	Rf- 0.68	Rf- 0.13
1	15	Detected Rf- 0.61	Detected Rf- 0.46	Detected Rf- 0.68	Detected Rf- 0.12

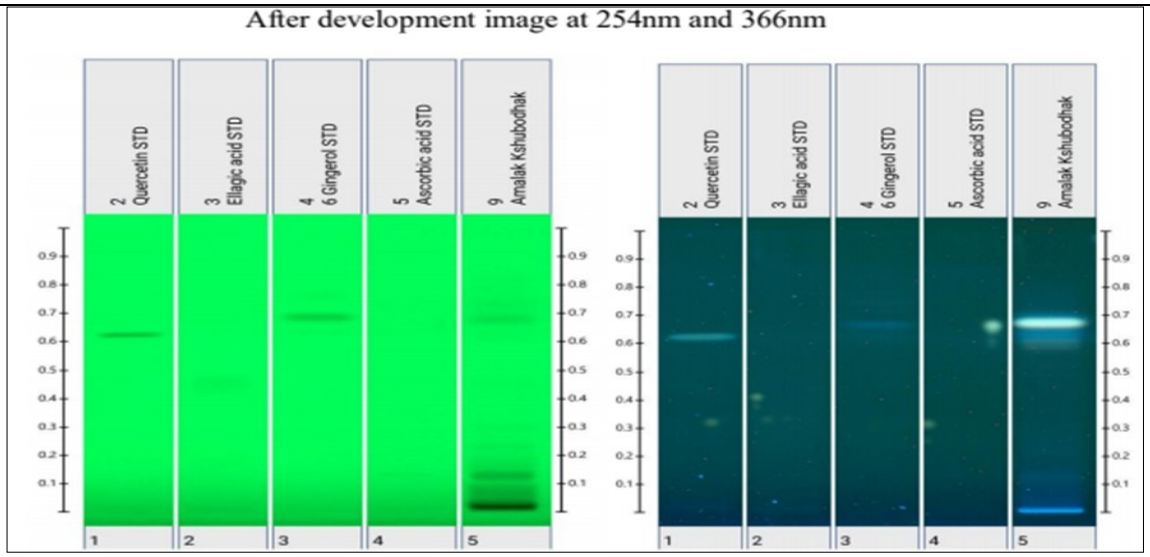

Fig. 2. HPTLC development image of standards and *Amlak kshudbodhak* at 254 nm and 366 nm.

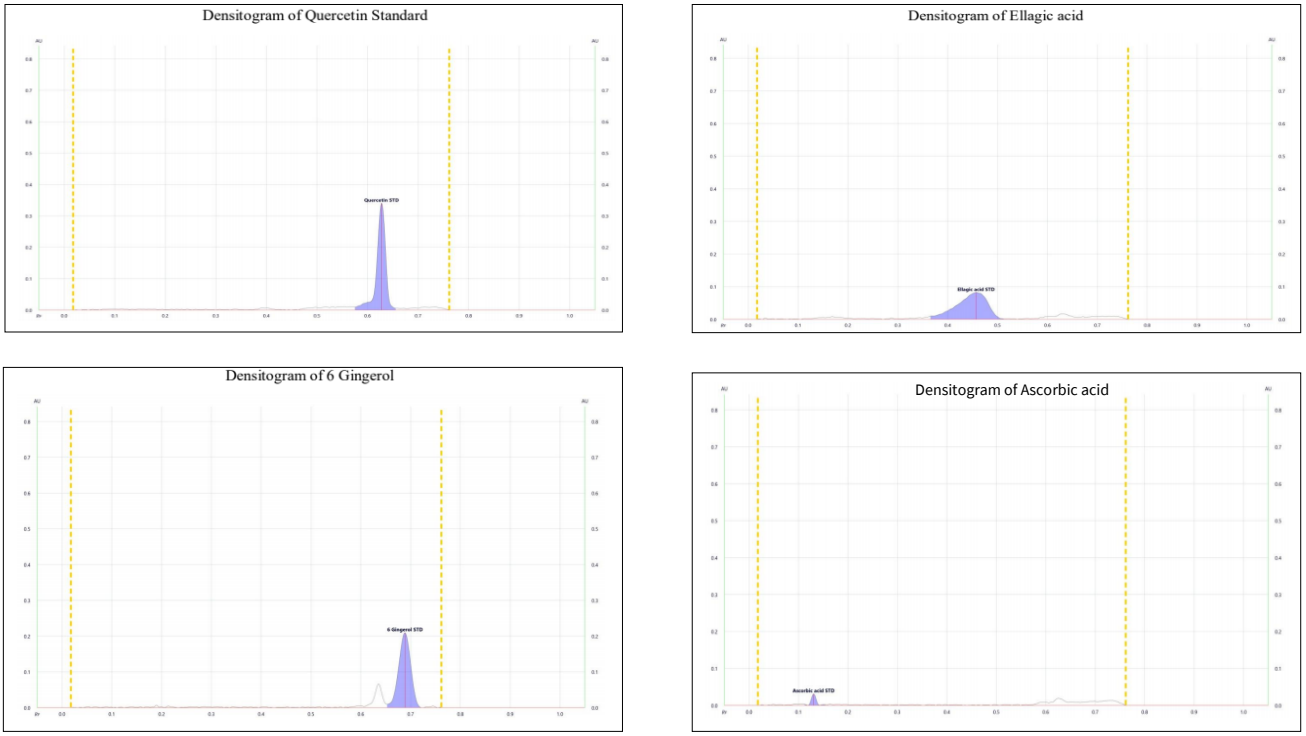

Fig. 3. HPTLC densitometry of quercetin, ellagic acid, 6-gingerol and ascorbic acid standards.

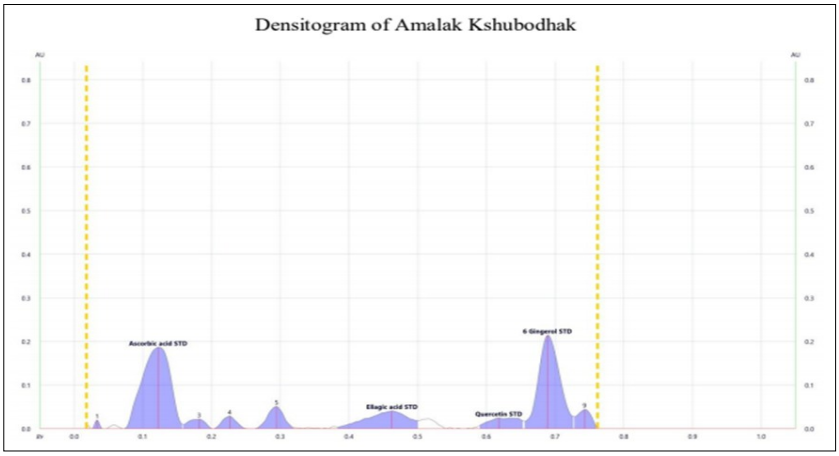

Fig. 4. HPTLC densitometry of *Amlak kshudbodhak.*

be determined. The TLC plates visualized under white light and UV at wavelengths of 254 nm and 366 nm show bands of separated compounds (Fig. 2).

Total phenolic content and total flavonoid content

The total phenolic content of AK was calculated using the linear regression equation of the tannic acid standard calibration curve using the Folin-Ciocalteu method. A food recipe extract was evaluated for its total phenolic content in mg of tannic acid equivalents per g dry weight. AK of HA extract has higher phenolic content (223.59 ± 7.8 mg TAE/g) as followed by AQ (192.51 ± 0.03 mg TAE/g) and AL extract (219.91 ± 6.26 mg TAE/g). The flavonoid content of AK was estimated using aluminium chloride. The total flavonoid content of HA extract of AK contains higher flavonoid content (32.64 ± 1.72 mg QAE/g), as compared to AQ (25.6 ± 2.26 mg QAE/g) and AL extract (28.29 ± 2.09 mg QAE/g).

DPPH assay

Based on the results, the purple colour of the solution decreased as a concentration-dependent response to AL, AQ and HA extracts of AK inhibited the formation of DPPH radicals (Table 3, 4 and Fig. 5). According to a linear regression analysis of concentration and percent inhibition, DPPH scavenging is concentration dependent. The regression coefficients for AL, AQ and HA extracts of amlak were 0.971, 0.975 and 0.978, respectively. Amlak extracts containing AL, AQ and HA had IC_{50} values of 92.94 µg/mL, 76.94 µg/mL and 83.45 µg/mL, respectively.

FRAP assay

A concentration-dependent reduction of ferricyanide to ferrocyanide was demonstrated by a rise in green absorbance at 700 nm for the standard ascorbic acid, AQ, HA and AL extracts of AK. The concentration versus absorbance is plotted for butylated hydroxytoluene (BHT) compared to extracts of AK at concentrations between 3.9 and 1000 µg/ml (Table 5, 6 and Fig. 6).

$ABTS^{\cdot+}$ assay

Table 7-10 and Fig. 7 indicates that extracts of AK at concentrations ranging from 40 to 400 µg/mL scavenged $ABTS^{\cdot+}$ radicals, as evidenced by a concentration-dependent decrease in solution blue. There was a concentration-dependent scavenging of $ABTS^{\cdot+}$ by AL, AQ and HA extracts of AK with regression coefficients of 0.98, 0.99 and 0.99, respectively. Based on regression curve analysis, the IC_{50} for AL, AQ and HA extracts was determined to be 142.23 µg/mL, 179.70 µg/mL and 176.85 µg/mL, respectively.

Powder microscopic observations

Microscopic evaluation of AK observed that the powder characters of 4 ingredients (*P. emblica* L., *Piper longum* L, *Z. officinale,* roscoe, sesame oil or cow ghee) are present in final food recipe (Table 11 and Fig. 8).

Physicochemical studies

Physico-chemical properties of the ingredients are shown in order to evaluate purity, quality and adulteration of food recipes (Table 12). Total ash, acid insoluble ash, water soluble extractive value, alcohol soluble extractive value and loss on drying are the determining factors.

Aflatoxin testing

Aflatoxin was determined by high-performance liquid chromatography (HPLC) with fluorescence detection (FLD) to be below the limit of quantification. AK samples were processed for aflatoxin (B1, B2, G1 and G2) as given in Table 13.

Test for heavy metals

Heavy metal contamination was carried out using inductively coupled plasma-mass spectrometry (ICP-MS) and atomic absorption spectroscopy (AAS). The results of heavy metal testing of the AK showed that Pb, As, Hg and Cd concentrations were below the limit of quantification (Table 14).

Test for mineral content

As shown in Table 15, AK was processed to determine the presence of essential minerals such as Fe, Ca, Na, K, Mg, Cu, Mn, P, Cr and Zn. The results revealed that the mineral content in AK was found in substantial quantity.

Pesticides content

All pesticides detected by LC-MS/MS and GC-MS/MS in AK were below detection limits (Table 16).

Discussion

In traditional herbal medicine, *P. emblica* (amla) has been used as a treatment for rheumatoid diseases, gonorrhoea, asthma, haemorrhage, jaundice, dyspepsia, nausea, constipation, diarrhoea, diabetes mellitus, coronary heart disease and various cancers (21). There have been numerous studies showing that amla has hypoglycaemic, anti-inflammatory, anti-hyperglycaemic, anti-hyperlipidemic and antioxidant properties (22-24). These properties may be explained by the fact that amla fruit is rich in vitamins C, tannins, polyphenols, fiber, minerals, proteins and amino acids (25). Among the antioxidants contained in amla fruits are tannins, ellagitannins, chebulagic acid, corilagin, geraniin and elaeocarpusin (26). Due

Table 2. HPTLC densitometric quantification of peak area comparison with standards

S. No.	Max RF	Height	Height %	Start RF	Start height	End RF	End height	Area	Area %	Assigned to
1	0.033	0.0196	3.10	0.026	0.0000	0.042	0.0000	0.00015	0.60	Not assigned
2	0.122	0.1867	29.55	0.072	0.0000	0.158	0.0093	0.00902	36.36	Ascorbic acid STD
3	0.182	0.0218	3.45	0.158	0.0093	0.201	0.0000	0.00064	2.57	Not assigned
4	0.226	0.0288	4.56	0.203	0.0000	0.253	0.0000	0.00067	2.71	Not assigned
5	0.294	0.0512	8.11	0.263	0.0000	0.321	0.0019	0.00138	5.58	Not assigned
6	0.463	0.0408	6.46	0.383	0.0036	0.500	0.0183	0.00285	11.52	Ellagic acid STD
7	0.618	0.0245	3.88	0.590	0.0071	0.653	0.0182	0.00128	5.16	Quercetin STD
8	0.689	0.2413	33.93	0.656	0.0172	0.728	0.0275	0.00777	31.32	6-gingerol STD
9	0.743	0.0440	6.96	0.728	0.0275	0.761	0.0007	0.00104	4.19	Not assigned

Table 3. DPPH scavenging activity of standard ascorbic acid

Ascorbic acid (µg/ml)	Scavenging activity (%)	IC_{50} (µg/ml)
2.5	17.69 ± 1.60	
5	25.37 ± 1.70	
10	31.21 ± 2.20	
12.5	43.18 ± 2.10	
15	57.45 ± 0.48	
17.5	61.86 ± 0.66	
20	68.20 ± 0.65	14.47 ± 0.94
22.5	70.52 ± 1.01	
25	74.70 ± 1.20	
27.5	80.61 ± 1.65	
30	91.26 ± 0.94	

Note: Values given as mean ± SD of three experiments in each group.

Table 4. DPPH scavenging activity of *Amlak kshudbodhak* aqueous (AQ), alcoholic (AL) and hydroalcoholic (HA) extract

Amlak kshudbodhak	**Scavenging activity (%)**		
(µg/mL)	**HA**	**AQ**	**AL**
20	13.33 ± 1.20	13.21 ± 0.20	12.43 ± 1.72
40	25.40 ± 0.20	27.91 ± 2.27	27.02 ± 2.24
60	38.73 ± 2.27	38.48 ± 3.20	41.04 ± 2.40
80	47.62 ± 1.21	50.53 ± 4.29	50.58 ± 5.71
100	58.52 ± 3.20	62.68 ± 4.27	58.82 ± 7.11
120	70.48 ± 2.20	66.60 ± 2.24	67.20 ± 6.78
140	73.54 ± 4.20	70.19 ± 2.27	70.23 ± 5.20
160	77.25 ± 6.21	72.62 ± 6.60	72.40 ± 6.25
180	79.47 ± 4.22	79.92 ± 7.20	80.78 ± 8.24
200	86.14 ± 6.33	87.10 ± 8.22	84.10 ± 4.32
IC_{50} (µg/mL)	83.45 ± 4.58	76.94 ± 6.36	92.94 ± 7.25

Note: Values are given as mean ± SD of three experiments in each group.

Table 5. Reducing power activity of standard-butylated hydroxytoluene (BHT)

BHT (µg/ml)	Scavenging activity (%)	IC_{50} (µg/mL)
3.9	1.97 ± 0.05	
7.8	4.50 ± 0.08	142.05 ± 0.01
15.6	8.33 ± 0.05	
31.25	15.23 ± 0.04	
62.5	27.33 ± 0.01	
125	44.53 ± 0.02	142.05 ± 0.01
250	68.10 ± 0.03	
500	100.33 ± 0.05	
1000	114.87 ± 0.10	

Note: Values given as mean ± SD of three experiments in each group.

Table 6. Reducing power activity of *Amlak kshudbodhak*

***Amlak kshudbodhak* (µg/ml)**	**AL**	**HA**	**AQ**
3.9	0.46 ± 0.03	4.34 ± 0.01	1.38 ± 0.02
7.8	6.93 ± 0.04	10.87 ± 0.02	8.51 ± 0.02
15.6	12.78 ± 0.03	18.19 ± 0.01	17.47 ± 0.02
31.25	25.99 ± 0.05	33.33 ± 0.01	30.87 ± 0.02
62.5	42.13 ± 0.06	50.99 ± 0.02	47.50 ± 0.02
125	59.36 ± 0.02	65.05 ± 0.04	64.55 ± 0.03
250	74.72 ± 0.04	78.39 ± 0.02	77.38 ± 0.02
500	85.33 ± 0.02	88.20 ± 0.04	87.45 ± 0.02
1000	88.80 ± 0.05	90.83 ± 0.06	88.25 ± 0.08
IC_{50} (µg/mL)	88.49 ± 1.05	64.04 ± 3.05	70.70 ± 5.05

Note: Values given as mean ± SD of three experiments in each group. AQ: aqueous, AL: alcoholic and HA: hydroalcoholic extract.

Table 7. $ABTS^{\bullet+}$ radical scavenging activity of ascorbic acid

Ascorbic acid (µg/mL)	Scavenging activity (%)	IC_{50} (µg/mL)
20	5.40 ± 0.49	
40	16.15 ± 3.47	
60	27.38 ± 2.58	
80	43.24 ± 1.90	
100	59.98 ± 10.63	
120	69.05 ± 4.15	20.73 ± 1.02
140	82.73 ± 5.19	
160	92.60 ± 0.20	
180	92.89 ± 0.21	
200	92.25 ± 0.22	

Note: Values given as mean ± SD of three experiments in each group.

Table 8. $ABTS^{\bullet+}$ scavenging activity of alcoholic (AL) extract of *Amlak kshudbodhak*

***Amlak kshudbodhak* (µg/ml)**	**Scavenging activity (%)**	**IC_{50} (µg/ml)**
60	26.40 ±1.89	
120	45.60 ± 2.96	
180	62.08 ±1.07	
240	77.19 ± 0.63	142.23 ± 0.28
300	86.49 ±1.12	
360	91.86 ± 0.48	
420	92.11 ± 0.28	

Note: Values are given as mean ± SD of three experiments in each group.

Table 9. $ABTS^{\bullet+}$ scavenging activity of aqueous (AQ) extract of *Amlak kshudbodhak*

***Amlak kshudbodhak* (µg/ml)**	**Scavenging activity (%)**	**IC_{50} (µg/ml)**
40	15.06 ± 2.11	
80	23.75 ± 3.58	
120	34.74 ± 5.29	
160	48.69 ± 1.06	
200	58.10 ± 4.77	179.70 ± 0.22
240	65.96 ± 4.29	
280	74.50 ± 2.32	
320	84.46 ± 3.33	
360	88.26 ± 1.23	
400	91.00 ± 0.22	

Note: Values are given as mean ± SD of three experiments in each group.

Table 10. $ABTS^{\bullet+}$ scavenging activity of hydro alcoholic (HA) extract of *Amlak kshudbodhak*

***Amlak kshudbodhak* (µg/ml)**	**Scavenging activity (%)**	**IC_{50} (µg/ml)**
40	16.18 ± 1.93	
80	26.88 ± 3.46	
120	35.90 ± 1.48	
160	48.51 ± 2.96	
200	59.84 ± 1.26	176.85 ± 0.08
240	64.81 ± 5.59	
280	75.87 ± 3.93	
320	82.29 ± 3.20	
360	87.14 ± 1.95	
400	91.02 ± 0.08	

Note: Values are given as mean ± SD of three experiments in each group.

Table 11. Microscopic observation of *Amlak kshudbodhak*

S. No.	**Raw ingredient**	**Findings**
1	*P. emblica* L.	1. Polygonal, thin and straight walled cells from epicarp 2. Fibres with pits 3. Broken fragments of tracheids and lignified spiral thickenings 4. Stone cell 5. Elongated, irregular parenchymatous mesocarpic cells
2	*Piper longum* L.	1. Slightly elongated, polygonal shaped cell 2. Lignified, rounded to elongated stone cells 3. Volatile oil globule 4. Simple rounded starch grains 5. Brown matter
3	*Zingiber officinale* Roscoe	1. Parenchyma cells from cortical region embedded with dense starch grains 2. Elongated- septate fiber 3. Spiral and reticulate vessel 4. Oleo resin embedded within parenchymatous cells 5. Starch grains with eccentric hilum
4	Sesame oil	1. Fixed oil globules

Table 12. Physico-chemical parameters of *Amlak kshudbodhak*

S. No.	**Parameters**	**Observation**
1	Total ash	4.46 ± 0.03
2	Acid insoluble ash	1.49 ± 0.01
3	Water soluble extractive value	14.40 ± 1.30
4	Alcohol soluble extractive value	20.27 ± 0.46
5	Loss on drying (LOD)	37.67 ± 0.29
6	pH value (10 % solution)	3.60 ± 0.00

Table 13. Aflatoxin content of *Amlak kshudbodhak*

S. No.	Name of aflatoxin	Results (mg/kg)	LOQ (mg/kg)	FSSAI (µg/kg)
1	Aflatoxin B1	BLQ	0.001	10
2	Aflatoxin B2	BLQ	0.001	10
3	Aflatoxin G1	BLQ	0.001	10
4	Aflatoxin G2	BLQ	0.001	10
5	Total aflatoxin	BLQ	-	20

Note: BLQ: below limit of quantification, LOQ: limit of quantification.

Table 14. Heavy metal content of *Amlak kshudbodhak*

S. No.	Name of elements	Result (mg/kg)	LOQ (mg/kg)	FSSAI (mg/kg)
1	Arsenic	BLQ	0.1	0.1
2	Cadmium	BLQ	0.1	0.1
3	Chromium	BLQ	0.25	1.0
4	Lead	BLQ	0.1	0.02
5	Mercury	BLQ	0.03	0.1

Note: LOQ: limit of quantification.

Table 15. Essential mineral content of *Amlak kshudbodhak*

S. No.	Name of elements	Result (mg/kg)	LOQ (mg/kg)	FSSAI (mg/kg)
1	Copper	1.48	0.25	5.0
2	Zinc	1.015	0.25	0.01
3	Calcium	371.7	0.25	5.0
4	Sodium	4245	0.25	2.0
5	Manganese	2.71	0.25	0.01
6	Magnesium	152.7	0.25	0.01
7	Iron	9.62	0.25	2.0
8	potassium	1576.0	0.25	3.0
9	phosphorous	2495.0	0.25	0.01

Note: LOQ: Limit of quantification.

Table 16. Pesticides content of *Amlak kshudbodhak*

S. No.	Name of pesticide	Result (mg/kg)	LOQ (mg/kg)	Equipment used	FSSAI (mg/kg)
1	Aldrin (aldrin and dieldrin combined expressed as dieldrin)	BDL	0.01	GC-MS/MS	0.01
2	Benalaxyl including other mixtures of constituent isomers including benalaxyl-M (sum of isomers)	BDL	0.01	LC-MS/MS	0.01
3	Bifenazate (sum of bifenazate plus bifenazate-diazene expressed as bifenazate) (F)	BDL	0.01	LC-MS/MS	0.01
4	Chlorpyrifos	BDL	0.01	GC-MS/MS	0.01
5	Chlorpyrifos-methyl	BDL	0.01	GC-MS/MS	0.01
6	Cypermethrin (including other mixtures of constituent isomers, sum of isomers)	BDL	0.01	GC-MS/MS	0.01
7	DDT (all isomers, sum of p.p'-DDT, o,p'-DDT, p.p-DDE and p,p'-TDE (DDD) expressed as DDT)	BDL	0.01	GC-MS/MS	0.01
8	Diazinon	BDL	0.01	LC-MS/MS	0.01
9	Heptachlor (sum of heptachlor and heptachlor epoxide expressed as heptachlor)	BDL	0.01	LC-MS/MS	0.01
10	Malathion (sum of malathion and malaoxon expressed as malathion)	BDL	0.01	LC-MS/MS	0.01
11	Methoxy fenazide	BDL	0.01	LC-MS/MS	0.01
12	Parathion-methyl (sum of parathion-methyl and paraoxon- methyl expressed as parathion- methyl)	BDL	0.01	GC-MS/MS	0.01
13	Permethrin (sum of isomers)	BDL	0.01	GC-MS/MS	0.01
14	Phorate (sum of phorate, its oxygen analogue and their sulfones expressed as phorate)	BDL	0.01	LC-MS/MS	0.01

Note: LOQ- Limit of quantification.

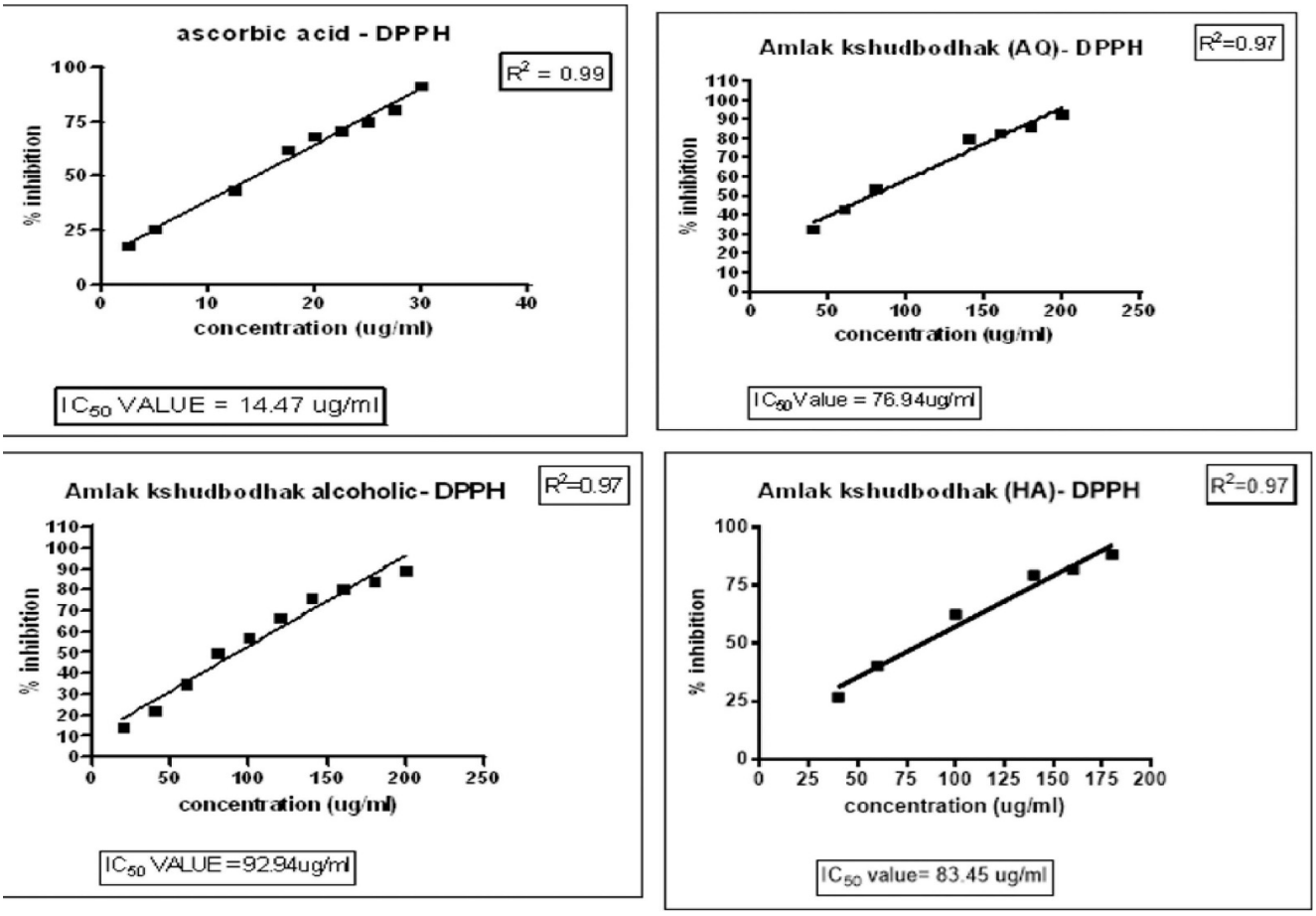

Fig. 5. Changes in the levels of DPPH radical scavenging activity of standard ascorbic acid and different concentrations of aqueous (AQ), alcoholic (AL) and hydroalcoholic (HA) extracts of *Amlak kshudbodhak.*

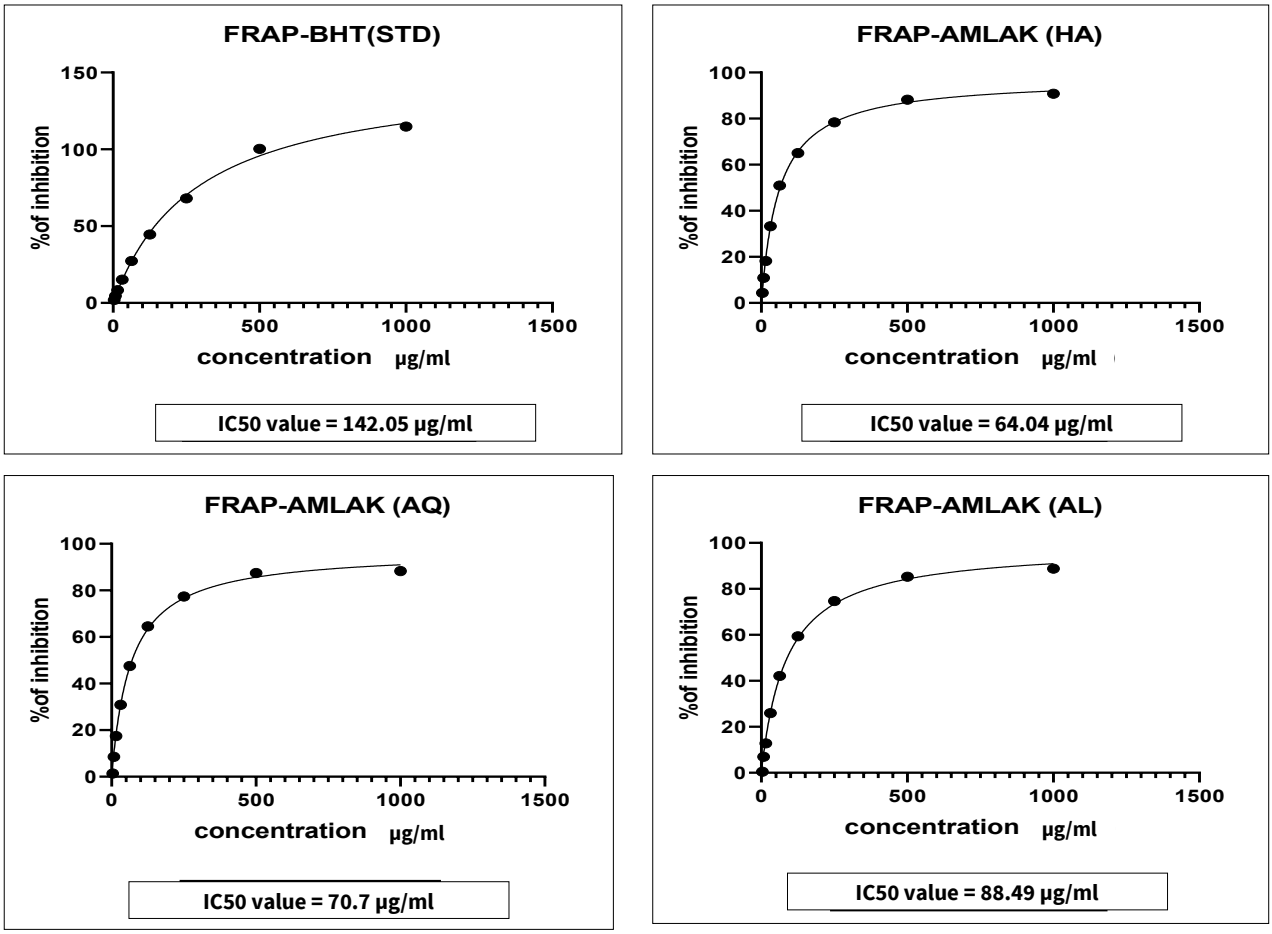

Fig. 6. Changes in the levels of FRAP assays of different concentrations of butylated hydroxy toluene (BHT) and different concentrations of aqueous (AQ), alcoholic and hydroalcoholic (HA) extracts of *Amlak kshudbodhak.*

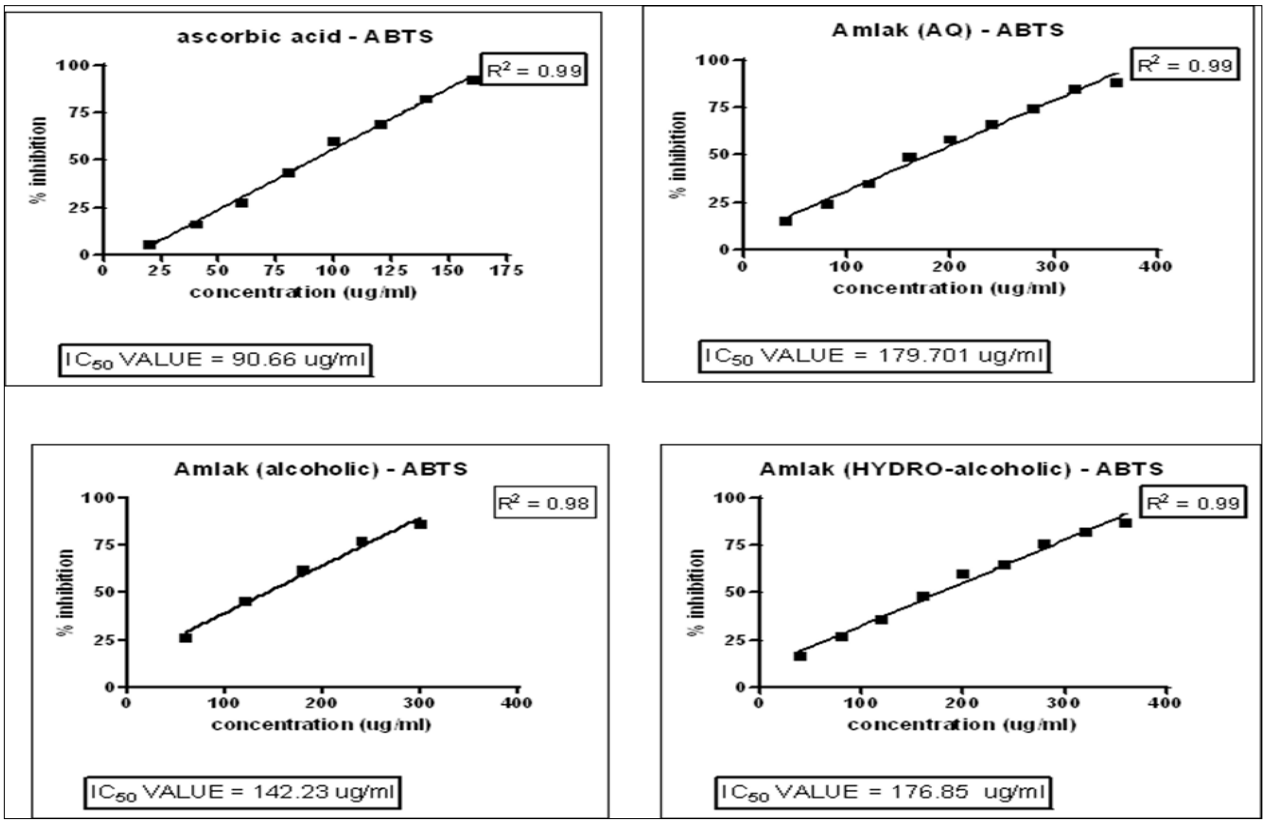

Fig. 7. Changes in the levels of ABTS$^{\bullet+}$ radical scavenging assays of different concentrations of ascorbic acid and different concentrations of aqueous (AQ), alcoholic and hydroalcoholic (HA) extracts of *Amlak kshudbodhak.*

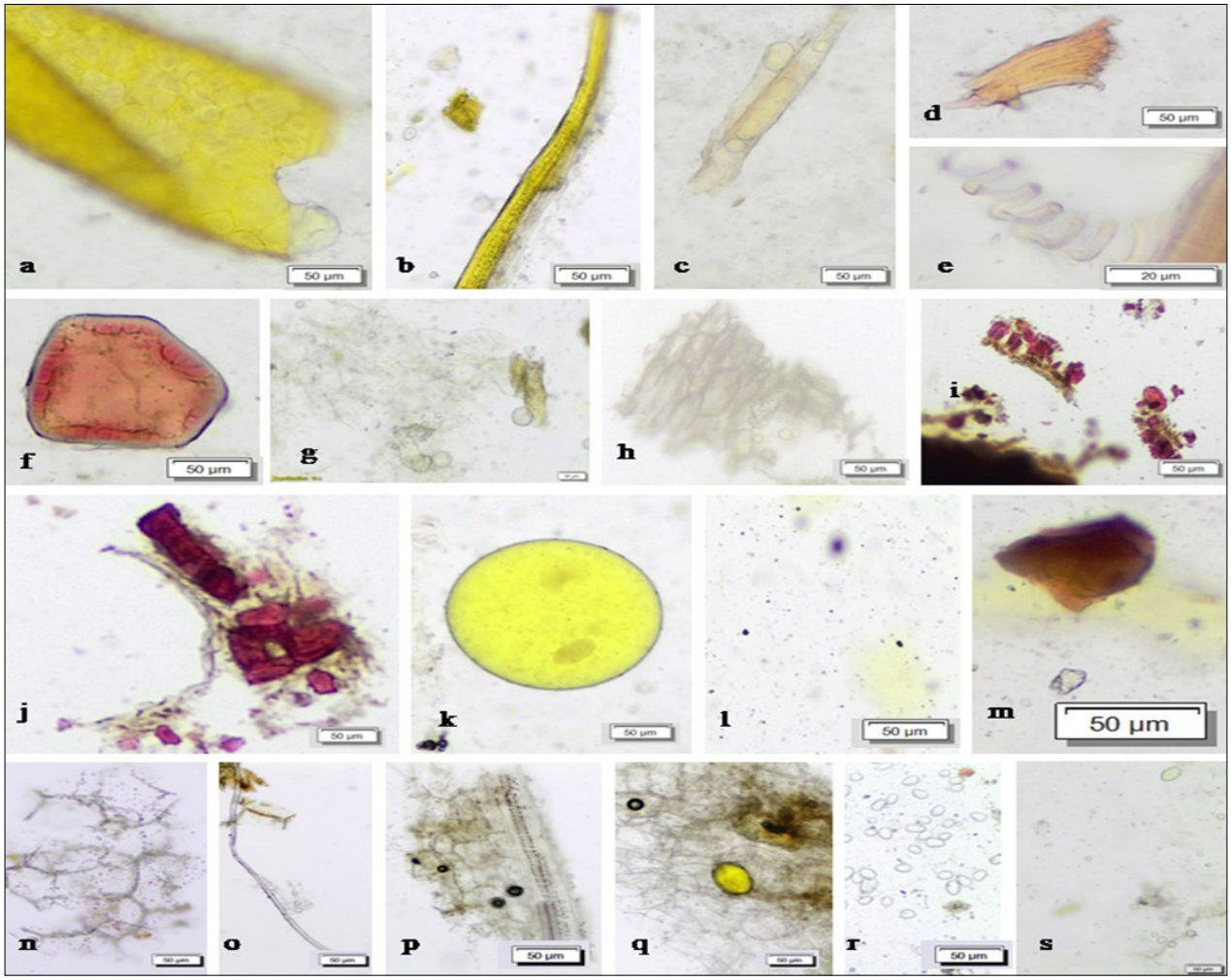

Fig. 8. Powder microscopy of *Amlak kshudbodhak* showing: a. epicarp, b. fiber, c. fiber, d. tracheids, e. spiral thickening, f, brachysclerieds stone cell, g. parenchymatous mesocarpic cells, h. epicarpic cells, i. stone cells, j. stone cells, k. oil globule, l. starch grains, m. brown matter, n. parenchyma cells embedded with starch grains, o. fibers, p. spiral thickening of vessel, q. reticulate thickening of vessels, r. oleo-resins, s. starch grains. Structures a to g belonging to *amla*; h to m belonging long pepper powder; n to s belonging to dry ginger.

to their antioxidant properties, these plants also promote vascular health by improving blood flow, acting as anti-coagulants and preventing blood clots, causing a warming sensation. There are several mechanisms involved in the activities associated with amla, including its role in immunity and digestion. The mechanisms by which amla exerts each of its activities are not fully understood and they may be simultaneously mediated by multiple components. An anti-inflammatory, anti-thrombosis, anticoagulant and anti-platelet effect of amla may prevent a variety of vascular disorders (27).

This study evaluated total flavonoids and total phenolic content, *in vitro* radical scavenging assays, HPTLC profiles, microscopic characteristics, physicochemical studies, testing for aflatoxin, heavy metals, essential minerals and pesticides. As a result of quantitative HPTLC analysis, ellagic acid (0.02 %), quercetin (0.01 %), ascorbic acid (0.89 %) and 6-gingerol (0.05 %) were identified as four active constituents. AK is composed primarily of ascorbic acid with a percentage of 0.89, which serves as a co-factor, co-enzyme complements, co-substrate and powerful antioxidant in numerous reactions and metabolic processes (28). A second major constituent of AK extract is 6-gingerol, which exhibits antioxidant, anti-inflammatory, anticancer, neuroprotective, anti-obesity and anti-diabetic properties (29). Third major compound, ellagic acid, has a percentage of 0.02 and is found naturally in fruits and vegetables. It inhibits lipid peroxidation and enhances antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase to scavenge free radicals. In addition, it stabilizes vitamin E and folic acid and promotes iron absorption (30). Quercetin is the fourth major compound and it is a flavonoid found in many fruits. Quercetin protects biomolecules from oxidative damage and protects cells from oxidative stress (31).

It was also determined that AK's exhibits phenolic content was 223.59 mg TAE/g, while its flavonoid content was 32.64 mg QAE/g. A variety of phytochemicals found in nutrients and food recipes, such as flavonoids, are known for their antioxidant, anti-cancer, anti-bacterial, cardioprotective, anti-inflammatory, immune system-enhancing, UV-radiation protection and interesting medicinal and pharmaceutical properties (32).

The DPPH, FRAP and $ABTS^{\cdot+}$ assays, flavonoids and phenolic compounds were found in the food extract, including ellagic acid, quercetin, ascorbic acid and 6-gingerol. These compounds stabilize hydroxyl radicals by donating hydrogen electrons, thus resulting in relatively stable radicals. As a result, the free hydroxyl group on the aromatic ring is responsible for its antioxidant properties.

Microscopic evaluation showing some diagnostic characters which includes abundant fixed oil globules of sesame oil and cow *ghee*. Polygonal, thin and straight walled cells from epicarp, fibers with pits, broken fragments of tracheids and lignified spiral thickenings, stone cell and elongated, irregular parenchymatous mesocarpic cells (*P. emblica*); slightly elongated, polygonal shaped cells, lignified, rounded to elongated stone cells, volatile oil globule, simple rounded starch grains and brown matter seen in powder (*Piper longum*); parenchyma cells from cortical region embedded with dense starch grains, elongated-septate fiber, spiral and reticulate vessel, oleo resin embedded within parenchymatous cells, starch grains with eccentric hilum (*Z. officinale*).

A physicochemical analysis revealed that the AL, HA and AQ extracts for alcohol and water-soluble extractive values contained more polar compounds. There were no aflatoxins, heavy metals and pesticide residues detected in the AK recipe. As a result of the food preparation process, phenolic and flavonoids were found to contribute to the highest nutritional value of AK and pesticides and toxic substances were not detected.

Conclusion

AK demonstrated significant antioxidant properties in various *in vitro* assays, effectively scavenging free radicals. The AL extracts of AK showed significantly higher activity in DPPH, FRAP and $ABTS^{\cdot+}$ radical scavenging assays compared to the AQ and HA extracts. Essential minerals, phenolic and flavonoid content were detected and microscopical studies indicated that seaweed oil, cow *ghee*, *P. emblica* and *Piper longum* were present in the sample. The prepared food recipe has the highest nutritional value and is beneficial for humankind. It has not been detected for aflatoxin, pesticide residue and heavy metals in the AK. It may be recommended for patients with malabsorption syndrome, communicable diseases and reduced hepatitis risk. There are limitations to AK and dosage is not always scientifically validated. Traditional recipes may not have a standardized shelf life. Non-standard preparation and storage can result in microbial contamination. Clinical trials will be necessary to determine the effectiveness of the formulation in treating anorexia, malnutrition and digestive illnesses. Furthermore, understanding its interaction with digestive enzymes, gut microbiota and neurochemical appetite pathways will be crucial for validating its therapeutic potential.

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Authors' contributions

AMG designed the project. SM and RHK drafted the manuscript. SBP and SPB performed the experimentation. AJ and MJ were prepared the food recipe. RA, SN, PM, VKL and RN corrected and improved the manuscript. All the authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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