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ORIGINAL ARTICLE

Chemico-nutritional characterization and anti-inflammatory potential of Chirabilva (*Holoptelea integrifolia* Roxb.) seed: alternate source of protein supplement and fatty acids

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Abstract Exploring unconventional protein sources can be an alternative strategy to meet the deficiency. The seeds of Chirabilva (*Holoptelea integrifolia* Roxb., Family- Ulmaceae) are eaten raw by the ethnic communities of Southeast Asian countries. The present study assessed the chemical, nutritional, and biological potential of the seeds (HIS) and pericarp (HISP) of *H. integrifolia.* The seeds contain mainly fixed and very few essential oils. The fixed oil of HIS is composed primarily of unsaturated oleic (47%) and saturated palmitic (37%) acids. The HIS are exceptional due to a high content of lipid (50%), protein (24%), carbohydrates (19%), fiber (4%), and anti-nutritional components within permissible limits. The high content (in mg/Kg) of phosphorus (6000), magnesium (422), Calcium (279), and essential nutrients (Ni, Co, Zn, Fe, Cu, Mn, and Cr) in the range of (0.04–6.69) were observed. The moderate antioxidant potential of HISP was evident in single electron transfer in-vitro assays. Moreover, HISP extract and HIS solvent-extracted fixed oil showed anti-inflammatory action in lipopolysaccharide-induced HaCaT cells by significantly attenuating pro-inflammatory cytokines (TNF-α) without causing cytotoxicity. Results support de-oiled HIS cake as an alternative source of a high-protein diet and its oil with anti-inflammatory attributes for topical applications.

Keywords Protein alternatives · Plant natural products · Micro-nutrient analysis · High energy edible seed · Anti-inflammatory potential

Abbreviations

ANFs	Anti-nutritional factors
HISP	*Holoptelea integrifolia* seed
HIS	*Holoptelea integrifolia* pericarp
RDA	Recommended dietary allowances
SFA	Saturated fatty acids
MUFA	Monosaturated fatty acids
PUFA	Polyunsaturated fatty acids

Introduction

Worldwide, more than 820 million have insufficient food, and many more pose the risk of premature death and morbidity due to unhealthy diets. It is reported that among 14,000 edible plant species, only 150–200 are consumed. While rice, maize, and wheat mainly contribute 60% of the calories consumed. Despite adequate food supplies, malnutrition remains a major challenge for India. This is because 46.6 million children under five are undernourished, and around 14.4 million are obese or overweight. (Raghunathan et al. 2021). The report of the EAT Lancet-Commission highlighted that the Indian population consumes more simple carbohydrates and fewer proteins and complex carbohydrates in their diets, heavily dependent on rice and wheat than fruits and vegetables.

Many underused plant species with excellent nutritional profiles are part of the traditional diet of countries, viz.,

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Brazil, India, Indonesia, China, Mexico, and West Africa (Schulz et al. 2020). Including a traditional diet could be an alternative strategy for transforming conventional grain and meat into healthy diets to achieve the goals of UN Sustainable Development. Worldwide, the valorization of traditional food diets using unconventional edible medicinal plants has attracted the attention of many investigations. However, these unconventional traditional medicinal foods are part of a healthy diet in many countries like India, Brazil, and China. Initially, the usage of medicinal plants was confined to traditional medicinal systems of developing countries and is now disseminated worldwide. Afro-Asian regions' culture and culinary experiences offer ample opportunities to learn new ways of preparing functional food and healthy diets. The interest in traditional medicinal plants is growing exponentially with the exploration of a more unique and sustainable source of secondary metabolites in addition to complex carbohydrates, dietary fibers, essential fatty acids, minerals, proteins, vitamins for the health benefits with application to pharmaceutical, and functional food more recently in sports foods.

Indian elm (*Holoptelea integrifolia* Roxb.) is locally known as 'Chirabilva'. It is dispersed up to 2000 feet altitude. It is predominantly found in all parts of India and tropical regions of the northern hemisphere. Indian elm is drought-resistant, effortlessly grows on sandy and loam to gravelly subsoil, and its resilient climate nature makes it a preferential choice for agro-forestry. Its fruits supplement nutritional needs, brain tonic, physical disability, polyurea, and urinary disorders by Indian tribal communities. The seeds are consumed raw by ethnic minorities and remain underutilized by most people. However, there is documented evidence of the use of *H. integrifolia* in traditional Indian medicine and ethnic groups. Different people have used it for various health benefits over the centuries.

Recently, an anti-oxidant and protective action on DNA damage by methanolic extract of *H. integrifolia* leaves has been reported (Mondal and Bandyopadhyay 2023). There is limited information on the nutritional value of *H. integrifolia* seed (HIS) and its oil (Chauhan et al. 2010). Some phytochemicals beneficial for health, mainly triterpenes, are reported from its seed (Srivastava 2020). The seeds of *H. integrifolia* have about 50% fixed oil content and a reasonable amount of saturated and unsaturated fatty acids, protein, fiber, energy, and mineral content (Srivastava 2020). Vegetable oils play an important role in food, a healthy diet, and nutrition as a source of vitamins and essential unsaturated fatty acids. Therefore, exploring the possibility of unconventional edible food sources of vegetable oils has been carriedout. The present research investigation will unfolds insights into the functional and velorization aspects of *H. integrifolia* seed in future. The fatty acid profile, anti-oxidant potential, anti-nutritional factors, HIS have not been studied systematically so far. The current study demonstrate the importance of H. integrifolia seed as an alterenate source of complex carbohydrate and protein supplement to attain nutritional benefits and health well being.

Material and methods

Plant sample

Ripen fruits and feathery seeds of *H. integrifolia* were collected during month of April 2019 from the campus of Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, Uttar Pradesh, India, Lucknow, India (latitude 26° 53′ 41″ N, longitude 80° 59′ 0′). Nature of the soil was sandy loam with low content of carbon. Plant samples were authenticated by the Taxonomist of the Institute with the assignment of a voucher specimen (CIMAP-8294). The seeds and pericarp were manually separated and stored in an air-tight plastic box at room temperature in a dark and dry place until analysis. Subsequently, seeds were pulverized in the mechanical grinder (HL1683/04, 600W, Philips India Limited, India) and stored in the refrigerator at 4°C until use.

Reagents, standard solutions, and reference material

The analytical grade reagent solution of HNO3 (65%, Merck, KGaA, Germany) and H_2O_2 (30%, M/s Merck Specialties, Pvt. Ltd. India) were used to digest seed samples. Ultrapure Type-I water (resistivity 18 MΩ cm^{-1}) was prepared with an ELGA system (ELGA, High Wycombe, Bucks, England). Standard stock certified reference solution (1000 µg/mL) of as (CGAs-1), Hg (CGHg-1), and multi-elements (IV-Stock-4), all from Inorganic ventures, USA, were used for macro- and microelements profile analysis. The working solutions of heavy metals (0.01–5 µg/mL) and macronutrients (0.05–500 µg/mL) were prepared from the stock and stored in decontaminated polyethylene bottles. Analytical grade ethanol 95% (Fisher Scientific, USA) was used for the extract preparation of HISP.

Physicochemical analysis of seed

The physicochemical parameters of HI seed, viz. seed density, hydration capacity, hydration index, swelling capacity, and swelling index, were evaluated following the reported method with some minor modifications (Martínez-Preciado et al. 2020). The seed color was subjectively determined. The cotyledon, endosperm, and seed coat proportions were calculated using the average measure of 100 g of seed.

Density

In a measuring cylinder (250 mL), 100 mL of distilled water (100 mL) was poured containing 100 g of seeds. The seeds were sunk entirely below the water, and the displaced water volume was recorded.

Hydration capacity

Hundred-gram seeds were taken to count the total number of seeds. Afterward, the seeds were mixed with water (100 mL) in the measuring cylinder. The measuring cylinder was closed with the glass lid and kept overnight at room temperature. The seeds were drained after 1 day (24 h). The extra water was discarded, and separated swollen seeds were reweighing.

$$\text{Hydration capacity per seed} = \frac{\text{Weight of soaked seeds} - \text{Weight of seed before soaking}}{\text{Number of seeds}}$$

Hydration index

It was evaluated by the formula given below:

$$\text{Hydration index} = (\text{Hydration capacity of seeds}) / (\text{Weight of one seed}).$$

Swelling capacity

Hundred gram of seeds were weighed, and the total number of seeds were counted. Seed (100 g) was poured into a measuring cylinder to record the change in the volume before and after soaking. Swelling capacity/seed was estimated by the given formula:

$$\text{Swelling capacity} = \frac{(\text{Volume after soaking} - \text{Volume before soaking})}{\text{Number of seeds}}$$

$$\text{Swelling index} = (\text{Swelling capacity of seeds}) / (\text{Volume of one seed}).$$

Proximate composition of seed and vitamin analysis

Proximate composition analysis, such as moisture content, total fat, crude fiber, crude protein, and total ash of *H. integrifolia* seeds, was investigated following the standard AOAC method (AOAC 2012). The standard equation determined the total carbohydrate content, i.e., 100-(% sum of moisture, ash, lipid, and protein). The calorific value of the seeds (kcal/100 g) was calculated and estimated by the described protocol. Ascorbic acid (vitamin C) content was evaluated colorimetrically at 760 nm. The standard method of fats and oil analysis was used for β-carotene analysis (FSSAI 2016).

Amylose determination

Amylose content in HIS seed was evaluated by following the reported method (Kumar et al. 2019). Briefly, HIS powder (100 mg) was added into 0.5 N KOH (10 mL) solution, and suspension vortexed. Then the sample was transferred to a volumetric flask, and the volume was made up to 100 mL. Thereafter, an aliquot of this solution (10 mL) was taken into another 100-mL flask, and 0.1 N HCl (5.0 mL) and 0.5 mL of iodine reagent were added. The final volume was made up to 50 mL. The absorbance was recorded at 625 nm. The quantification of amylose was done using amylose, and amylopectin blends standard calibration curve.

Mineral and metals analysis

Accurately weighed (0.2 g) powdered *H. integrifolia* seeds (HIS) material was taken into 50 mL polypropylene digest tubes. Two milliliters of HNO_3 were added, and the mixture was left overnight to imbibe. Before microwave digestion, 2.0 mL of H_2O_2 was added. Then samples were digested using a high-pressure microwave system (Multiwave PRO, Anton Paar, USA) with an optimized temperature profile. Briefly, the temperature was first raised ambient to 155 °C and held for 10 min; 175 °C for 10 min; 195 °C for 30 min. Samples were cooled to room temperature and then diluted up to 50 mL with ultrapure deionized water. Inductively coupled plasma with atomic emission spectrometry (ICP-AES, Perkin Elmer, and Optima 5300 V) evaluated the mineral and trace elements. The phosphorus was determined by a flow-to-inject analyzer (FIAstar 5000, Foss Tecator AB, Sweden). At the same time, a flame emission photometer was used to estimate potassium and sodium.

Anti-nutritional factors analysis

Quantitative determination of hydrogen cyanide, oxalates, total phenolics, tannins, saponins, phytic acid, and alkaloids was carried out following the standard method of AOAC for hydrogen cyanide and oxalates (AOAC 2012). The earlier methods were used to determine total phenolics, tannins, and saponins (Sánchez-Rangel et al. 2013).

Sample preparation, chemical characterization, and biological activity

HIS fixed oil extraction and its physicochemical characterization

The feathery seed of *H. integrifolia* was divided manually into two parts, i.e., seed (cotyledon-HIS) and pericarp

(HISP). HIS were powdered and 100 g taken in round bottom 1000 mL flask filtration and extracted with petroleum ether (3 × 500 mL) by sonication for 30 min at 35 °C. Filtrates were collected and dried under a vacuum. The fixed oil was collected in an amber-colored bottle and stored at 4 °C until analysis. Physicochemical parameters of the HIS fixed oil viz. optical rotation by polarimeter (SEPA-300, Horiba, Tokyo, Japan), specific gravity by specific gravity meter (DA-500, KEM, Japan), and refractive index by refractometer (Atago RX-7000α Tokyo, Japan) were evaluated following the standard procedure (FSSAI 2016). The saponification value, peroxide value, Iodine value, and the acid value was estimated by titration method using potassium hydroxide, 0.5N hydrochloric acid, and phenolphthalein indicator (FSSAI 2016).

HIS fatty acid methyl esters (FAME) by GC-FID

Methylation of HIS oil was done to convert into transmethylated fatty acid methyl esters prior to the GC analysis following the method with minor modification (Suri et al. 2019). Twenty-five milliliter of the reaction mixture (methanol:toluene:sulphuric acid—20:10:1, v/v/v) was added to the 1.0 mL of HIS seed oil in a closed flask connected with the condenser and heated for 2 h. on a heating mantle. After cooling, liquid–liquid partitioning with n-hexane was performed, and the hexane solution was dried under a vacuum. The dried oil sample was treated with anhydrous sodium sulfate before GC analysis. FAME content of HIS was determined using GC-FID system Instrument (Perkin Elmer Auto system XL), equipped with Equity-5 (60 m × 0.32 mm i.d.; 0.25 µm film thickness). H_2 was used in gaseous form as a carrier with a 1.0 mL per min (constant flow) flow rate. The temperature of the column was programmed and ranged from 70 to 250 °C, programmed at 3 °C/min, and 250–320 °C, programmed at 5 °C/min. 280 °C and 300 °C temperatures are set as injector and detector temperatures. The split ratio of the injected sample (0.02 µL) was 1:50. Characterization of fatty acids was accomplished by RT (retention time) matching using FAME standard mixture (Sigma-Aldrich, USA) and RRI (relative retention indices) to n-alkanes homologous series (C6-C28 hydrocarbons, Polyscience Corp., Niles IL). The relative fatty acid composition of the results of three independent reactions is reported.

HISP extract preparation and its HPLC profile

Five hundred grams of powdered (mesh size 120) HISP was extracted with ethanol (95%) under sonication (3 × 500 mL) for 45 min by maintaining the temperature at 45 °C. The resulting extracts were pooled and dried under a vacuum. An HPLC profile of HISP was also developed to ensure the quality of the extract showing the anti-inflammatory action at very low concentrations. The HISP extract was analyzed by reverse phase HPLC using Phenomenex Luna C_{18} column (4.6 × 250 mm, 5 µm) and linear gradient mobile phase of solvent-A (water) and Solvent-B (acetonitrile) as start with 10% B; 30% B at 5 min; 75% B 7.0 min, 85% B at 10 min, 10% B at 15 min and maintained till the end of the run time i.e., 20 min. The flow rate was maintained at 1.0 mL/min throughout the analysis. An injection volume of 20 µL was injected, and data was recorded with PDA at 190–800 nm. The simultaneous quantification of five targeted phytochemicals was done at 220 nm wavelength.

Anti-oxidant potential of HIS defatted seed powder, HISP extract, and HIS oil

The preliminary phytochemical identification and antioxidant potential in terms of DPPH, ABTS•+ and FRAP assays and total phenolic content were evaluated following the reported methods (Jyotshna et al. 2018). The ascorbic acid was used for comparative potential assessment. The stock solution of ethanol extract of defatted seed powder and its pericarp (1000 mg/mL) while solvent extracted oil was diluted in DMSO before analysis using standard spectrophotometric methods.

DPPH assay Freshly prepared stock solution of 0.1 M DPPH in Tris HCl buffer (pH 7.4) was mixed with varying concentrations of extracts/oil (1–500 µg/mL) vortexed and incubated at 37 °C in the dark for 30 min for reaction. The discoloration capacity of extract/oil was measured at 517 nm. DMSO was used as a reaction blank. The results were presented as µg IC_{50}/mL.

ABTS assay The varying extracts/oil (1–500 µg/mL) were mixed with $ABTS^{+\cdot}$ reagent [14 mM ABTS•+: 4.9 mM $K_2S_2O_8$—1:1, v/v] solution, incubated in the dark for 10 min at 37 °C, thereafter absorbance taken at 734 nm. The IC_{50} values were calculated.

FRAP assay Freshly prepared FRAP reagent [300 mM acetate buffer (pH3.6): 20 mM $FeCl_3$: 10 mM TPTZ solution: 10:1:1, v/v/v] was used as per the reported method (Benzie and Strain 1999). The varying extracts/oil (1–500 µg/mL) were mixed with FRAP reagent was incubated at 37 °C for 30 min. Then absorbance was measured at 593 nm. The antioxidant potential of extracts/oil was presented in terms of $FeSO_4$ equivalent.

Total phenolics Sample solutions of extracts/oil in varying dilutions (1–500 µg/mL) were added with reagent [2N Folin–Ciocalteu reagent: 7.5% concentration of Na_2CO_3: 1:9, v/v]. The solution was incubated for 90 min at 37 °C. The absorbance was measured at 765 nm against a blank reagent. The content of total phenolics was calculated as gallic acid equivalent (GAE).

Cell toxicity assessment

Cytotoxic study of the *H. integrifolia* seeds (HIS) fixed oil and the ethanol extract of its pericarp (HISP) was done by using the earlier reported method (Srivastava et al. 2019) with minor modification. In brief, human keratinocyte cells (NCCS, Pune, India) at 0.5×10^6 live cells/mL density were utilized for the experimental assay. Cells were treated with 1% DMSO. Samples of HIS fixed oil (0.1 and 1% v/v) and HISP extract (1 and 10 µg/mL) are incubated for 24 h at 37 °C in 5% CO_2. Twenty microliters of MTT solution (5 mg/mL in PBS) were mixed per well after incubation and kept for 4 h, followed by solubilization of cell for 10 min in 100 µL DMSO and media removal. The absorbance was recorded at 570 nm using a microplate ELISA reader (SpectraMax 190, Molecular Devices Inc., USA). The survival (%) and cell cytotoxicity were calculated by applying the formula.

$$Survival(\%) = \frac{OD_{sample} - OD_{zero-day}}{OD_{control} - OD_{zero-day}} \times 100$$

$$Cytotoxicity(\%) = 100 - Survival(\%)$$

Anti-inflammatory evaluation (in-vitro)

HIS fixed oil at two concentrations (0.1 and 1%) was treated with human keratinocyte cells (0.5×10^6 live cells/mL), followed by *lipopolysaccharide* stimulation for 16 h. The pro-inflammatory cytokine TNF-α (Tumour necrosis factor α) in the supernatant of cell culture was evaluated by the use of human-specific enzyme immunoassay (EIA) kits (BD Biosciences, USA) according to the earlier reported method (Singh et al. 2014). In brief, the 100 µL of TNF-α capture antibody/well was used for ELISA plates coating and left for incubation at 4 °C overnight and followed by blocking of the well by 200 µL per well assay diluents. 100 µL of the standard and supernatant of the culture was mixed into the washed wells and kept for incubation at room temp. (22–25 °C) for 2 h. Followed by incubation, each well was washed 5 times with wash buffer. After that, the detecting solution (detection antibody and streptavidin–horseradish–peroxidase conjugate), 100 µL, was mixed/well. The wells were rewashed by wash buffer and 100 µL of substrate solution tetramethylbenzidine (TMB) was mixed. Lastly, 2N H_2SO_4 stop solution (50 µL) was added after 30 min. to stop the reaction. The absorbance was recorded at 450 nm and 570 nm. The color density was recorded at 570 nm and subtracted from the 450 nm color density. The TNF-α value was presented in picograms per milliliter.

Statistical analysis

Results are presented as mean ± SEM (standard error of the mean) of experiments performed in triplicate. One-way ANOVA was applied to test for statistical significance at $p < 0.05$. The data analysis was performed using GraphPad Prism 5 (San Diego, CA, USA) software.

Result and discussion

Physicochemical characteristics

The physicochemical parameters of *H. integrifolia* seeds, viz. average seed weight, seed-coat ratio, cotyledon ratio, density, hydration capacity, hydration index, swelling capacity, swelling index, and amylose content (Table 1) are reported for the first time in the study. The swelling capacity, hydration index, & hydration capacity of *H. integrifolia* seeds are comparable with the seeds of *Sesbania* (Hossain and Becker 2001), which are consumed in roasted form occasionally by rural populations in India. The low hydration index is similar to millets. In contrast, the seed density and swelling index are relatively higher than other seeds of cereals, pulses, and oilseeds (Mathur et al. 2020). The low water absorption capacity of HIS may be due to the restricted permeability of its seed coat and harder cotyledons. It is well established that the water-swelling absorbing capacity of seeds governs by various factors, e.g., cell wall structure, composition, and compactness of cells. The low amount of amylose (1.08%) indicates lower adhesiveness and softer gel formation. The amylose content of the starch is known to be linked with gel firmness through synaeresis of water. The amylose in starch, protein, and fiber is the key factor for the flour's pasting properties (Yadav et al. 2018).

Table 1 Physicochemical properties of the *H. integrifolia* seeds

Parameter	*H. integrifolia* seeds
Seed color	Brown
Seed weight, g (average of 100 seeds)	0.02 ± 0.01
Seed coat percent	16.44 ± 0.03
Cotyledon (% whole seed)	83.56 ± 0.04
Density (g/mL)	0.71 ± 0.01
Hydration capacity (g/seed)	0.02 ± 0.01
Hydration index	0.90 ± 0.02
Swelling capacity (mL/seed)	0.05 ± 0.03
Swelling index	0.84 ± 0.02
Amylose (%)	1.08 ± 0.06

Proximate composition, anti-nutritional factors, and mineral analysis

The phytochemical content in functional food is crucial for efficacy in terms of health benefits and safety point of view. Standard chemical tests for the screening of phytochemicals were performed in *H. integrifolia* seeds powder (seed waste after solvent extraction) and ethanol extract of its pericarp HISP. Results showed that HIS seed powder is devoid of alkaloids, saponins, and tannins and could be considered relatively safe than HISP. The joint codex committee (WHO/FAO) program on food standards of oils & fat has recommended the saponification and iodine values for edible oil in the range of 186–194 mg KOH/g and 80–100 mg/g, respectively (Srivastava 2020). The saponification value reveals the character of the fatty acid of the sample. The saponification (192.20 ± 1.36 mg KOH/g) and iodine (91.36 ± 2.05 mg/g) values of HIS oil are within the acceptable range and comparable to other edible oils like Mustard, Arachis, Sunflower, and Safflower oil as per the FAO/WHO recommendations (Table 2). The lower iodine value of HIS oil may provide excellent stability and prevent oxidative deterioration in storage. In contrast, the acid value is generally used to know the edibility of the oil. The high acid (3.36 mg KOH/g) and peroxide value (28 ± 0.38 mill equivalents oxygen/kg) has exceeded the FAO/WHO permitted value of 0.6 mg KOH/g and 10 mill-equivalents oxygen/kg for edible oils (Table 2). The values are matching with the reported data for Niger oil used in Ethiopia as edible oil (Negash et al. 2019). However, blending other edible oil, e.g., soybean, olive, and canola oils, is highly recommended in the HIS oil for better palatability and edibility.

Proximate analysis results have shown that appreciable amounts of fat (49.86%), carbohydrates (19.56%), and protein (23.57%) were present in the HIS. The seeds were also fiber-rich (4.25%) (Table 3). Moisture content and dry matter analysis of seeds directly affect the nutritional content. Lower moisture (3.54%) content is beneficial to increase the shelf life and fit for long time storage (Table 3). The dietary anti-oxidants (Vitamin-C 2.65 mg/100 g and β-carotene 2.10 mg/100 g) in dried seed powder (Table 3) suggest that about 50% of the recommended daily allowances (RDA) of

Table 2 Preliminary phytochemical screening and antioxidant potential of defatted seed powder, pericarp extracts and solvent extracted fixed oil of *H. integrifolia* seeds

Parameters	Test/unit	Inference		
		HIS defatted extract	HISP extract	HIS Oil
Phytochemical tests				
Flavonoids	Shinoda test	+	+	+
Alkaloids	Dragandrof and Meyer tests	−	+	−
Saponins	Froth formation in H_2O	−	+	−
Tannins	Vanillin HCl	−	+	−
Carbohydrate	Molisch's Test	+	+	−
Proteins	Ninhydrin	+	+	−
Mucilage & gum	With 95% alcohol	−	+	−
Waxes	With alcoholic KOH	−	−	−
Phytochemical assessment				
Acid value	mgKOH/g	NR	NR	3.36 ± 0.27
Iodine value	mg/g	NR	NR	91.36 ± 2.05
Saponification value	mg KOH/g	NR	NR	192.20 ± 1.36
Peroxide value	milliequivalents	NR	NR	28 ± 0.38
Optical rotation (21.7 °C)	–	NR	NR	− 0.0800
Refractive index (20 °C)	–	NR	NR	1.46513
Specific gravity (25 °C)	–	NR	NR	0.9113
Anti-oxidant potential				
DPPH	μg IC_{50}/mL	> 100	50.43	80.42
ABTS	μg IC_{50}/mL	> 100	88.81	> 100
FRAP	mgFSE/100 g	10.15	23.41	3.24
TP	mgGAE/100 g	1.32	2.35	0.15

HIS, *H. integrifolia* seeds; HISP, *H. integrifolia* seeds pericarp; +Positive test showing the presence; − negative test showing the absence; DPPH-(2, 2′-diphenyl-1-picrylhydrazyl; ABTS, (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid; FRAP, Ferric reducing antioxidant power; TP, Total phenolic; IC_{50}, Concentration showing 50% inhibition; FSE, Ferrous sulphate equivalent; GAE, Gallic acid equivalent; NR, Not relevent

β-carotene can be met by the consumption of 100 g HIS. The updated RDA value of β-carotene for the Indians is 4.8 mg/day (ICMR 2020). Results projected that the defatted *H. integrifolia* seeds could be an economical and alternative source of high-energy protein supplement foods to improve the nutritional quality of weaning food for infants and toddlers to cope with protein-energy malnourishment. However, a future in-depth study is warranted to unfold protein quality by amino acid analysis.

The estimation of anti-nutritional factors (ANFs) in *H. integrifolia* seeds is presented in Table 3. The anti-nutritional factors were found within the permissible limit in the raw seed. The concentration level of these anti-nutritional factors is safe to consume for human beings. The ANFs are significant for plant defense, and some are also responsible for biological activity. Moreover, alkaloids possess several biological activities like anticancer, antimalarial, antiarrhythmic, and antihypertensive effects. At the same time, saponin inhibits the absorption of several nutrients. Oxalate and phytic acid amount affect the utilization of iron, zinc, and calcium because metabolically active forms of minerals are only absorbed in the body (Šimić et al. 2009).

Table 3 Nutritional and anti-nutritional composition of *H. integrifolia* seeds

Parameter	Values (on dry weight basis)	RDA-Indian (men/women)
Proximate analysis	g/100 g	–
Ash content	3.47 ± 0.33	
Moisture content	3.54 ± 0.27	
Crude fat	49.86 ± 0.73	
Crude protein	23.57 ± 0.42	
Crude fiber	4.25 ± 0.48	
Total carbohydrate	19.56 ± 1.32	
Calorific value (kcal/100 g)	604.26 ± 2.73	
Elemental composition	ppm	mg/day
Potassium	6800 ± 21	3750/3225
Calcium	279.25 ± 1.03	600
Magnesium	421.80 ± 3.25	340/310
Iron	6.69 ± 1.07	17/21
Zinc	4.11 ± 0.23	12/10
Manganese	1.69 ± 1.12	4.0
Copper	0.33 ± 0.38	1.7
Cobalt	0.21 ± 0.07	–
Nickel	0.04 ± .04	–
Phosphorus	5670 ± 20	600
Aluminum	ND	–
Chromium	0.10 ± 0.03	50 µg
Dietary antioxidants	mg/100 g	
Ascorbic acid (Vitamin C	2.65 mg/100 g	
β-carotene	2.10 mg/100 g	
Ant-nutrient factors	mg/100 g	–
Saponins	0.62 ± 0.02	
Phytic acid	2.44 ± 0.05	
Alkaloids	2.40 ± 0.01	
Tannins	0.35 ± 0.02	
Oxalate	3.01 ± 0.02	
Total phenolics	12.7 ± 0.71	
Total cyanide	ND	

RDA Recomonded daily allowance, *ND* Not detected

HIS contains some essential nutrients such as calcium, magnesium, and phosphorus in appreciable concentration with fair amounts of nickel, cobalt, zinc, iron, copper, manganese, and chromium, which acts as a mineral anti-oxidant (Table 3). While heavy metals, viz. aluminum, cadmium, arsenic, lead, & mercury, are absent or present below the detection limit except chromium (0.10 ppm). Eleven essential minerals and six metals of HIS were also compared with recommended dietary allowances (RDA) (Table 3). The proximate and mineral analysis of HIS seeds reveals their on the consumption of 100 g HIS/day could met the about 1/5, 1/8 and 1 RDA value of potessium, magnesium and phosphorus, respectively.

FAME of HIS oil and HISP chemical characterization

Solvent extraction HIS has yielded a high yield of 49.86% v/w odorless, pale yellow viscous oil. The oil yield was relatively higher, with the previous findings of 25.2–49.63% from different locations in India (Chauhan et al. 2010). The fatty acid composition of *HIS* seed oil was analyzed by converting it into trans-methylated fatty acid methyl esters followed by capillary GC-FID method. The oleic acid was the main constituent, followed by palmitic acid > stearic > linoleic > myristic > arachidic > and elaidic acid (Table 4). The total saturated fatty acids (ΣSFA) content was 47.49%, with palmitic (36.89%) and stearic acid (5.58%) as major lipids. Similar content of monosaturated fatty acids (ΣMUFA) 47.47% mainly due to the major presence of oleic (46.46%) and minor elaidic (1.01%) acids. At the same time, a very lower amount of polyunsaturated fatty acids (ΣPUFA) content was observed due to the presence of linolenic acid (5.04%). HIS oil results are different from previous reports of high linolenic acid (~60%) content (Das 2002). The study suggested that keeping low cholesterol levels in plasma and liver sum of PUFA and MUFA/SFA ratio should not exceed 2. According to American Health Association, the ratio of SFA: MUFA: PUFA (1:1.5:1) is key in maintaining the ideal LDL/HDL ratio in both normal and hypercholesteremia individuals.. ΣSFA and ΣMUFA in an almost equal ratio in the HIS have the potential for energy-rich nutritional and health benefits with oleic acid (C18:1) as major MUFA and lower SFA.

Table 4 *H. integrifolia* seed fixed oil fatty acid profile

Fatty acid	Carbon number and unsaturation	Methyl ester	Content (%)	RI (Cal.)*	RI (Rep.)**	Methods of identification***
Myristic acid	C14:0	Methyl myristate	3.86	1725	1723	MS, RI, SS
Palmitic acid	C16:0	Methyl palmitate	36.89	1927	1924	MS, RI, SS
Linoleic acid	C18:2	Methyl linoleate	5.04	2096	2093	MS, RI, SS
Oleic acid	C18:1 cis (n9)	Methyl oleate	46.46	2104	2103	MS, RI, SS
Elaidic acid	C18:1 trans (n9)	Methyl elaidiate	1.01	2107	2109	MS, RI, SS
Stearic acid	C18:0	Methyl stearate	5.58	2127	2124	MS, RI, SS
Arachidic acid	C20:0	Methyl arachidate	1.16	2328	2324	MS, RI, SS
ΣSFA			47.49			
ΣMUFA			47.47			
ΣPUFA			5.04			

*RI (Retention indices), Average calculated retention index for Elite-5 MS column; **RI (Rep.), Reported retention index; ***Methods of identification: SS, Standard Samples, by comparing retention time; MS, Mass spectra comparison with the mass libraries; RI, Retention indices; SFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids and PUFA, Polyunsaturated fatty acids

The chemical investigations on the seed showed the presence of β-sitosterol-D-glucoside, α, and β-sitosterol, Lupeol, Fridel-1-en-3-one, β-amyrin, Friedlin, Epifriedlinol, stigmasterol, tannins, proteins, fatty acids (Srivastava 2020). These compounds are devoid of chromophoric group and could not be detected in the HPLC–PDA analysis. In the absence of any known reference chemical(s) from *H. integrifolia*, efforts were made to ensure the quality of the HISP extract by chemical fingerprint at 220, 254, and 310 nm (Fig. 1). The content (in µg/g) of minor phytochemicals viz., gallic acid (5.8), rutin (73), tannic acid (230), betullinic acid (1100), and kaempferol (410) was determined using dully characterized reference compounds with purity > 95% available in the laboratory. However, the quantitation of the major peak of the unknown compound appeared at a retention time of 10.1 min with characteristic UV maxima at 237 nm, and 307 nm could not be achieved. It is the first report demonstrating the quantitation of key phytochemicals in *H. integrifolia* seed pericarp.

Anti-oxidant potentials

The anti-oxidant pintail of HIS seed oil, the seed powder extract after solvent extraction, and the pericarp extract prepared in ethanol were evaluated for their anti-oxidant potential using DPPH, ABTS, FRAP, and total phenolic

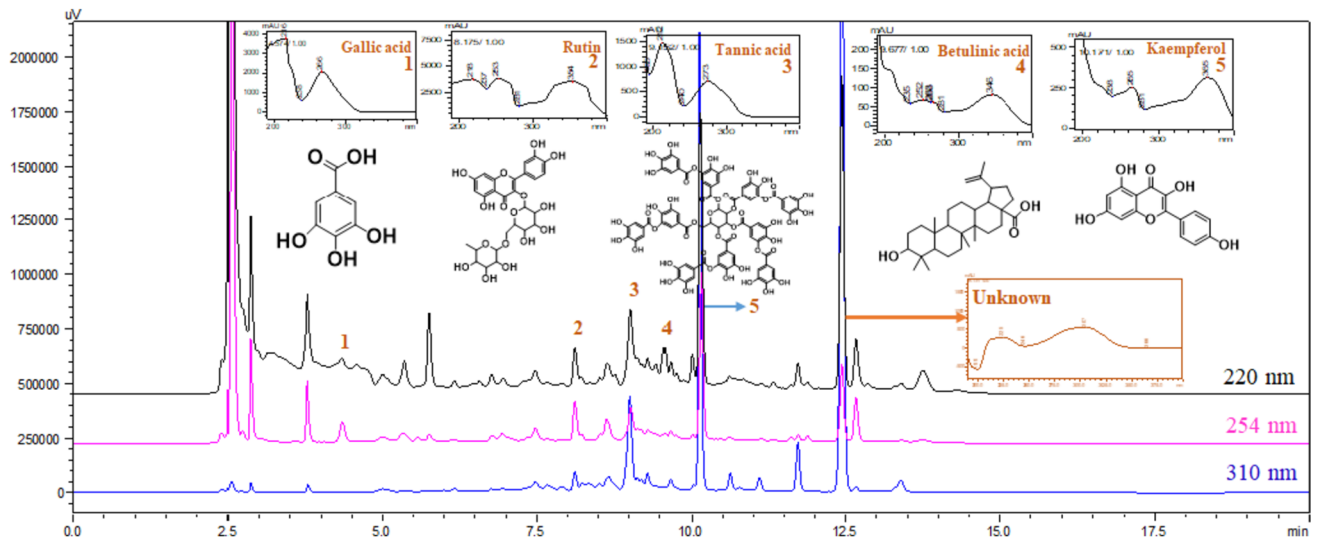

Fig. 1 HPLC profile of *H. integrifolia* seed pericarp ethanol extract (500 mg/mL) showing the presence of gallic acid, rutin, tannic acid, betullinic acid, kaempferol and unidentified compounds

assessment (Table 2). The low anti-oxidant potential of HIS oil and the defatted HIS powder is evident by higher IC_{50} values of 80 and > 100 µg/mL in DPPH and ABTS assays. The ferric-reducing anti-oxidant power of *H. integrifolia* seed (defatted), pericarp, and solvent-extracted oil followed the order—pericarp > seed defatted powder > seed oil. A relatively higher total phenolics content in HISP extract than HIS defatted extract and oil indicated the presence of low-molecular phytochemicals, e.g., phenolic acids, the group of flavonoids. The HPLC profile of HISP extract also supports that compounds of a phenolic nature are available but in low concentration. The present study demonstrates that anti-oxidants are mainly confined in the pericarp part of the seed, while very few remain as extracted seed powder after solvent extraction of fixed edible oil.

Anti-inflammatory potential

Exploration of new sources for valorization in the raw food or utilization of waste parts in terms of their beneficial health effects is a new strategy for niche food development and cosmeceuticals. The pro-inflammatory cytokine production (TNF-α) is increased significantly to magnify the pro-inflammation response. We have studied the inflammatory cytokines modulation level by using HIS seed oil and its waste part, i.e., HISP ethanol extract. LPS-induced HaCaT (human keratinocyte) cells, the secreted levels of TNF-α were significantly ($p < 0.05$) inhibited by HISP extract at both 1.0 µg/mL and 10 µg/mL while HIS fixed oil only at higher concentration (1.0%) (Fig. 2b) without causing any cytotoxicity (Fig. 2a). The pro-inflammatory cytokines overproduction may worsen the condition of several inflammatory diseases. Therefore, controlled cytokine production could help reduce inflammatory disease outcomes (Singh et al. 2014). The results support the previous anti-inflammatory finding of methanol extract of HIS induced by carrageenan paw edema in rats (Affan et al. 2015).

It is well-established that palmitic acid causes triglyceride accumulation, and its overloading leads to liver damage mediated by mitochondrial dysfunction. However, the protective action of oleic acid against palmitic acid-induced lipotoxicity in β-cells has been demonstrated through inhibition of ROS production, endoplasmic reticulum (ER) stress, apoptosis, inflammation, and promotion of insulin secretion (Nemecz et al. 2019). Despite some controversies on free fatty acids (FFA), increasing evidence designates the role of oleic acid in controlling blood pressure, cancer progression, and inflammatory diseases (Granado-Casas and Mauricio 2019). A further in-vivo study is planned to unfold the HISP compound responsible for the anti-inflammatory potential and its mechanism of action.

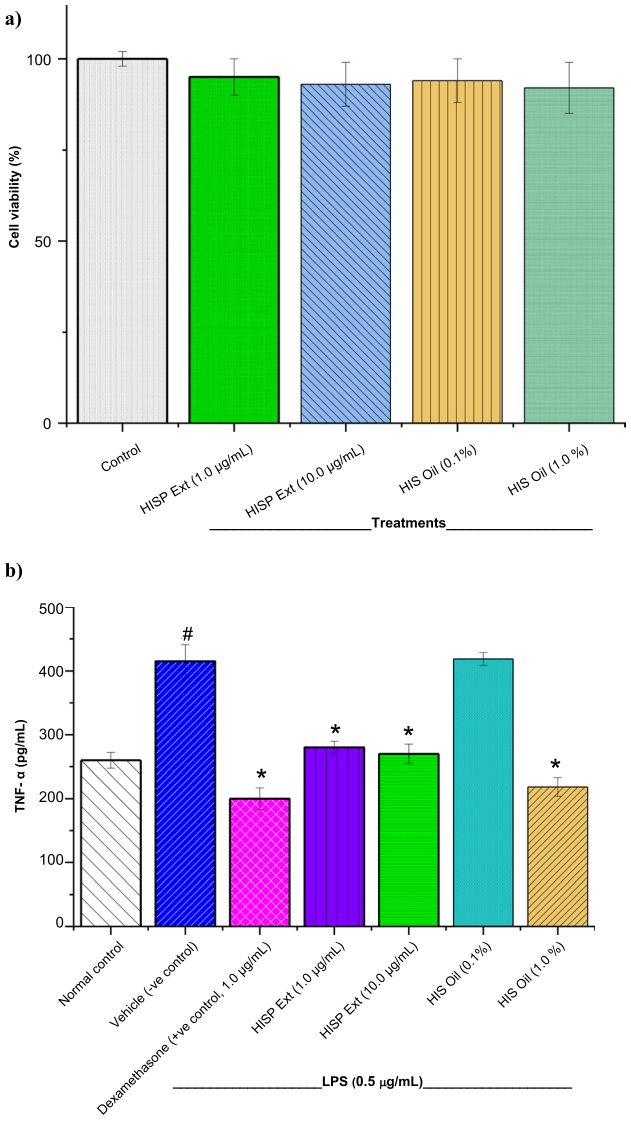

Fig. 2 **a** Effect of *H. integrifolia* seed (HIS) on **a** cell viability in HaCaT cells using MTT assay and **b** anti-inflammation potential in LPS induced HaCaT cells. #Normal control versus vehicle (− ve control); *vehicle (− ve control) versus treatment

In-vitro safety assessment

Results of the cell viability assay were recorded regarding significant changes in the live cell population percentage in HaCaT cells using MTT assay. It is evident that no significant changes were found in the live-cell population ($p < 0.05$) of the treatments of HIS oil (0.1% and 1.0%) and HISP (1.0 and 10 µg/mL) as compared to normal cells (Fig. 2a). Results supports the biocompatibility and non-toxic nature

of HIS oil and HISP extract for safe use in nutritional and functional applications.

Conclusion

The essential micronutrients, protein, and carbohydrate content in de-oiled seed cake *H. integrifolia* demonstrate that it can be an alternate ingredient for a low-calorie diet. Additionally, the consumption of 100 g HIS/day could met the about 1/2, 1/5, 1/8 and 1 RDA value of β-carotene, potessium, magnesium and phosphorus, respectively The present study has also demonstrated that anti-oxidants were mainly intacted in the pericarp part of the seed, and did not extracted out during fixed oil separation using solvents. The ethanolic extract of *H. integrifolia* seed pericarp (HISP) and its oils which was rich in oleic acid has exhibited anti-inflammatory activities by reducing pro-inflammatory cytokine (TNF-α). The HIS oil could not deteriorate in storage from oxidation due to lower iodine value storage. The total saturated fatty acids (ΣSFA), monosaturated fatty acids (ΣMUFA) and polyunsaturated fatty acids (ΣPUFA) content was 47.49%, 47.47%, and 5.04%, respectively. The low content PUFA of and almost equal amount MUFA and SFA could be useful in maintaining the ideal LDL/HDL ratio in both normal and hypercholesteremia individuals. The defatted *H. integrifolia* seeds could be an economic and new alternative source of energy rich protein supplement foods to improve the nutritional status of the vegetarian diet.

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Data availability Data sharing does not apply to this article as no new data were produced in the present study.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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