Phyto-constituents profiling of Luffa echinata and in vitro assessment of antioxidant, anti-diabetic, anticancer and anti-acetylcholine esterase activities

Suraj B. Patel, Savaliram G. Ghane

Plant Physiology Laboratory, Department of Botany, Shivaji University, Kolhapur 416 004, Maharashtra, India

Abstract

Luffa echinata Roxb. is one of the neglected medicinal plants. It is an important source of bioactive metabolites and used in several Ayurvedic formulations. In the present analysis, mature leaves and fruits were extracted with acetone, ethanol, acetonitrile, methanol and water. Phytochemicals like total phenolic (TPC), flavonoid (TFC), tannin (TTC), alkaloid (TAC) and terpenoid (TTEC) content were analysed. Further, antioxidant (AOX) activities like 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, 2,2'-azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid (ABTS) radical scavenging, metal chelating activity (MC), ferric reducing antioxidant power (FRAP) and phosphomolybdenum assay (PMA) were studied. Highest TPC and TFC (189.57 ± 1.9 mg TAE/g extract, 30.48 ± 0.7 mg CE/g extract, respectively) were reported from acetone extract of the leaves. Ethanolic fruit extract showed the highest TTC (13.79 ± 0.2 mg CE/g extract). Acetone and acetonitrile fruit extract revealed maximum TTEC (602.79 ± 3.5 mg UAE/g extract) and moderate TAC (19.96 ± 0.9 mg GE/g extract), respectively. In AOX, highest DPPH (50.52 ± 0.03 mg AAE/g extract) and ABTS (26.78 ± 0.03 mg TE/g extract) radical scavenging reported in methanolic extract of fruit; however, acetone extract of leaf showed highest FRAP (376.89 ± 1.95 mg Fe(II)/g extract) and PMA (326.54 ± 4.73 mg AAE/g extract). In contrast, aqueous extract of leaf and fruit revealed highest metal chelating activity (41.67 ± 0.49 mg EDTA/g extract). In anti-diabetic studies, acetone extract of leaves and fruits exhibited appreciable inhibition of α-amylase (83.33%) and α-glycosidase (77.42%) enzymes. Similarly, acetyl cholinesterase (AChE) inhibition was highest in water (88.91%) and acetone (81.87%) extracts of leaf and fruits. Fruit extracts showed potent anticancer activity against breast (MCF-7) and colon (HT-29) cancer cell lines (LC50 329.36 and 385.17 µg/mL, respectively). RP-HPLC analysis revealed highest cucurbitacin B (CuB) (196.24 ± 1.4 mg/g DW), followed by cucurbitacin I (CuI) and cucurbitacin E (CuE) in the fruits (57.14 ± 4.9 and 2.03 ± 0.03 mg/g DW, respectively). GC-MS analysis revealed presence of bioactive compounds from various groups. Based on the present findings, it was revealed that the fruit and leaf of L. echinata can be used as potent bioresource for natural antioxidants, anti-diabetic, and anticancer drug.

1. Introduction

The plant kingdom is blessed with large amount of biologically active compounds like phenolics, flavonoids, terpenes, ascorbate acid, tocopherols, carotenoids are collectively called as an antioxidant. All the plant parts like root, stem, leaf, fruit, seed and flowers show presence of naturally occurring antioxidants (Gámez-Meza et al., 2009). These antioxidants have ability to protect plant cell from the damage caused by unstable molecule called as reactive oxygen species (ROS). ROS are a group of compounds which are...
derived from metabolism of oxygen led to biochemical and physiological lesions and often results in metabolic impairment and finally cell death (Li et al., 2011). They also act as mediator of many diseases including cancer, premature ageing, progestin-mediated inflammatory processes, hypertension and heart disease (Souri et al., 2007). Synthetic antioxidants like butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinones and gallic acid esters have been suspected to cause or promote negative health effects (Patel et al., 2018).

Family Cucurbitaceae Juss. contain high economic value as it is a major source of food and possess pharmacological properties like antiulcer, antiadipic, analgesic, nephro-protective and anticancer (Patel et al., 2020). In India, 31 genera and 94 species of family cucurbitaceae were reported (Renner and Pandey, 2013). Ribosome inactivating proteins (MAP30, luffin A and B) and anti-HIV activity have been reported in some species, hence these plants serve as an important source of anti-retroviral drugs (Modi and Kumar, 2013).

Terpenoids are versatile group from bioactive compounds widely used in pharmaceutical, cosmetic and food industries (Jaeger and Cuny, 2016). Terpenoids are used not only to treat various health related issues but also in flavours, fragrances, spices as well as food additives (Schempp et al., 2017). Members of Cucurbitaceae contain cucurbitacins that possess keto-, hydroxy- and acetoxy-groups and are broadly divided into twelve categories (A-T). Since ancient times, cucurbitacins are used in folk medicines due to broad applicability in pharmacological activities like anticancer, cytotoxic, anti-inflammatory, anti-fertility, diuretics and cardiovasucular protective. It is a good inhibitor of JAK2 (STAT3; a common oncogenic signalling pathway (Abdelwahab et al., 2011, Eyol et al., 2016). Cucurbitacins have chemo-preventive role against several human cancer cell lines including breast, lung, uterine, cervix, brain, liver, skin and prostate cancers. Some toxic effects like increase in the capillary permeability irritate the intestinal mucosa, and strongly increase intestinal motility have been reported (Eyol et al., 2016).

_Luffa echinata_ Roxb. is commonly called as bitter sponge gourd or Bristly luffa and used in traditional Ayurvedic medicines. The plant possesses various medicinal properties such as laxative, analgesic and used to treat bronchitis, piles, jaundice, and vaginal discharge. Fruits are effective in biliary, intestinal colic and putrid fever. Whole plant is also used as chronic bronchitis, anti-helminthic, emetic, stomachic, nephritis and abortifacient (Kirtikar and Basu, 1933; Nadkarni and Nadkarni, 1976; Kumar et al., 2012). Many reports showed the potency of fruit against jaundice and also exhibited diuretic and antihypertensive effects (Modi and Kumar, 2013). Pharmacological studies reported that fruits and seeds are anti-hepatic and anti-antihelminthic (Murthy et al., 2011). Ethanolic extract revealed presence of alkaloids, carbohydrates, proteins, glycosides, flavonoids, sterols, triterpenes, flavonates, reducing sugars and tannins (Kailasai et al., 2011). Potent bioactive compounds like CuB, elatetin (CuE), elatetin-2-glucoside, isocucurbitacin B, β-sitosterol, echinatol-A and -B, chrysoyl-7-glucoside, chrysoyl-7-epiglucoside, echinatol A, echinatol B, echinatin have been reported from the fruits (Seshadri and Vidyasewaran, 1971; Ahmed et al., 2001). Datiscacin (cucurbititin-20-acetate); a bitter compound known to have antitumor activity has been reported from _L. echinata_ (Ahmad et al., 1994). Similarly, flavonoids (luteolin-7-glucoside and chrysoeriol-7-glucoside), triterpenes, fatty acids, and saponin (gypsogenin) have been also isolated from seeds (Modi and Kumar, 2013).

Extraction must be nontoxic, eco-friendly, fast and cost effective. Both conventional and non-conventional extraction methods are preferred in which microwave assisted extraction (MAE) is widely used technique for the extraction of bioactive compounds. In this method, solvent and sample mixture heated by using energy of microwave radiation that increase diffusivity of phytochemicals. MAE has several advantages over the classical extraction methods. It consumes less time and quantity of solvent for extraction (Aires, 2017). Hence, MAE was preferred in the present investigation.

Cancer is one of the most distractive common diseases leading to loss of many life forms. It has been estimated that total death due to cancer will rise to 10 million in 2020 and over 16 million in 2050 (Jemal et al., 2011). To overcome this problem several treatments like chemotherapy, radiotherapy and synthetic drugs are preferred but showed several ill effects on human health. Hence, there is an urgent need to focus on alternative therapies which cure cancer (Ochwang’i et al., 2014). Diabetes is also one of severe life threatening disease. It was estimated that by the year 2030, total 7.8% of world population will be affected by this disease (Whiting et al., 2011). Diabetes leads to cardiovascular diseases, premature death, kidney failure and depression. Hence, it is the need of time to investigate natural α-amylase and α-glucosidase inhibitors (Patel et al., 2020). Alzheimer’s disease (AD) is considered as one of most common form of dementia, and characterized by slow degradation of neurological functions. Acetylcholine esterase inhibitors (AChEIs) have ability to increase acetylcholine in between synaptic region, resulting into restoration of deficient cholinergic neurotransmission (Ghane et al., 2018; Attar and Ghane, 2019). Several compounds from the genus _Luffa_ have been identified as AChEIs (Feitosa et al., 2011).

Literature survey revealed that very little information available on the potent bioactivities from _L. echinata_. Hence, in present investigation, mature leaf and fruits were sequentially extracted using several solvents to investigate in vitro antioxidant, anti-diabetic, acetylcholine esterase inhibitory and anticancer potential. In addition, potent bioactive compounds were separated and identified using RP-HPLC, GC–MS, and LC–MS.

2. Materials and methods

2.1. Preparation of extract

Mature leaves and fruits of _L. echinata_ were collected in November 2017, from Shahada town of Nandurbar district, Maharashtra, India. Location lies in between 1°32′34.6″N and 74°29′33.1″E. Plant materials were dried in hot air oven for 72 h at 60 °C and powdered by using electric mill. The powdered material (5 g) was sequentially extracted with 50 mL of respective solvents (acetone, acetonitrile, ethanol, methanol and water) using microwave oven (Samsung CE1350L, Thailand at 900 W power) for period of 180 s. Extracts were centrifuged at 6000 rpm for 10 min, and supernatants were collected in petridish. After complete evaporation, the residue was re-dissolved in 4 mL of respective solvent (acetone, acetonitrile, ethanol, methanol and water), filtered using bacterial filter (6 μm), stored at 4 °C and used for further analysis.

2.2. Determination of total phenolic content (TPC)

TPC was determined according to the method adopted by Patel et al. (2020) with minor modifications. Briefly, the sample (40 μL from 5 mg/mL stock) and standard tannic acid were introduced into a 5 mL test tube. Pre-diluted (1:10) 1 mL Folin reagent and 0.8 mL of sodium carbonate (7.5% w/v) were added, mixed and incubated for 60 min at room temperature. Post-incubation, absorption of reaction mixture was taken at 765 nm using UV–Vis Spectrophotometer (Jasco, V-730, Japan). TPC was expressed as mg of tannic acid equivalents (TAE) per gram extract.

2.3. Determination of total flavonoid content (TFC)

TFC was determined as per our earlier protocol (Ghane et al., 2018). Plant extract (50 μL) was treated with 75 μL of (5% w/v)
NaNO₂, after 6 min incubation, 150 µL (10% w/v) AlCl₃ was added. Post-incubation, 75 µL of distilled water was used to make up volume and finally 800 µL of 1 M NaOH was added. Absorbance of the reaction mixture was taken immediately at 510 nm. Standard catechin was used to plot calibration curve and results were expressed as mg of catechin equivalent (CE)/g of plant extract.

2.4. Determination of total tannin content (TTC)

The vanillin-HCl method with minor modifications was used to determine total tannin content (Attar and Ghane, 2017). Briefly, plant extract or standard catechin (100 µL) and 1 mL reagent (4% vanillin and 8% conc. HCl; 1:1 in methanol) were mixed and incubated at room temperature for 20 min and absorbance were taken at 500 nm. All the results were expressed as mg catechin equivalent (CE)/g extract.

2.5. Total terpenoid content (TTEC)

To determine TTEC, the method adopted by earlier researchers was used (Patel et al., 2018). In test tube, 20 µL plant extract or standard ursolic acid (5–40 µg) and 150 µL acidic vanillin reagent (5 g vanillin in 100 mL glacial acetic acid) was taken and mixed. Perchloric acid (500 µL) was added to the reaction mixture and heated in water bath for 45 min at 60 °C. Further, all the reaction mixtures were placed on an ice bath and brought to room temperature and 2.25 mL GAA was added. Absorbance was taken at 548 nm and results were expressed as mg ursolic acid equivalent (UAEC)/g extract.

2.6. Total alkaloid content (TAC)

TAC was determined as per method reported by Ghane et al. (2018). Briefly, 69.8 mg of bromocresol green was dissolved in 3 mL 2 N NaOH and 5 mL distilled water, heated and diluted for 1000 mL using distilled water. Plant extract (100 µL from 5 mg/mL stock), 1 mL bromocresol green and 1 mL sodium phosphate buffer was mixed and reaction mixture was extracted using 2 mL chloroform and absorbance of chloroform layer was measured at 470 nm. Galanthamine (20–120 µL) was used as standard and content were expressed as mg galanthamine equivalent (GE)/g extract.

2.7. Antioxidant assay

2.7.1. DPPH radical scavenging activity (DPPH)

The activity was performed as per Patel et al. (2020). Stock solution of DPPH was prepared by dissolving 25 mg DPPH in 1000 mL methanol and kept it in refrigerator until further use. Working solution of DPPH and 30 µL (5 mg/mL stock) plant extract or standard ascorbic acid (mg/mL) were taken in test tube. This reaction kept at dark for 30 min and absorbance of reaction was measured at 515 nm. Control was prepared by using 50 µL methanol in place of the plant sample. Results were expressed in mg of ascorbic acid equivalent (AAE)/g extract.

2.7.2. Ferric reducing antioxidant property assay (FRAP)

FRAP assay was performed according Patel et al. (2020). Briefly, FRAP reagent was prepared by mixing acetic buffer solution (pH 3.6), 10 µM TPTZ in 40 µM HCl, and 20 µM FeCl₃ at ratio of 10:1:1 (v/v/v). Plant extract (20 µL from 5 mg/mL stock) and 1 mL reagent was mixed, incubated at 37 °C in water bath for 30 min, and absorbance was read at 593 nm. Standard FeSO₄·7H₂O (20–120 mg/mL) was used to plot curve. Results were expressed in mg Fe (II) equivalent/g extract.

2.7.3. Phosphomolybdate assay (PMA)

The method given by Prieto et al. (1999) was adopted with some minor modifications. In this assay, 20 µL plant extract (5 mg/mL stock) or standard ascorbic acid (20–120 µg/mL) was mixed with phosphomolybdate reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Then reaction mixture was incubated in water bath at 90 °C for 90 min, cooled to room temperature and absorbance was measured at 695 nm. The antioxidant capacity was expressed as mg of ascorbic acid equivalents (AAE)/g extract.

2.7.4. Metal chelating activity (MC)

To determine total antioxidant capacity of L. echinata extracts, metal chelating assay was performed as per our previous reports (Attar and Ghane, 2019). In brief, 50 µL of 2 mM FeCl₂ was added to 40 µL (5 mg/mL stock) extract or standard EDTA (20–120 µg/mL). The reaction was started by the addition of 100 µL of 5 mM ferrozine solution and 650 µL distilled water was added to make up the volume. The mixture was vigorously shaken and left to stand at room temperature for 10 min. The absorbance of the solution was thereafter measured at 562 nm. Na₂-EDTA was used as positive control. The results were expressed as mg EDTA equivalent (EE)/g extract.

2.7.5. ABTS radical scavenging activity (ABTS)

ABTS radical cation decolourization assay performed according to our earlier protocols (Patel et al., 2018). ABTS radical cation (ABTS) was produced by reacting ABTS solution with 2.45 mM potassium persulphate (1:1 ratio) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. ABTS solution was diluted in methanol (1:89 ratio) to obtain an absorbance 0.7 (±0.02) at 734 nm. In test tube, 1 mL ABTS solution and 20 µL of extract (5 mg/mL stock) or standard was taken, incubated at 30 °C and absorbance taken at 730 nm exactly after 30 min using methanol as blank. All the solutions were used on the day of preparation. The results were expressed as mg trolox equivalent (TE)/g extract.

2.8. Anti-diabetic activity

2.8.1. α-Amylase inhibition activity

This activity was performed by using earlier published protocols with some minor modifications (Ghane et al., 2018). Plant extract (100 µL) was mixed with 111 µL α-amylase solution (18 units) and 0.02 M sodium phosphate buffer (pH 6.9) and final volume was made to 1 mL. This reaction mixture was then incubated at 25 °C for 10 min, 500 µL 1% starch solution was added and reaction mixture was incubated 25 °C about 30 min. Post incubation, 0.5 mL dinitrosalicylic acid reagent (1 g 3, 5-dinitrosalicylic acid in 20 mL of 2 M NaOH + 30 g Rochelle salt + 50 mL distilled water, all the contents were dissolved and final volume made up to 100 mL with distilled water) was used for interruption of the reaction. All the test tubes were incubated in boiling water bath (100 °C, 5 min) and cooled to room temperature. Finally, reaction mixture was diluted 5 times with distilled water and absorbance was taken at 540 nm. Control was prepared by using sodium phosphate buffer. Acarbose was used as a positive control. α-Amylase inhibition was calculated on a percent basis.

2.8.2. α-Glucosidase inhibition activity

α-Glucosidase inhibition activity was studied as per Ghane et al. (2018). Briefly, 20 µL plant extract was mixed with 100 µL α-glucosidase solution (1 U/mL), 0.1 mol/L phosphate buffer (pH 6.9) and volume made to 500 µL. After 5 min of pre-incubation at 25 °C, 100 µL p-nitrophenyl-α-D-glucopyranoside (5 mmol/L) solution was added and reaction mixture was incubated (10 min, 25 °C). Reaction was terminated by adding 1 mL of 0.1 M Na₂CO₃.
and absorbance was measured at 405 nm. The control was without any extract and acarbose was used as a positive control. Inhibition of α-glucosidase was calculated on a percent basis.

2.9. Acetylcholine esterase (AChE) inhibitory activity

AChE inhibition was studied as per Ghane et al. (2018). Known volume of plant extract (10 μL) was mixed with 15 μL (0.04 unit) of AChE solution. Sodium phosphate buffer (0.1 M pH 8.0) was added to make 800 μL reaction volume and incubated for 15 min at room temperature. Post-incubation, 60 μL of 0.5 mM 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) was added and reaction was initiated by adding 20 μL acetyl thiocyanide iodide (0.71 mM). After 30 min of incubation, absorbance of the reaction was measured at 412 nm on UV–visible spectrophotometer. Galanthamine hydrobromide was used as a standard control. AChE inhibitory activity was calculated on a percent basis.

2.10. Anticancer activity

Anticancer activity was studied as per Patel et al. (2020). Breast (MCF-7) and colon (HT-29) cancer cell lines were grown on RPMI 1640 medium which contain 10% fetal bovine serum and 2 mM L-glutamine. All the selected cells were inoculated into 96 well microtiter plates (100 μL) and plates were incubated at 37 °C temperature, 5% CO2, 95% air and 100% relative humidity for 24 h. All the extracts were solubilized in dimethyl sulfoxide (100 μg/mL) and diluted using distilled water to obtain 1 mg/mL stock. Extracts were diluted to 100–800 μg/mL with the medium and 10 μL of these different drug dilutions were added to the appropriate microtiter wells. Post-incoculation, all the plates were incubated for 48 h, and 50 μL cold TCA (25% w/v) was used to stop the reaction and incubated for 60 min at 4 °C. Further, supernatant was discarded and washed with tap water. Sulfurphodamine B (0.4%, 50 μL) solution was mixed in each plate and incubated for 20 min. Washing five times with residual dye was removed from well 1% acetic acid followed by air drying. Bound stain was then eluted with 10 mM nitrobenzoic acid (DTNB) was added and reaction was initiated by adding 20 μL of 0.5 mM 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) was added and reaction was initiated by adding 20 μL acetyl thiocyanide iodide (0.71 mM). After 30 min of incubation, absorbance of the reaction was measured at 412 nm on UV–visible spectrophotometer. Galanthamine hydrobromide was used as a standard control. AChE inhibitory activity was calculated on a percent basis.

2.11. Analysis of cucurbitacins, phenolics and other metabolites by RP-HPLC, GC–MS and HR-LCMS

2.11.1. RP-HPLC analysis of cucurbitacins

Three tetracyclic tri-terpenes viz. cucurbitacin I (Cul), cucurbitacin B (Cub) and cucurbitacin E (Cue) were quantified using RP-HPLC apparatus equipped with quaternary pump, autosampler, Hiber C18 column (5 μm, 250–4.6 mm) and UV detector (UV 2070) (Jasco Model No. LC-2000 Plus). Mobile phase consists of acetonitrile, water and methanol (32:35:33, v/v/v) and flow rate was 1 mL/min (Patel et al., 2020). Injection volume was kept at 20 μL. All the peaks were monitored at 230 nm with residual dye was removed from well 1% acetic acid followed by air drying. Bound stain was then eluted with 10 mM nitrobenzoic acid (DTNB) was added and reaction was initiated by adding 20 μL acetyl thiocyanide iodide (0.71 mM). After 30 min of incubation, absorbance of the reaction was measured at 412 nm on UV–visible spectrophotometer. Galanthamine hydrobromide was used as a standard control. AChE inhibitory activity was calculated on a percent basis.

2.11.2. RP-HPLC analysis of phenolics

RP-HPLC analysis of phenolics was performed using Jasco chromatographic system equipped with quaternary pump, autosam-
ponent analysis (PCA) was performed using Minitab ver. 18 software.

3. Result

3.1. Phytochemical analysis

In phytochemicals, TPC, TFC, TTC, TAC and TTEC were determined and results are depicted in Table 1. TPC was dependent on the plant parts and solvents used in the present study and values ranged between 189.56 and 38.70 mg TAE/g extract. Acetone extract of leaves (189.56 ± 1.9 mg TAE/g extract) and methanol extract of fruits (104.85 ± 0.61 mg TAE/g extract) exhibited highest TPC, while aqueous extract of both leaf and fruit denoted lowermost phenolic content (58.04 ± 0.86 and 38.69 ± 1.84 mg TAE/g extract, respectively). In TFC, acetone extract of leaf and fruit represent inflated TFC (30.48 ± 0.72 and 11.27 ± 0.50 mg CE/g extract, respectively). Alike TPC, least TFC was noted in aqueous extract of leaf and fruits (1.81 ± 0.52 and 0.057 ± 0.02 mg CE/g extract, respectively). In case of TTC, all the analysed extracts failed to show significant tannin content (13.78 to 2.80 mg CE/g extract), of which, methanol extract of leaf and fruit exhibited the highest TTC (13.78 ± 0.2 and 7.87 ± 0.15 mg CE/g extract, respectively) (Table 1). Higliest TTEC was documented from acetone extract of leaf and fruit (541.0 ± 2.23 and 602.7 ± 3.5 mg UAE/g extract, respectively). Similarly, acetonitrile leaf extract and acetonitrile fruit extract revealed highest TAC (18.99 ± 0.8 and 19.96 ± 0.9 mg GE/g extract, respectively) (Table 1).

3.2. Antioxidant assays

Antioxidant potential from the leaf and fruit extracts of L. echinata was estimated by measuring DPPH, ABTS, FRAP, PMA, and MC activities (Fig. 1). Among tested extracts, methanol leaf and fruit extracts showed promising DPPH radical scavenging activity (Fig. 1a) (42.35 ± 0.74 and 50.52 ± 0.03 mg AAE/g extract, respectively). In contrast, least responses were noted from aqueous extracts of leaf and fruit (10.22 ± 0.43 and 25.35 ± 0.69 mg AAE/g extract, respectively). In ABTS radical scavenging activity, methanolic extract of leaf and fruit exhibited remarkable activity (24.50 ± 0.39 and 26.78 ± 0.03 mg TE/g extract, respectively), whereas lowest activity was found in acetonitrile leaf and acetonitrile fruit extract (11.07 ± 0.18 and 14.48 ± 0.39 mg TE/g extract respectively) (Fig. 1b). Promising FRAP activity was observed in all the tested extracts wherein highest reduction was reported in aceton

3.3. Chemometric analysis

Relationship between phytochemicals and antioxidant activities from leaf and fruit extracts was studied (Fig. 2). Multivariate analysis of leaf extract revealed 84.1% total variability wherein component 1 contributed 63.8% variability. Among all the variables studied, TPC, TFC, TTC, TAC, DPPH, ABTS, FRAP, PMA and MC enjoyed positive dominance of component 1 with the coefficients 0.568, 0.415, 0.365, 0.345 and 0.390, respectively (Fig. 2a). Similarly, component 2 was mainly dominated by DPPH, ABTS, FRAP and PMA with the loading values 0.299, 0.538, 0.338 and 0.174, respectively. From Fig. 2a, it was cleared that only methanol solvent along with DPPH, ABTS, FRAP and PMA occupied positive plane of both the components. Similarly, variables from the fruits extracts were subjected to PCA and it was found that component first explained 53.1% variability out of 82.9% (Fig. 2b). Component 1 was dominated mainly due to TFC (0.284), TAC (0.309), TTC (0.345), TPC (0.390) and PMA (0.365). Among all the variables studied, TPC, TFC, TAC, TTEC, DPPH, ABTS, FRAP and PMA enjoyed positive side of component 2. In both the components, strong relationship between the phytochemicals (TPC, TFC, TTC and TAC) and antioxidant activities (DPPH, FRAP, ABTS and PMA) was observed from methanol and acetonitrile extracts of the fruits (Fig. 2b).

3.4. Bioactivities

Anti-diabetic, anti-acetylcholine esterase (AChE) and anticanic activities were performed and results are presented in Tables 1 and 2. Anti-diabetic activity of the selected extracts was performed by α-amylase and α-glucosidase inhibition and showed significant variation in the results. Acetonitrile leaf and acetonitrile fruit extracts

| Solvent | Plant part | Phytochemicals Bioactivities |
|---------|------------|-------------------------------|
|         |            | TPCa | TFCb | TTCb | TTECc | TACd |
| Acetone | Leaf       | 189.57 ± 1.9a | 30.48 ± 0.7 | 8.18 ± 0.1b | 541.09 ± 2.2 | 53.17 ± 3.9d |
|         | Fruit      | 95.72 ± 5.8b | 11.28 ± 0.5c | 5.76 ± 0.4d | 602.79 ± 3.5 | 76.59 ± 3.5ab |
| Acetonitrile | Leaf  | 70.87 ± 1.4a | 13.55 ± 0.3b | 13.11 ± 0.7e | 305.12 ± 2.8 | 83.33 ± 3.9b |
|         | Fruit      | 101.67 ± 1.9b | 6.64 ± 0.2a | 4.09 ± 0.2a | 84.96 ± 2.9 | 76.59 ± 3.5ab |
| Ethanol | Leaf       | 79.28 ± 2.1a | 2.69 ± 0.2a | 3.71 ± 0.07 | 84.65 ± 0.9 | 54.37 ± 1.1d |
|         | Fruit      | 145.43 ± 2.1b | 13.96 ± 0.8a | 13.79 ± 0.2a | 112.25 ± 2.2 | 61.51 ± 1.1a |
| Methanol | Leaf      | 104.86 ± 0.6c | 6.42 ± 0.1a | 7.88 ± 0.1b | 89.30 ± 4.1 | 67.86 ± 0.3b |
|         | Fruit      | 58.04 ± 0.8a | 1.81 ± 0.5 | 8.18 ± 0.2b | 88.91 ± 0.9 | 11.79 ± 1.5b |

Values were the means of three replicates ± Standard Error (SE). Mean value with different alphabets in column showed statistically significant differences (p < 0.05) according to Duncan multiple range test.

a (mg TAE/g extract), b (mg CE/g extract), c (mg UAE/g extract), d (mg GE/g extract). TAE: Tannic acid equivalent, CE: Catechin equivalent, UAE: Ursolic acid equivalent, GE: Galantamine equivalent. c % inhibition at standard acarbose at 100 μg – 36.84%, acetylcholine esterase inhibition at standard galanthamine (3 μg) – 32.41%. ND – Not defined.
showed highest \( \alpha \)-amylase inhibitory activity (83.33 ± 3.96 and 76.58 ± 3.57\%, respectively), while aqueous extract of leaf and fruit showed poor activity (Table 1). Acetonitrile extract of leaves and fruits revealed the highest inhibition of \( \alpha \)-glucosidase enzyme (64.64 ± 1.55 and 77.41 ± 1.43\%, respectively). In contrast, acetone extract of leaves and aqueous fruit extract explained the lowermost inhibitory activity (15.11 ± 0.76 and 11.79 ± 1.55\%, respectively) (Table 1). Strong AChE inhibition was reported in aqueous leaf extract (88.91 ± 0.90\%) and acetone fruit extract (81.87 ± 0.9\%). Anticancer activity was evaluated against human breast (MCF-7) and colon (HT-29) cancer cell lines and results are presented in Table 2 and supplementary Figure 1. Growth inhibition was directly proportional to concentration of extract tested. Fruit extract of \( \textit{L. echinata} \) showed highest cytostatic (TGI-161.58 mg/mL) and cytotoxic (LC\(_{50}\) 329.36 mg/mL) activity against MCF-7 cell line when compared to standard adriamycin (GI\(_{50}\) < 10 \( \mu \)g/mL, TGI 104.27 \( \mu \)g/mL, LC\(_{50}\) 385.17 \( \mu \)g/mL). Similarly, efficient activity reported against HT-29 cell line. The extract also showed remarkable anticancer activity (GI\(_{50}\) < 10 \( \mu \)g/mL, TGI-3.95 \( \mu \)g/mL, LC\(_{50}\) 159.98 \( \mu \)g/mL) against adriamycin (GI\(_{50}\) < 10, TGI 0.880, LC\(_{50}\) 63.20 \( \mu \)g/mL) (Table 2; Supplementary Fig. 1).

### 3.5. Detection of cucurbitacins, phenolics and other bioactives

Important bioactive metabolites were separated by using RP-HPLC, GC–MS, and LC-MS. RP-HPLC analysis revealed presence of three cucurbitacins (Cul, CuB and CuE) (Fig. 3, Supplementary Fig. 2). Fruits extracts divulged presence of all the three cucurbitacins; wherein, CuB (196.24 ± 1.4 mg/g DW) content was highest followed by CuE (57.14 ± 4.9 mg/g DW) and Cul (2.03 ± 0.03 mg/g DW) (Fig. 3b). Similarly, leaf extract showed presence of only two cucurbitacins viz. CuB (2.19 ± 0.17 mg/g DW) and CuE (2.97 ± 0.54 mg/g DW) (Fig. 3c). Fruit extract revealed presence of phenolics where GA (1.26 ± 0.07 mg/g DW) was found in highest quantities followed by CA (0.40 ± 0.04 mg/g DW), VA (0.14 ± 0.01 mg/g DW), CHLA (0.039 ± 0.006 mg/g DW) and COA (0.005 ± 0.00 mg/g DW). Content of all the phenolics noted comparatively less from the leaves wherein GA was observed in higher (1.26 ± 0.07 mg/g DW).
Table 2

In vitro anticancer activity of *Luffa echinata* fruit against human cancer cell lines.

|         | MCF-7 | HT-29 |
|---------|-------|-------|
|         | GI_{50} (µg/mL) | TGI (µg/mL) | LC_{50} (µg/mL) | GI_{50} (µg/mL) | TGI (µg/mL) | LC_{50} (µg/mL) |
| Standard adriamycin | <10 | 104.27 | 385.17 | <10 | 0.880 | 63.20 |
| *Luffa echinata* fruit | <10 | 161.58 | 329.36 | <10 | 3.95 | 159.98 |

TGI - concentration of drug causing total inhibition of cell growth, LC_{50} - concentration of drug causing 50% cell death, GI_{50} - concentration of drug cause 50% of maximal inhibition of cell proliferation.
mg/g DW) quantities (Supplementary Table 1 and Supplementary Fig. 3). By using GC–MS, bioactive metabolites were separated and identified from methanol extract. In the present study, total 24 compounds belong to fatty acids, phenols, lipids, volatiles, aldehydes, and triterpenes were detected and identified on the basis of chromatogram peak area, molecular weight, molecular formula, and derived compounds. Compounds such as 9,12-octadecadienoic acid, 18-nonadecenoic acid, hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, n-hexadecanoic acid, octadecanoic acid, squalene were identified (Supplementary Table 1). LC-MS analysis revealed presence of total 30 major compounds from the fruit extract (Table 3, Supplementary Fig. 4). Detected compounds were mainly from terpenoids (6), phenolics (2), flavonoids (6), alkaloids (3), and fatty acids (13) categories.

Fig. 3. RP-HPLC chromatogram (a) Std. 1-CuI, 2-CuR, 3-CuE; (b) Cucurbitacins in L. echinata fruit; (c) Cucurbitacins in L. echinata leaf.
Several important metabolites like cinnacassiol E (398.19 g/mol), cinnacassiol C₄ (382.19 g/mol), hymenoxon (282.14 g/mol), clerodin (434.23 g/mol), [6]-shogaol, maritimetin, gartanin, rotenone, hispidulin, berbamunine, bebeermesine, daphnoline, dimethyl adipate, traumatic acid, palmitic acid and petroselinic acid were reported (Table 3, Supplementary Fig. 4).

4. Discussion

*Luffa echinata* is rich source of bioactive compounds and hence used in pharmaceutical and cosmetic industries (Kirtikar and Basu, 1933). The presence of massive and important bioactivities relies on bioactive composites particularly phytochemicals and antioxidants. For that purpose validation and documentation of bioactive metabolites is very much essential (Ghane et al., 2018; Attar and Ghane, 2019; Patel et al., 2020). Phytochemical analysis of *L. echinata* exhibited diversified components from various extracts of leaf and fruits. Acetone and methanol extracts were found superior for phytochemical extraction, suggesting a greater content of polar and fruits. Acetone and methanol extracts were found superior for phytochemical extraction, suggesting a greater content of polar

### Table 3

The compounds detected in fruit extract of *Luffa echinata* by LCMS–ESI-Q-TOF-MS.

| Sr. No. | Category         | Name of Compound       | Molecular Formula | RT     | M/Z     | Mass    |
|--------|------------------|------------------------|-------------------|--------|---------|---------|
| 1      | Triterpenoids    | Cinnacassiol E<sup>a</sup> | C₂₀H₃₀O₇  | 4.59   | 381.1918| 382.199 |
| 2      | Hymenoxon<sup>a</sup> | C₁₅H₂₂O₅  | 12.58  | 281.1386| 282.146 |
| 3      | Ibuprofen acyl glucuronide<sup>a</sup> | C₂₀H₂₃O₆  | 13.31  | 397.1581| 398.156 |
| 4      | Cinnacassiol C₃<sup>a</sup> | C₂₀H₂₃O₇  | 4.58   | 395.1502| 396.1573|
| 5      | Clerodin<sup>a</sup> | C₂₀H₂₄O₇  | 4.58   | 397.1581| 398.156 |
| 6      | Nigakikatone E<sup>a</sup> | C₁₅H₂₄O₇  | 2.77   | 449.2182| 450.2255|
| 7      | Phenols<sup>b</sup> | [6]-Shogaol<sup>a</sup> | C₁₅H₂₂O₅  | 13.36  | 275.1642| 276.1713|
| 8      | Maritimetin<sup>a</sup> | C₁₅H₂₄O₇  | 20.1   | 285.0391| 286.0464|
| 9      | Flavonoids<sup>b</sup> | Gartanin<sup>a</sup> | C₂₀H₂₄O₇  | 34.22  | 395.1502| 396.1573|
| 10     | Rotenone<sup>a</sup> | C₂₁H₂₂O₇  | 34.81  | 393.1346| 394.1416|
| 11     | Aguin<sup>a</sup> | C₂⁰H₂₄O₇  | 3.28   | 563.1402| 564.1476|
| 12     | Meloneside A<sup>a</sup> | C₂₁H₂₄O₇  | 3.31   | 593.151 | 594.1583|
| 13     | Galangin<sup>a</sup> | C₂₁H₂₄O₇  | 6.52   | 269.0457| 270.053 |
| 14     | Hispidulin<sup>a</sup> | C₂₁H₂₄O₇  | 6.73   | 299.0564| 300.0637|
| 15     | Alkaloids<sup>a</sup> | Berbamunine<sup>a</sup> | C₂⁰H₂₄O₇ | 26.63  | 595.2819| 596.2885|
| 16     | Bebeermesine<sup>a</sup> | C₂₁H₂₄O₇  | 28.38  | 593.2663| 594.2733|
| 17     | Daphnoline<sup>a</sup> | C₂₁H₂₄O₇  | 30.82  | 579.2596| 580.2576|
| 18     | Fatty acid<sup>a</sup> | Methyl N-(a-methylbutyryl)glycine<sup>a</sup> | C₁₇H₂₄O₇  | 17.41  | 187.0971| 188.1044|
| 19     | Dimethyl adipate<sup>a</sup> | C₂₂H₂₆O₈  | 15.14  | 173.0815| 174.0887|
| 20     | Traumatic Acid<sup>a</sup> | C₂₁H₂₂O₈  | 24.1   | 227.1278| 228.1353|
| 21     | Chaulmoogric acid<sup>a</sup> | C₂₃H₂₆O₈  | 42.73  | 279.2343| 280.2416|
| 22     | Di(2-ethylhexyl) adipate<sup>a</sup> | C₂₃H₂₆O₈  | 43.04  | 369.3007| 370.308 |
| 23     | Palmitic acid<sup>a</sup> | C₁₇H₃₄O₂  | 47.03  | 281.2496| 282.2569|
| 24     | Traumatic Acid<sup>a</sup> | C₂₁H₂₂O₈  | 46.44  | 255.2335| 256.2407|
| 25     | Di(2-ethylhexyl) adipate<sup>a</sup> | C₂₃H₂₆O₈  | 47.03  | 281.2496| 282.2569|
| 26     | Fatty acid<sup>a</sup> | Methyl N-(a-methylbutyryl)glycine<sup>a</sup> | C₁₇H₂₄O₇  | 17.41  | 187.0971| 188.1044|
| 27     | Traumatic Acid<sup>a</sup> | C₂₁H₂₂O₈  | 47.03  | 255.2335| 256.2407|
| 28     | (+)-9,10-Dihydroic acid<sup>a</sup> | C₁₇H₂₄O₇  | 27.42  | 313.2392| 314.2465|
| 29     | Sebacic acid<sup>a</sup> | C₁₇H₃₄O₂  | 29.92  | 201.1135| 202.1208|

<sup>a</sup> Isocratic system (acetonitrile: methanol: water; 32:35:33).
<sup>b</sup> Gradient system (0.01% formic acid: acetonitrile; 65:35).
best. Hence, polar solvents could be preferred to separate antidiabetic drugs from *L. echinata*. Kushawaha et al. (2016) determined similar findings from hot water extract of *Cucurbita maxima* seeds. Acetylcholinesterase inhibitors are the medications that prevent the breakdown of acetylcholine in human body. Acetylcholine is one of the chemicals responsible for communication in between nerve cells and brain. Its reduced level initiates symptoms of Alzheimer’s disease (Khadri et al., 2010). Leaf and fruit extracts showed presence of significant AChE inhibitory activity. Similar findings have been reported by Ghane et al. (2018) in different species of *Crinum*. Anticancer activity of methanolic fruit extract was tested against human breast (MCF-7) and colon (HT-29) cancer cell lines and findings showed presence of promising anticancer compounds in *L. echinata*. Patel et al. (2020) reported higher inhibitory activity from the methanolic fruit extract of *D. palmatus* when tested against MCF-7 and HT-29 cell lines (IC_{50} 44.27 and 68.31 µg/ml, respectively) that could be due to the presence of cucurbitacins.

Antioxidants are responsible for detoxification of reactive oxygen intermediates in plant system (Patel et al., 2018; Patel et al., 2020). Therefore, improved antioxidant status can minimize the risk of developing free radical induced disease. Antioxidants are determined spectrophotometrically by exploiting ability to reduce fluorescent or oxidizing agent, and this change in colour is correlated with antioxidant activity (Siddeeg et al., 2020). Antioxidants are commonly screened by using DPPH, ABTS, FRAP, phosphomolybdate, metal chelating etc. assays (Ghane et al., 2018; Patel et al., 2018; Attar and Ghane, 2019; Patel et al., 2020). DPPH is a stable free radical which accepts electrons and hydrogen radical from antioxidant compound. A solution of DPPH radicals is converted into DPPH-H (diphenylhydrazine) molecules having low colour intensity. The discoulouration of DPPH solution due to the extract represents radical scavenging activity (Aksoy et al., 2013). Similar findings were reported in *L. cylindrica* and *Lagenaria siceraria* (Sharma et al., 2012; Attar and Ghane, 2019). ABTS cation radical was developed by reaction of potassium per sulphate with ABTS salt. Loss of nitrogen atom from ABTS responsible for the formation of free radicals. Nitrogen quenches hydrogen atom and results into decoulourisation of solution. In FRAP assay, antioxidants react with a ferric tripopyridyldiazine (Fe^{3+}-TPTZ) complex which produces blue coloured ferrous tripopyridyldiazine (Fe^{2+}-TPTZ) and its reducing potential is measured at 593 nm (Patel et al., 2018; Attar and Ghane, 2019). In phosphomolybdate assay, there was production of green phosphate/Mo(V) complex due to presence of antioxidant and phosphate ions were reduced. By using spectrophotometer green phosphate/Mo(V) complex was measured (Prieto et al., 1999). Metal ions like iron which stimulates lipid per-oxidation by Fenton reaction as well as responsible for decomposition of lipid hydro peroxides into peroxyl and alkoxyl radicals that can perpetuate the chain reaction. Chelating activity is significant which reduces the concentration of the transition metal that catalyzes lipid peroxidation (Mohan et al., 2012). Highest DPPH, ABTS, FRAP, PMA, and MC were recorded from methanol, acetone and aqueous extracts and hence could be the best solvents for maximizing the recovery of antioxidants (Ghane et al., 2018; Patel et al., 2018; Attar and Ghane, 2019). Antioxidant activities showed positive correlation with analysed phytochemicals, this might indicate that these phytochemicals are main contributor to antioxidant activity in the examined extracts (Attar and Ghane, 2019).

Further, correlation between phytochemicals and bioactivities from five extracting solvents was determined by principal component analysis (PCA). PCA is the most common statistical method used to analyse relationship between numbers of variables to compress original data into small factors with minimum loss of information. Data with suppressed or hidden information of all the variables increase the efficiency of statistical technique and computed variables could be intercorrelated in PCA (Garcia et al., 2019). From the PCA data, it could be inferred that methanol and acetone extracts found responsible for the potent antioxidant activities. These solvents could be used for the extraction of natural antioxidants from *L. echinata*. Our results agreed with Attar and Ghane (2019), who tested fruit parts of *L. siceraria* for determination of variation in the chemical profile. Ghane et al. (2018) observed similar pattern of attraction in between phytochemicals and antioxidants of methanol extract of *Crinum* species. Similarly, Gupta et al. (2018) demonstrated variation in chemical profile of different plant parts of *Citrullus colocynthis*.

HPLC analysis confirmed the presence of three tetracyclic triterpenes i.e. cucurbitacins in methanolic extract of leaves and fruits. Fruits represented all the three cucurbitacins (Cul, CuB and CuE), while leaf acquired only CuB and CuE. Similarly fruit extract acquire phenolics (GA, CA, VA, CHLA and COA) in significant amount. Terpenes as well as phenolics are also known to possess promising antioxidant potential (Celaya et al., 2016; Attar and Ghane, 2018; Patel et al., 2020). Additionally, GC–MS and LC–MS analysis revealed presence of compounds like octadecadienoic acid, nonadecenoic acid, hexadecanoic acid, squalene, cinncassiol E, clerodin, galangin, hispidulin, maritimetin, and bebeerines etc. were known to possess anticancer, anti diabetic, antimicrobial, antioxidant, anti-inflammatory, antimitogenic activities (De Freitas et al., 2017; Kou et al., 2018; Oetari et al., 2019; Yin et al., 2008). Compounds detected in RP-HPLC, GC–MS and LC–MS analysis could be responsible to exhibit potent bioactivities in *L. echinata*.

5. Conclusion

Present study revealed that fruit and leaf extract of *L. echinata* exhibited promising antioxidant activities that could be due to the presence of phenolics, flavonoids and terpenoids. Acetone and methanol were found to be the best solvents for maximizing the recovery of antioxidants. Acetone and acetonitrile extracts revealed promising anti diabetic (α-amylase, α-glucosidase inhibitory) and anti-acetylcholine esterase activities. Methanol extract of fruit was found effective against tested cancer cell lines viz. MCF-7 and HT-29. RP-HPLC study showed presence of tetracyclic tri-terpenes called cucurbitacins viz. Cul, CuB and CuE and five phenolics (GA, CA, VA, CHLA and COA). In LC-MS and GC–MS analysis also revealed diverse array of compounds belongs to terpenoids, phenolics, alkaldoids and fatty acids. All the detected compounds could be responsible for the potent antioxidant, anti diabetic, anti-acetylcholine esterase and anticancer activities. We conclude that fruits of *L. echinata* can be used for the management of diabetes, neurological disorder and cancer through development of novel drugs.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Authors are thankful to Science and Engineering Board (SERB), New Delhi, India for financial assistance (Sanction no. SB/EMEQ-460/2014). DST–FIST and UGC-DRS-SAP programs are duly acknowledged. We are also grateful to Anti-cancer drug screening facility (ACDSF), Advanced Centre for Treatment, Research & Education in Cancer, (ACTREC), Tata Memorial Centre, Mumbai, India for their help in anticancer activities.
Tiwari, A.K., Rao, J.M., 2002. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. Curr. Sci. 83, 30–38. https://www.jstor.org/stable/24106071.

Whiting, D.R., Guariguata, L., Weil, C., Shaw, J., 2011. IDF Diabetes Atlas: Global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Res. Clin. Pract. 94, 311–321. https://doi.org/10.1016/j.diabres.2011.10.029.

World Health Organization (WHO). Diabetes. https://www.who.int/Home/Newsroom/Fact_sheets/Detail/Diabetes/ (accessed 18 November 2020).

Yin, J., Xing, H., Ye, J., 2008. Efficacy of berberine in patients with type 2 diabetes mellitus. Metabolism. 57, 712–717. https://doi.org/10.1016/j.metabol.2008.01.013.