Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2015; 4(1): 87-94
Received: 18-03-2015
Accepted: 02-04-2015

Nazia Khatoon
Food Processing Technology Laboratory, University School of Biotechnology, Guru Gobind Singh Indraprastha University, Sector-16 C, Dwarka, New Delhi-110078, India.

Rajinder K. Gupta
Food Processing Technology Laboratory, University School of Biotechnology, Guru Gobind Singh Indraprastha University, Sector-16 C, Dwarka, New Delhi-110078, India.
Email: rkg67ap@yahoo.com

Yogesh K. Tyagi
University School of Basic and Applied Sciences, Guru Gobind Singh Indraprastha University, Sector-16 C, Dwarka, New Delhi-110078, India.
Email: drytyagi@gmail.com

Correspondence:
Yogesh K. Tyagi
University School of Basic and Applied Sciences, Guru Gobind Singh Indraprastha University, Sector-16 C, Dwarka, New Delhi-110078, India.
Email: email: rkg67ap@yahoo.com, and drytyagi@gmail.com
Contact No. +91-11-25302408

Nutraceutical potential and phytochemical screening of *Buchanania lanzan*, an underutilized exotic Indian nut and its use as a source of functional food

Nazia Khatoon, Rajinder K. Gupta, Yogesh K. Tyagi

Abstract
The seeds of an underutilized exotic fruit Chironji, *Buchanania lanzan* (Anacardiaceae) were investigated for their nutritional, phytochemical and antioxidant properties to understand its nutraceutical potential. The nutritional analysis showed good protein content (43.24 %), moderate carbohydrate content (12.96 %), and high amount of crude fiber (18.50 %), fat (38 %) and also high calorie value (229.99kCal). ICP-OES analysis has shown these edible seeds contain Iron (4.8mg/100g), Phosphorous (593mg/100g), Magnesium (275mg/100g) and Calcium (70mg/100g) in considerable amount and Manganese, Copper, Barium, Aluminium and Boron in trace amounts. A thorough physiochemical characterization of the seeds demonstrates that it as an active source of phenolics, natural antioxidants and minerals. Total phenolics and flavanoid content in petroleum ether extract (PEE), dichloromethane extract (DCME), methanolic extract (ME) and ethanol and water extract (EWE) were 5.78, 6.73, 10.05 and 13.42 µg GAE mg^{-1} and 13.74, 7.08, 5.21 and 11.41 µg CE mg^{-1} respectively. Antioxidant activities were carried out using FRAP assay and the results reveal that EWE and ME showed the highest antioxidant activity 13.42 and 10.05 µg $BHTg^{-1}$ respectively. Moreover EWE and ME showed antibacterial activity against *S. flexneri* and *B. cereus* strain. Antifungal assay was also carried against three test organisms. GC/MS Screening confirms the presence of considerable amounts of fatty acids, plant sterols and phenolic compounds. The oil of seeds was extracted by cold pressing and analyzed for the fatty acid profile which revealed the presence of polyunsaturated fatty acid such as Linolenic Acid (ω-3) and Linoleic Acid (ω-6) monounsaturated fatty acid such as Oleic Acid (ω-9). The seeds provide opportunities to develop value added products, dietary supplements and phytotherapeutic compounds.

Keywords: Phenolics, Flavonoid, GC/MS, *Buchanania lanzan*, Anacardiaceae.

1. Introduction
There is a lot more fruits and nuts in India, which are underutilized and ignored besides having lots more essential components, Chironji (*Buchanania lanzan*) is one of them. There is a need to explore such fruits and nuts for nutritional purposes. Chironji also known as char, achar, charoli and priyal. The tree is almost evergreen and grown in the tropical deciduous forests of Northern, Western and Central India, mostly in the states of Chhattisgarh, Jharkhand, Madhya Pradesh, Varanasi and Mirzapur districts of Uttar Pradesh Siddiqui ZM *et al.* [1]. The charoli seeds are lentil-sized, slightly flattened, and has an almond-like flavor and eaten in raw or roasted form. Oil is also used to treat skin diseases, remove spots and blemishes from the face. Instead of having enormous health and medicinal benefits very little literature is available on nutritional significance. All parts of this plant root, leaves, gum, bark and fruits have various medicinal applications. Mehta *et.al* demonstrated that the methanolic extract of *B. lanzan* kernel exhibited anti-inflammatory activity. Seeds are also medicinally valuable; contribute in ayurvedic and unani medicine as a nervine tonic, anticough and antileprotic Gupta M *et al*. [2]. Cell mediated immunity (CMI) and humoral immunity was significantly stimulated by *Buchanania lanzan* kernel Puri A *el al*. [3]. Sushma *et al* reported that methanolic leaves extract of *B. lanzan* possess antidiabetic, antihyperlipidemic and antioxidant activity. Ethanolic and methanolic extract of *Buchanania lanzan* Spreng roots has shown good antidiarrheal activity and significant wound healing activity respectively Kodati D *et al*. [4]. Ethanolic extract of *Buchanania lanzan* Spreng barks reduces chromosomal damage and oxidative stress Jain R *et al*. [5]. *Buchanania lanzan,* is included in the Red Data Book published by the International Union for Conservation of Nature and Natural Resources (IUCN) which include all medicinally valuable plants. This plant has also socio-economical benefits; it provides income to tribal people. This plant requires urgent conservation efforts because of extinction probability, the main reason is over-exploitation and no proper method of harvesting.

There is a need for development of proper technology for harvesting and educational program for tribal people related to development of *Buchanania lanzan* tree Kumar J *et al*. [6]. Natural antioxidants (free radical scavengers) are vastly used in the food industry to intensify the sensory, health-promoting, or retaining quality of foods Gulcin I *et al.* [7]. Studies show that consumption of food high in antioxidants as being prevented against certain types of cancer and may also reduce the risk of cardiovascular and cerebrovascular events. Oxidative damage of cellular and biomolecules (protein, lipid and nuclic acid) has been effectively reduced by antioxidants.

2. Materials and Methods

2.1 Reagents

All chemicals and solvents used in the sample preparation were analytical grade and purchased from local suppliers of SRL, Rankem labs and CDH. All the reference standards such as rutin, gallic acid, catechin, ascorbic acid were acquired from Sigma- Aldrich (Sigma- Aldrich, St. Louis, MO, USA). All media were purchased from SRL (Sisco Research Laboratories, Delhi, India).

2.2 Materials

Seeds of *Buchanania lanzan* were procured from a grocery store in Delhi and analyzed for its quality and adulteration of unwanted materials. They were screened manually for its quality.

2.3 Sample preparation- *Buchanania lanzan* seeds were crushed to very fine with the help of pestle and mortar and stored in an airtight container at 4 ºC for experimental purpose. Oil of seeds was obtained through cold press method and stored in the dark.

2.4 Extraction

Extraction was carried out by a soxhlet extraction method using three different solvents petroleum ether, dichloromethane and methanol sequentially and on shaker using ethanol-water (50:50). For the soxhlet extraction 100 g of crushed seeds were taken into soxhlet apparatus in a thimble and 300 ml of solvent, run sequentially for 4 cycles. Obtained solvents were concentrated on water bath. For the extraction on shaker, 100g of the sample with 100ml solvent were taken in a reagent bottle (60 °C, 140 rpm) and left on shaker for 48 hours with successive filtration. Then, the crude viscous extract was procured by concentrating the filtrates using a hot air oven. After evaporation of organic solvents all extracts were stored at -20 ºC until analysis.

2.5 Estimation of Nutritional constituents

The *Buchanania lanzan* seeds were analyzed for moisture, ash, protein, fat and carbohydrate content. Estimation of both moisture and ash content were done by gravimetrical method at 103 ºC to 104 ºC and ≤ 525ºC respectively. Protein content was obtained using Kjeldal method Ref. 976.05 Arlington, VA. [8]. Crude fat content of raw sample screened by using petroleum ether as an extracting medium. A gravimetric method was applied for screening of total dietary fiber content of fat and moisture free sample Arlington VA [8]. Total carbohydrates and energy content of the seeds were calculated using formulae:

Carbohydrate content = {100- moisture (%) –protein content (% dry weight) –crude fat (% dry weight) – ash (% dry weight)}

The calorific value in kilocalories (kcal) was obtained by following a system of Atwater, namely:

Energy (kcal) = (3.36 X % protein) + (3.60 X % carbohydrate) + (8.37 X % fat)

All major and minor mineral elements in the inspected material were obtained by using Optima 2100 DV ICP-OES (Perkin-Elmer, USA), for the response of the instrument a certified multi-element standard solution was used as a standard (Anton Paar Ltd., Hertford, UK) as per Ref 956.52 (AOAC, 2005).

2.6 Phytochemical analysis

Determination of total phenolics, flavanoids, alkaloids, tannins and saponins were done for phytochemical analysis of the seeds.

2.6.1 Total Phenolics content

The whole phenolics contents were estimated according to the Folin-Ciocalteau method given by McDonald S *et al*. [9]. An aliquot of the extract (100 µl) was blended with Folin Ciocalteu's reagent (250 µl), vortex and incubated at room temperature (RT) for 5 minutes. Sodium bicarbonate (1.5 ml of 20 %) was added to the reaction mixture and incubated again at RT for two hours. Absorbance was taken at 765 nm using a UV-Vis spectrophotometer. The total phenolic contents were expressed in µg of Gallic acid equivalents (GAE) per 1 mg of the extract Soni N *et al*. [10].

2.6.2 Total Flavanoid content

The aluminium Chloride calorimetric method was applied to determine total flavanoid content Chang C *et* al. [11]. Catechin (1 mg/ml) was used as a standard for preparing the calibration curve. Sample (250 µl) was diluted with distilled water (4.5 ml), followed by (0.03 ml, 5%) Sodium nitrate solution was added. After an incubation of 5 minutes, (0.03 ml, 10%) aluminium chloride was added at 25 ºC. After 5 minutes, NaOH (0.2 ml of 1M) was added into the reaction mixture. Final total volume adjusted up to 10 ml using distilled water, vortex and the observance was assessed instantly at 510 nm.

2.6.3 Total Tannin Content

5gm of sample was dissolved in distilled water (500 ml), and then incubate on shaker at 30 ºC, 140 rpm for 1hr. Then filtered the solution and taken 5 ml of filtrate in a test tube. Added $FeCl_3$ (3 ml of 0.1M) in potassium ferrocyanide (0.1N HCl+0. 008M) and the absorbance measured at 605 nm within 10 minutes.

2.6.4 Saponins content

Saponins were estimated gravimetrically according to the methods described by Obadoni & Ochuko *et al.* [12]. Raw sample powder (5g) was mixed with aqueous ethanol (50 ml of 20%). The Reaction mixture (RM) was heated over a hot water bath for 4 h (at 55 °C) with continuous stirring. The RM was filtered and the obtained residue re-extracted with other aqueous ethanol (50 ml of 20%). The combined RM was reduced to one-fourth of original volume over a water bath (90 °C). The concentrate was taken into a separating funnel and diethyl ether (20 ml) was added and shaken thoroughly for purification and this procedure done twice. The ether layer was discarded while the aqueous layer was recovered. n-butanol (15 ml) was added and the combined RM were washed twice with aqueous sodium chloride (10 ml of 5%). The samples

were dried into the oven till constant weight and the saponins were measured in percentage Mehra M *et al* [13].

2.6.5 Crude alkaloids determination
The crude alkaloid content was estimated gravimetrically Herborne JB. [14]. Raw sample (2.5 g) were taken and mixed with 100 ml of 10% acetic acid in ethanol solution and incubated for 4 hours at RT. The solution was filtered and concentrated to one fourth of original volume using a water bath. Concentrated ammonium hydroxide was added drop by drop to the RM until the precipitation occurred. The precipitate was collected and washed using dilute ammonium hydroxide and filtered. The crude alkaloid was weighed and calculated in percentage.

2.6.6 Antioxidant Potential
The antioxidant potential can be assessed by finding their free radicals scavenging capacity or their potential to reduce the compounds using the FRAP assay.

2.6.6.1 Ferric reducing activity power (FRAP) assay
The assay was performed according to the methodology of Benzie and Strain [15]. For performing FRAP assay, FRAP reagent was freshly made by combining TPTZ solution, $FeCl_3$ solution and acetate buffer in 1:1:10 proportion. An extract solution (100 µl) was mixed with FRAP (900 µl) reagent. After the mixture placed at 37 ºC for 4 min, the observance was taken at 593 nm against the blank. BHT was taken as the standard. The results were obtained as µg BHT equivalent per mg sample Mehra M, et *al.* [13].

2.7 Antibacterial activity
All the extracts were screened for antibacterial activities by an agar well diffusion method proposed by Kirby Bauer. Three Gram-positive bacteria and two Gram negative bacterial test pathogens were inoculated into nutrient broth. The Nutrient Agar (NA) plates were made (incubated at 37 °C for 24 h) for analysis. Extracts were reconstituted to a final concentration of 100 mg/ml. The bacterial inoculums (100 µl) were inoculated on Nutrient agar plates by spreading. Well of 6 mm was punched in the center of petri plates with the sterile cork borer & the extract was loaded into the well and incubated at 37 ºC for 24 h. The antibacterial activities were screened by measuring the diameter (mm) of the zone of inhibition. DMSO was used as control in one of the wells.

2.8 Antifungal activities
Antifungal activity was performed on PDA plate by well diffusion method against three fungal strains *Geotrichum candidum*, *Aspergillus niger* & *Rhizopus stolonifer*. For this, well of 10mm was punched in the center of petri plates with the sterile cork borer and the extract was poured into the well, Fungal plugs of 0.8 mm were placed on both sides of the periphery of petri plate. These plates were incubated at 30ºC for proper fungal growth & antifungal activity of the extract was checked on 7^{th} & 8^{th} days of incubation.

2.9 Characterization of secondary metabolites using GC/MS
Mass spectrometric detector (MSD- an Agilent 5975B) was used in scan mode (m/z 35-1050). The automatic RTL screener software in combination with the Agilent NIST'05 library were used to screen volatile and semi volatiles Mehra M *et al*. [13].

2.10 Fatty Acid profile by GC analysis
Fatty acid compositions were determined successfully, according to the AOAC Official method by gas chromatography. In order to obtain the fatty acid composition by GC, Initially edible oil samples were subjected to a transesterification operation to convert triacylglycerol into fatty acid methyl esters. Standard solution containing a mixture of fatty acid methyl esters were injected to determine retention time. Finally the methyl esters were subjected to GC analyses by extracting from the reaction mixture with 10 ml of n-hexane. The whole procedure was executed in duplicate Barison A *et al.* [16].

3 Results and Discussion
With the aim of determining the nutritional and health promoting components in fruits of *Buchanania lanzan* and its potential as a neutraceutical, above mentioned experiments were conducted and the results of those experiments are as follows:

3.1 Nutritional composition
The analysis of seeds of *B. lanzan* has shown its potential nutritional significances. It has revealed that *B. lanzan* seeds are a rich source of protein (43.24%) and fat (38%) content. *B. lanzan* seeds were also high dietary fiber content (18.50%). Seeds contain moderate amounts of carbohydrate (12.96%) which is generally available as instant energy source. The caloric values of the seeds were also high (229.99kCal). Moisture content determination is very important because it directly affects the nutritional contents of the grains, fruits, vegetables, nuts and its keeping quality & stability etc. Proximal values were calculated and are depicted in the Table 1.
The mineral composition (mg/100g) shows Phosphorous (P), Calcium (Ca), Iron (Fe), Magnesium (Mg) and Zink (Zn) in highest amount followed by other trace mineral contents. Phosphorous play vital role in the maintenance of healthy bone and teeth and also plays important role in energy metabolism. A high Ca content is not only play mandatory role in providing rigidity to the skeleton but also vital for blood clotting and many other metabolic processes Reid IR *et al*. [17]. Magnesium is needed for enzyme action, strong bones and teeth, balanced hormones, a healthy nervous and cardiovascular system Soni N *et al.* [10]. Recent studies prove very vital role of magnesium in the prevention of cardiovascular diseases Del Gobbo LC, *et al*, Chiuve SE *et al* [18, 19]. These minerals play crucial role in body metabolism and also have vital therapeutic significances.

Table 1: Nutritional composition of seeds of *Buchanania lanzan* (g/100g)

COMPONENTS	PROXIMAL VALUE (%)
Ash	2.20
Moisture	3.6
Crude fat	38
Total protein	43.24
Total carbohydrate	12.96
Total Crude fiber	18.50
Energy value (kCal)	229.99

Table: 2 Total Mineral contents estimated in *B. lanzan* seeds

MINERAL	CONCENTRATION (mg/100g)
P	593
Sr	0.68
Ti	0.01
Zn	3.32
Al	0.3
B	0.6
Ba	0.15
Ca	70
Cu	1.15
Fe	4.8
Mg	275
Mn	1.6

3.3 Phytochemical Analysis

The phytochemical analysis revealed the presence of phenolics, flavanoid alkaloids, saponins and other secondary metabolites. Phytochemicals, mainly phenolics are considered to be the important bioactive compounds. Multiple biological effects of phenolics are related to its antioxidant activity. The extracts which contain different classes of polyphenols are not only attractive in Phytotherapy but also in the food industry. These are responsible for a wide range of physiological effects, such as anti-allergenic, anti-atherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardioprotective and vasodilatory effects Rodrigues S *et al.* [20]. The highest phenolics and flavanoids were found in ethanol-water and Petroleum ether extract respectively. The total tannin content was 0.00425 µg TA/mg of sample. The alkaloid content was found to be 3.5 (%). The amount of saponins was calculated and found to be 0.59 g per 100 g of seed sample. Alkaloid has many pharmacological significance like antihypertensive effects (many indole alkaloids), antiarrhythmic effect (quinidine, spareien), antimalarial activity (quinine), and anticancer actions. Saponins are known as anti-nutritional factors that can reduce the uptake of certain nutrients, including cholesterol and glucose at the gate through intra lumenal physicochemical interaction or other yet unidentified activity Igwe C *et al* [21].

Table 3: Phytochemical analysis of *Buchanania lanzan* seed extract

SOLVENTS	Total phenolic content (µg GAE mg^{-1})	Total Flavonoid Content (µg CE mg^{-1})
Petroleum ether	5.78	13.74
Dichloromethane	6.73	7.08
Methanol	10.05	5.21
Ethanol- water	13.42	11.413

3.4 Antioxidant potential

Polyphenolics are considered to function as antioxidants by various mechanisms like donating electrons, free radical scavenging by H-donation. Hence FRAP assay was performed to find out the potential of *Buchanania lanzan* seeds as a candidate for nutraceutical and determined its pharmacological significance.

3.4.1 FRAP assay

The ability of all the extract to reduce ferric ions was obtained by using the FRAP assay proposed by Benzie and Strain (1996). BHT (standard) used to plot a calibration curve, for this assay and values of BHT equivalents for the samples were calculated by extrapolation of the standard curve y = 0.0136x-0.0952, R^2 =0. 999. The values are tabulated below:

Table 4: FRAP assay for different extracts of *Buchanania lanzan* seeds extract.

SOLVENT	BHT equivalents (µg/1mg)
Petroleum ether	1.07
Dichloromethane	3.567
Methanol	11.13
Ethanol-Water	18.90

3.5 Antimicrobial activity

To evaluate the antibacterial activity of *Buchanania lanzan* seeds three gram positive (*Staphylococcus aureus, Staphylococcus epidermidis and Bacillus cereus*) and two gram negative (*E. coli, S. flexneri*) bacterial test pathogens were used. Results were analyzed by measuring the zones of inhibition. Inhibiting concentrations used for both samples were 100 mg/ml. The methanol and ethanol-water extracts showed significant zones of inhibition (in mm) for the bacterial test pathogens viz. *B. cereus and S. flexneri.*

Table 5: Antimicrobial Activity of *Buchanania lanzan* seeds Extract

Test Pathogens	Diameter of zone of inhibition (mm)			
Gram Positive	PETE	DCME	MEE	EWE
S. aureus	-	-	-	-
S. epidermidis	-	-	-	-
B. cereus	-	-	10	10
Gram Negative	-	-	-	-
E. coli	-	-	-	-
S. flexneri	-	-	8	8

3.6 Antifungal activities

The fungal growth inhibitory effects of different extracts were estimated by using the well diffusion method. Test for inhibitory activity was performed on PDA plates. The zone of inhibition was observed after 7 days. *Buchanania lanzan* seed extracts showed no antifungal activity against all three fungal strains.

3.7 Characterization of secondary metabolites of the extracts using GC/MS

Screening of different secondary metabolites was done using GC/MS of the fruit sample. The data obtained revealed the presence of abundance of valuable fatty acids, plant sterols like stigmasterol, γ-sitosterol, β-sitosterol and polyphenolic compounds. Plant sterols plays important role in prevention of cardiovascular diseases by lowering low density lipoprotein in serum. Plant sterol rich foods are effective against cardiovascular risk. These help to balance the cholesterol in the body. Tocopherol detected in petroleum ether fraction is known for its immune enhancement and health management activity. Phytosterol were present in all the extracts which contributes to reduce the human diseases, by cholesterol reducing and anticancer activities. Various essential fatty acids were detected in extracts of *Buchanania lanzan* seeds like oleic acid and eicosanoic acid. Oleic acid is associated with lower stroke incidence.

Table 6: List of compounds detected in Petroleum ether fraction of *Buchanania lanzan*

S. No.	Compound Detected	CAS#	Area (%)	R.T
1.	Oleic Acid	000112-80-1	37.57	22.164
2.	Tetradecanoic Acid	000544-63-8	0.45	18.149
3.	9-Octadecenamide	000301-02-0	2.36	23.869
4.	n-Hexadecanoic acid	000057-10-3	24.51	20.460
5.	9-Octadecenamide	000301-02-0	2.36	23.869
6.	γ-Tocopherol	007616-22-0	1.18	30.295
7.	Stigmasterol, 22, 23diydro	1000214-20-7	0.97	35.264
8.	γ-Sitosterol	000083-47-6	0.97	35.264
9.	β-Sitosterol	000083-46-5	0.97	35.264
10.	γ-Tocopherol	007616-22-0	1.18	30.295
11.	9-Octadecenoic Acid	000111-03-5	1.98	26.426
12.	Hexadecane	000544-76-3	0.13	16.276

Table 7: List of compound detected in Dichloromethane fraction of *Buchanania lanzan*

S. No.	Compound Detected	CAS#	Area (%)	RT
1.	Tetradecanoic acid	000544-63-8	0.25	18.138
2.	n-Hexadecanoic acid	000057-10-3	18.81	20.336
3.	Tetradecanoic acid	000544-63-8	18.81	20.336
4.	Oleic Acid	000112-80-1	36.10	22.052
5.	Octadec-9-enoic acid	1000190-13-7	36.10	22.052
6.	γ-Sitosterol	000083-47-6	3.96	35.252
7.	Stigmasterol, 22, 23-diydro-	1000214-20-7	3.96	35.252
8.	β-Sitosterol	000083-46-5	3.96	35.252

Table 8: List of compounds in methanolic fraction of *Buchanania lanzan*

S. No.	Compound Detected	CAS#	Area (%)	RT
1.	Phenol, 2,4-bis(1, 1-dimetyletyl)	000096-76-4	0.08	11.465
2.	1-Hexadecene	000629-73-2	0.21	14.459
3.	7-Hexadecene	035507-09-6	0.21	14.459
4.	Phenol, 2-(1-phenyletyl)-	004237-44-9	0.63	17.162
5.	1-Octadecene	000112-88-9	0.50	18.172
6.	5-Octadecene	007206-21-5	0.50	18.172
7.	Pentadecanoic acid, 14-methyl-, methyl ester	005129-60-2	0.48	19.798
8.	Hexadecanoic acid, methyl ester	005129-60-2	0.48	19.798
9.	n-Hexadecanoic acid	000057-10-3	12.69	20.415
10.	Tetrsdecanoic acid	000544-63-8	12.69	20.415
11.	Pyrene	000129-00-0	47.26	21.996
12.	Eicosanoic acid	000506-30-9	1.90	23.914
13.	1, 2-Benzenedicarboxylic acid, mono (2-etylexyl) ester	004376-20-9	16.93	25.461
14.	1, 2-Benzenedicarboxylic acid, diisooctyl ester	027554-26-3	16.93	25.461
15.	9-Octadecenoic acid (z) -, 2,3-diydroxypropyl ester	000111-03-5	2.76	26.504
16.	9-Octadecenoic acid (z) -, 2-hydroxy-1-(Hydroxymetyl) ethyl ester	003443-84-3	2.76	26.504
17.	Stigmasterol, 22, 23-diydro-	1000214-20-7	0.91	35.409
18.	y –Sitosterol	000083-47-6	0.91	35.409
19.	β-Sitosterol	000083-46-5	0.91	35.409
20.	Phenol, 2-(1-phenylethyl)-	001988-89-2	0.63	17.162
21.	Azulene, 1,4-dimethl-7-(1-methylethyle)-	000489-84-9	0.63	17.162
22.	Octadecanoic acid	000506-30-9	1.90	23.914
23.	Eicosanoic acid	000506-30-9	1.90	23.914
24.	Oleic acid, 3-hydroxypropyl ester	000821-17-0	4.02	24.430
25.	9-Octadecenoic acid (z) -, 2-hyroxypropyl ester	000821-17-0	4.02	24.430
26.	9-Octadecanoic acid (z) -, 2,3-dihydroxypropyl ester	000111-03-5	2.76	26.504

Table 9: List of Compounds in Ethanol-Water fraction of *Buchanania lanzan* seeds

S. No.	Compound Detected	CAS#	Area (%)	R.T
1.	Phenol, 2, 4-bi's (1,1-dimetyletyl)	000096-76-4	0.31	15.267
2.	Tetradecanoic Acid	000544-63-8	0.60	18.138
3.	n-Hexadecanoic Acid	000057-10-3	24.19	20.314
4.	Oleic Acid	000112-80-1	41.88	22.018
5.	Octadec-9-enoic Acid	1000190-13-7	41.88	22.018
6.	2, 6, 10, 14, 18, 22-Tetracosaex ane	000111-02-4	4.55	27.570
7.	γ- Sitosterol	000083-47-6	2.18	35.253
8.	Stigmasterol, 22,23-dihydro	1000214-20-7	2.18	35.253
9.	β-Sitosterol	000083-46-5	2.18	35.253
10.	2,6-Octadienal, 3,7-dimethyi	005392-40-5	0.06	11.86
11.	Hexadecanoic acid	023470-00-0	5.54	25.024
12.	Octadecanoic acid	000123-94-4	6.29	26.606
13.	Hexadecanoic acid, 2-hydroxy-1-(hydroxyl-methyl) ethyl ester	023470-00-0	5.54	25.024
14.	Octadecanoic acid, 2,3-dihydroxypropyl ester	000123-94-4	6.29	26.606

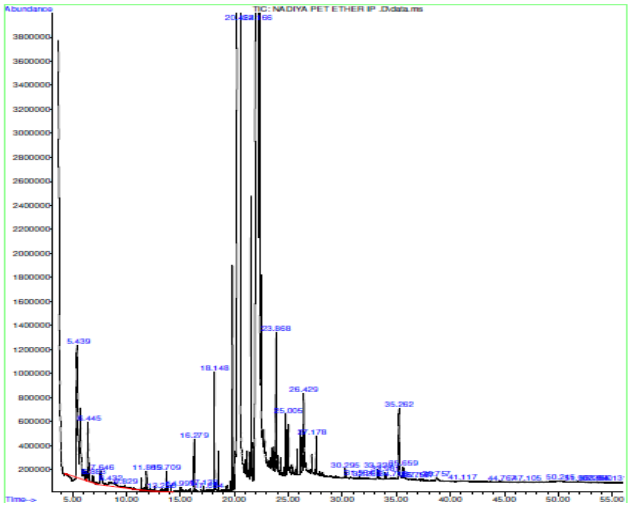

Fig 1: Chromatogram representing compounds present in Petroleum ether fraction

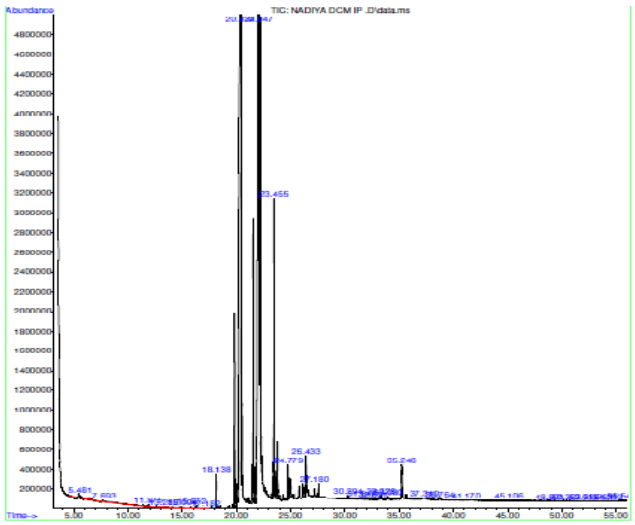

Fig 2: Chromatogram representing compounds present in Dichloromethane fraction

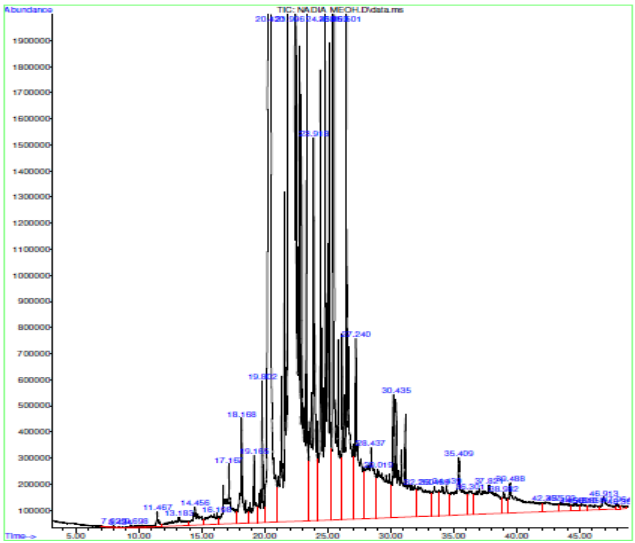

Fig 3: Chromatogram representing compounds present in Methanolic Fraction

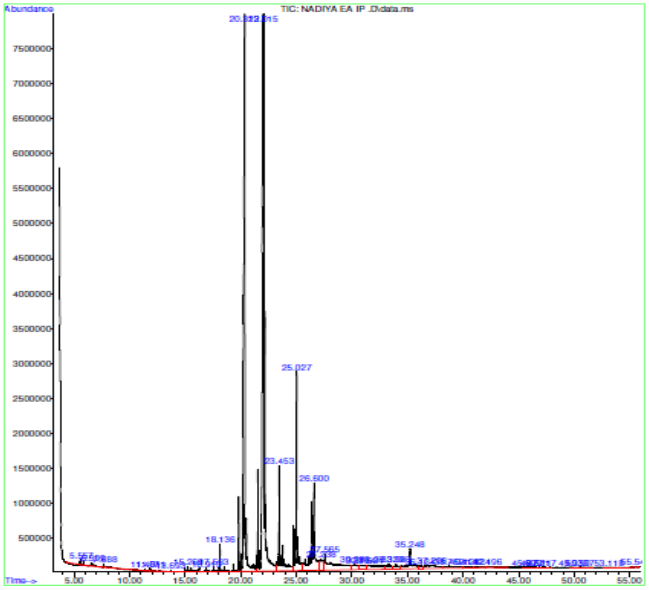

Fig 4: Chromatogram representing compounds present in Ethanol-water fraction

3.8 Fatty Acid Composition

Analysis of the results shows *Buchanania lanzan* oil (BLO) contains Monounsaturated fatty acids (MUFA 56.08%), Poly unsaturated fatty acids (PUFA 6.50%), Saturated fatty acids (SFA 37.40%) and Trans fatty acids (TFA 0.00%). Oil extracted by a cold percolation method contains more fatty acids as compared to the oil obtained from soxhlet extraction method. These fatty acids are important both nutritionally and medicinally. Elevated LDL-cholesterol is the primary factor for cardiovascular diseases, research shows that blood cholesterol and triglyceride levels decrease dramatically by diets high in MUFA versus SFA or trans fats Hu FB *et al*. [22]. BLO contains very high Oleic acid, which play an important role in the activation of different pathways of immune component cell and also have the ability to reduce inflammatory effect of long chain fatty acid Harvey KA *et al*.[23]. MUFA promotes healthy blood cholesterol and triglyceride concentrations, regulates body weight and composition, mediates blood pressure, and improves insulin sensitivity and glucose metabolism.

Table 10: Chemical constituents of cold pressed *Buchanania lanzan* oil

S. No	Chemical component	%
A	**Saturated Fatty Acid**	
1	Myristic Acid	0.41
2	Palmitic Acid	31.28
3	Stearic Acid	5.16
4	Arachidic Acid	0.45
5	Lignoseric Acid	0.10
B	**Monounsaturated fatty acids**	
1	Palmitoleic Acid (C16: 1)	0.76
2	Oleic Acid (C18: 1) (ω-9)	55.17
3	Cis 11-Eicosanoic Acid (C20:1) (ω-9)	0.16
C	**Polyunsaturated Fatty Acid**	
1	Linolenic Acid (C18: 3) (ω-3)	0.26
2	Linoleic Acid (C18: 2) (ω-6)	6.24

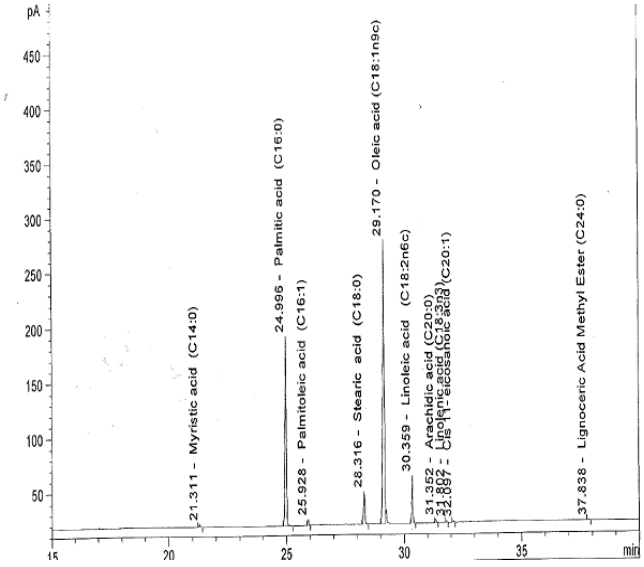

Fig 5: Mass Spectrum of *Buchanania lanzan* seeds oil Composition in table 10

4. Conclusion: Chemical composition of the *Buchanania lanzan* seeds demonstrates it as a potential source of protein, fat, dietary fiber, and energy. They were found to be very good source of Phosphorus, Calcium, Magnesium and Iron. Seeds of *B. lanzan* are potential source of, phytochemical, tocopherols, essential fatty acids like oleic acids, linoleic acid and linolenic acid. The nutritional and phytochemical composition of the seeds were demonstrated and found very rich source of bioactive components and can be a vital functional food, nutritional supplements and other value added products, which is currently underutilized. Phytochemical screening shows it is phytochemically very rich source of polyphenols, flavonoids, tannins, alkaloids and saponins. These polyphenols have been proven for its disease fighting power. Good antioxidant activities were found in *B. lanzan* seeds which show its ability of reducing oxidative stress. GC/MS profile shows, abundance of fatty acids, polyphenols, phytosterols, sitosterol, and stigmasterol in seed extract, these phytosterols are receiving so much attention because of their clinical and nutritional significances.

5. Acknowledgement
We are very grateful to the University Grants commission for the financial support under the Special Assistance Program (SAP).

6. References

1. Siddiqui MZ, Chowdhury AR, Prasad N, Thomas M. *Buchanania lanzan:* a species of enormous potentials. World Journal of Pharmaceutical Sciences 2014; 2(4):374-379.
2. Gupta M, Shaw BP. Use of medicinal plants in *Panchkarma* Ayurvedic therapy. Indian Journal of Traditional Knowledge 2009; 8(3):372-378.
3. Puri A, Sahai R, Singh KL, Saxena RP, Tandon JS, Saxena KC. Immunostimulant activity of dry fruits and plant materials used in the Indian traditional medical system for mothers after childbirth and invalids. Journal of Ethnopharmacol 2000; 71(1-2):89-92.
4. Kodati D, Surendra K, Patnaik A. Antidiarrhoeal activity of alcoholic extract of *Buchanania lanzan* Spreng. Roots. Pharmacologyonline 2010; 3:720-726.
5. Jain R, Jain SK. Effect of *Buchanania lanzan* Spreng. bark extract on cyclophosphamide induced genotoxicity and oxidative stress in mice. Asian Pacific Journal of Tropical Medicine 2012; 5(3):187-191.
6. Kumar J, Vangaiah PC, Srivastav PP, Bhowmika PK. Chirnji nut processing present practice and scope. Indian journal of traditional knowledge 2012; 11(1):202-204.
7. Gülçin I. Antioxidant properties of Resveratrol: A structure-activity insight. Innovative Food Science and Emerging Technologies 2010; 11(1):210-218.
8. Official method of analysis, AOAC, Association of official analytical chemistry, Edition 16, Arlington VA, Washington DC, USA, 1995.
9. McDonald S, Prenzler PD, Antolovish M, Robards K. Phenolic content and antioxidant activity of olive extract. Food Chemistry 2001; 73(1):73-84.
10. Soni N, Metha S, Satpathy G, Gupta RK. Estimation of nutritional, phytochemical, antioxidant and antibacterial activity of dried fig (*Ficus carica*). Journal of Pharmacognosy and Phytochemistry 2014; 3(2):158-165.
11. Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis 2002; 10(3):178-182.
12. Obadoni B, Ochuk PO. Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. Global Journal of Pure and Applied Sciences 2001; 8(2):203-208.
13. Mehra M, Pasricha V, Gupta RK. Estimation of nutritional, phytochemical and antioxidant activity of seeds of muskmelon (*Cucumis melo*) and watermelon (*Citrullus lanatus*) and nutritional analysis of their respective oil. Journal of Pharmacognosy and Phytochemistry 2015; 3(6):98-102.
14. Herborne JB. Phytochemical Methods, Chapman and Hall, London, 1973, 110-113.
15. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power the FRAP assay. Analytical Biotechemistry 1996; 239(1):70-76.
16. Barison A, Silva CW, Campos FR, Simonelli F, Lenz CA, Ferreira AG. A simple methodology for the determination of fatty acid composition in edible oils through 1H NMR spectroscopy**.** Magnetic resonance in chemistry 2010; 48(8):642-50.
17. Reid IR, Ames RW, Evans MC, Gamble GD, Sharpe SJ. Effect of calcium supplementation on bone loss in postmenopausal women. The New England Journal of Medicine 1993; 328(7)460-464.
18. Del Gobbo LC, Imamura F, Wu JH *et al*. circulating and dietary magnesium and risk of cardiovascular disease: a systematic review and meta-analysis of prospective studies. Americal Journal of Clinical Nutrition 2013; 98(1):160-173.
19. Chiuve SE, Sun Q, Curhan GC, Tylor EN, Spiegelman D, Willett WC *et al***.** Dietary and plasma magnesium and risk of coronary heart disease among women. Journal of the American Heart Association 2013; 2(2):000114.
20. Rodrigues S, Pinto ASG. Ultrasound extraction of phenolic compounds from coconut (*Cocos nucifera*) shell powder. Journal of food engineering 2007; 80(3)869-872.
21. Igwe C, Ojiako O, Nwaagu L, Onyeze G. Lipid Lowering Effect of Aqueous Leaf Extract of Spondias Mombin Linn. Internet Journal of Pharmacology 2007, 6(1).

22. Hu FB, Manson JE, Willett WC. Types of dietary fat and risk of coronary heart disease: A critical review. American journal of Epidemiology 2001; 20(1):5-19.
23. Harvey KA, Walker CL, Xu Z, Whitley P, Pavlina TM, Hise M *et al*. Oleic acid inhibits stearic acid-induced inhibition of cell growth and pro-inflammatory responses in human aortic endothelial cells. Journal Lipid Res 2010; 51(12):3470-80.