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Preliminary screening of nutraceutical potential of *Annona squamosa*, an underutilized exotic fruit of India and its use as a valuable source in functional foods.

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Abstract
The underutilized, exotic fruits of Shareefa, *Annona squamosa* (Annonaceae family) were investigated for their nutritional, phytochemical, antioxidant and antimicrobial potential. A thorough nutritional characterization of the edible fruit pulp demonstrated it to be a good source of phenolics compounds, natural antioxidants and minerals. It is a moderate source of copper, manganese followed by the other nutrients. It showed 1.25 g and 0.97 g 100 g $^{-1}$ DM alkaloids in pulp and seed sample respectively followed by saponins value. All assays were carried out in methanolic extracts of the fruit (fresh and dried pulp and seeds). Total phenolic, flavonoid and flavonol contents in pulp were obtained as 546.25 µg GAE (fresh) and 536.87 µg GAE (dried), 88 µg CE (fresh) and 205 µg CE (dried) and 156.19 µg (fresh) and 349.5 µg 100 mg $^{-1}$ respectively. The methanolic extract of Custard apple pulp obtained a good antioxidant potential of ABTS (IC_{50} = 5 mg ml $^{-1}$ for pulp extract) and FRAP (45.58 µg BHT 100 mg $^{-1}$ in dried pulp extract and 62.88 µg BHT 100 mg $^{-1}$ in seed extract respectively). GC/MS screening of the pulp extracts revealed the presence of fatty acids, alkanes, alkenes, alcohols, ketones and aldehydes, some of them having therapeutic use as well as components in food preparations such as 2-octanol which is used in flavorings and perfumes, D-mannitol is used as a sweetener for diabetic patients, Heptanoic acid is used in fragrances and as artificial flavors while in seeds, the presence of caryophyllene (sesquiterpene: a component of essential oils), diterpenes, phytols which are precursors of many forms of vitamin E, and sterols were revealed. Total phenolic, flavonoid and flavonol contents in seeds were obtained as 250.625 µg GAE, 339 µg CE and 605.71 µg 100 mg $^{-1}$ extract respectively.

Keywords: Nutritional, Phytochemicals, antioxidants, antimicrobial, GC/MS, ICP-OES.

1. Introduction
Fruits and vegetables have been consumed by humans since ancient times. Scientific investigations have proved that an increased consumption of fruits and vegetables is known to reduce instances of cancer and cardiovascular mortality (Oyebode, Walker and Mindwell, 2014) [1]. They contain substances known as phytochemicals which include polyphenols, coumarins, carotenoids, alkaloids, vitamins, minerals, etc. Several studies have revealed that antioxidant compounds play an important role in inhibition of hydrolytic and oxidative enzymes, anti-inflammatory action and various other biological or pharmacological activities in addition to free radical scavenging of harmful active oxygen species including O^{2-}, H_2O_2, $^{\cdot}OH$, and O_2 (Sachdeva, Karan and Singh, 2014) [2]. Antioxidants, by their very nature, are capable of producing free radicals before they can react and cause harm by their reaction as an inhibitor or activator for a number of mammalian enzyme systems, electron donors, metal chelators or scavengers of free oxygen radicals. Because oxidation is a naturally occurring process within the body, a balance with antioxidants must exist to maintain good health. Many researches indicate that the oxidative stress (OS) leading to free radical attack on neural cells contributes calamitous role to neuro-degeneration. Though oxygen is imperative for life, imbalanced metabolism and excess reactive oxygen species (ROS) generation end into a range of disorders such as Alzheimer's disease, Parkinson's disease, aging and many other neural disorders. (Bayani *et al*, 2009) [3].
Annona squamosa is a well-branched tree or shrub from the family *Annonaceae* that bears edible fruits called sugar-apples or custard apples. It tolerates a tropical lowland climate. In India, it is cultivated in Andhra Pradesh, Maharashtra, Karnataka, Bihar, Orissa, Assam and Tamil Nadu. Besides India, it is common in China, Philippines, Egypt and Central Africa. The round or heart-shape greenish yellow, ripened fruit is suspended on a thickened stalk, is 5 to 10

centimeters in diameter with many round protuberances and covered with a powdery bloom. Fruits are formed of loosely cohering or almost free carpels. The pulp is white tinged yellow, edible and sweetly aromatic. Each carpel contains an oblong, shiny and smooth, dark brown to black, 1.3 to 1.6 centimeters long seed. These contain anti-oxidants like Vitamin C, which helps to fight free radicals in our body. It is high in potassium and magnesium that protects our heart from cardiac disease and controls blood pressure. This fruit is also known to be great for eyes, and cures indigestion problems. It is important to include this fruit in daily diet, as the copper content helps to cure constipation, and helps to treat diarrhea and dysentery.

In view of the growing interest in these nutritional and polyphenolic compounds, there is a need to identify and quantify these important compounds in these exotic fruits to evaluate the potential nutritional and health benefits. However, there is not enough literature for the chemical composition of raw whole *Annona squamosa* pulp for its use as a valuable source of functional food. Delving deep into the composition and bioactive compounds present in this fruit can lead to a better understanding and appreciation of the pharmaceutical, nutraceutical and medicinal value that these fruits might offer and an increased consumption by the general public.

The custard apple pulp contains various chemical constituents such as alkaloids anonaine, higenamine, roemerine, noreorydine, corydine (have anticancer activity), norisocorydine, isocorydine, glaucine, fruit contain vitamin-c, α- and β-pinine, limonene, β-farnesene, β-sitosterol and rutin (Sharma and Chand, 2013) [4]. Roots possess anti-diabetic activity and contain an essential oil (0.15%); β caryophyllene α pinene, α-humulene, α Gurjunene. (Pandey., Barve 2011) [5].The bark contains annonaine, an alkaloid which is found to possess antimalarial activity. (Pandey, Barve., 2011) [5]. The aim of the present study was to evaluate the nutritional, chemical and phytochemical constituents, secondary metabolites and antioxidant potential of the underutilized exotic fruits of *A. squamosa* to use them as a potential candidate for nutraceuticals, functional foods and in various food preparations.

2. Materials and methods

2.1 Reagents

All analytical grade chemicals and solvents used in the sample preparation were purchased from local supplier of SRL, Rankem labs and CDH. The reference standards (rutin, gallic acid, catechin, ascorbic acid) were obtained from Sigma- Aldrich (Sigma- Aldrich, St. Louis, MO, USA). All media were purchased from SRL (Sisco Research Laboratories, Delhi, India) and cultures were obtained from IMTECH (IMTECH, Chandigarh, India). All the other chemicals; TPTZ (2,4,6- tripyridyl-s-triazine), and ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) were procured from Fluka (Fluka, Switzerland).

2.2 Materials

Fruits were collected from northern part (Delhi) of India in November 2013. Upon arrival in the laboratory, samples were dried/crushed and taken for immediate analysis of moisture, ash and other nutritional parameters.

2.3 Extraction

Ground fresh, oven-dried pulp and crushed seeds (100 g) were extracted by a slight modification of the method of Demiray *et al*., (2009) [6]. Sample was extracted using methanol (pulp) and petroleum ether (seeds) as the solvent. 100 g of the sample with 100 ml of solvent in a reagent bottle was incubated at 40 ^{0}C for two days with subsequent filtration. Then, the combined supernatants were filtered and filtrates were pooled and concentrated using hot air oven until a crude viscous extract was obtained.

After evaporation of organic solvents, these were stored at -20 ^{0}C till analysis.

2.4 Determination of nutritional constituents

Moisture and total ash content was determined by gravimetrical method at 103 ^{0}C to 104 ^{0}C (Ref. 935.29, AOAC, 1995) [7] and at ≤ 525 ^{0}C (Ref. 900.02A, AOAC, 1995) [7] respectively. The total nitrogen content was determined using the Kjeldahl method Ref. 976.05 (AOAC, 1995) [7] and the obtained nitrogen was transformed into protein content by multiplying the total nitrogen by a conversion factor of 6.25. Crude fat content was assessed using the AOAC method, Ref. 2003.06. The amount of total carbohydrates [12] was calculated with the following formula: total carbohydrates (% fresh weight) = 100 - moisture (%) - protein content (% fresh weight) - crude fat (% fresh weight) - ash (% fresh weight) and reported as total carbohydrates in g 100 $^{-1}$ g DM. The calorific value per 100 g of DM was calculated according to the system of Atwater, namely: kcal = (3.36 × % protein fresh weight) + (3.60 × % total carbohydrate fresh weight) + (8.37 × % fat fresh weight).

2.5 Phytochemical analysis

2.5.1 Crude alkaloids and saponins determination

These were determined gravimetrically as per the methods described by Herborne (1973) [8] and Obadoni & Ochuko (2001) [9] and the results were expressed as g 100 g $^{-1}$ DM.

2.5.2 Total Phenolic content

Total phenolic content was determined as per the method described by Singleton and Rossi (1965) [10]. Briefly, appropriate volumes of sample extracts were oxidized with Folin-Ciocalteu reagent and the reaction was neutralized with sodium carbonate. The results were expressed as gallic acid equivalents (GAE, µg 100 mg $^{-1}$ EY).

2.5.3 Total Flavonoid content

Total flavonoid content was determined by colorimetric method (Jia, Tang & Wu, 1999) [11]. Briefly 0.25 ml (100 mg ml $^{-1}$) of each extract was diluted with 4.5 ml of distilled water and 0.3 ml of 5 % $NaNO_2$ solution. After 5 min, 0.3 ml of 10% $AlCl_3$ was added and incubated for 5 min. Then, 2 ml of 1M NaOH was added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured immediately at 510 nm. The results were expressed as catechin equivalents (CE, µg 100 mg $^{-1}$ EY).

2.5.4 Flavonol content estimation

For estimation of the flavonol content, 0.25 ml of each extract (100 mg ml $^{-1}$) was added to 1 ml of ethanol followed by 1 ml of 2 % aluminium chloride solution with gentle mixing. The solution was then mixed with 3 ml of 5 % sodium acetate solution and incubated at 20 ^{0}C for 2.5 h (Miliauskas, Venskutonis & Beek, 2004)[12]. Absorbance was measured at 440 nm and expressed as rutin equivalents (RE, µg 100 mg $^{-1}$ EY).

2.5.5 Assessment of Antioxidant activities

Polyphenolics are known to function as antioxidants through a number of mechanisms including radical scavenging by H^+ donation, prevention of chain initiation by donating electrons or by binding transition metal ion catalysts (Yildirim *et al*., 2000) [13]. The antioxidant potential of phenolic compounds was measured by

assessing their radical scavenging potential using DPPH and $ABTS^{\cdot+}$ radical cation scavenging assay or their ability to reduce compounds by donating electrons using FRAP assay.

2.5.5.1 ABTS radical scavenging assay

The ability of the test sample to scavenge $ABTS^{+}$ radical cation was compared to Ascorbic acid standard. The total antioxidant activity of the *A. squamosa* extracts was evaluated according to the decolorization of the ABTS radical cation ($ABTS^{+}$) as percentage inhibition by Re *et al*, (1999) [14]. The cation was pre-generated by mixing 7 mM ABTS stock solution with 2.45 mM potassium persulfate and incubating for 12–16 h in the dark at room temperature until the reaction was complete and the absorbance was stable to 0.70 (± 0.02). Next, 1 ml was mixed with 10 µl of the test sample (0.05–10 mg ml^{-1}) and the absorbance was measured at 734 nm after 6 min. The percent inhibition was calculated and plotted as a function of the concentration of standard and sample to determine the ascorbic acid equivalent antioxidant concentration.

2.5.5.2 Ferric reducing activity power (FRAP) assay

The ability of the extract to reduce ferric ions was determined using the FRAP assay developed by Benzie and Strain (1996) [15]. Appropriate dilutions of extracts were prepared and 100 µl was mixed to 900 µl of FRAP reagent, vortexed and incubated at 37 ^{0}C for 4 min. The absorbance was measured at 593 nm and reported as BHT equivalents (µg 100 mg $^{-1}$ EY).

2.6 Chromatographic analysis

2.6.1 GCMSD profiling of methanolic extracts of *A. squamosa*

Extracts were diluted with DMSO (fresh and dried pulp) and Petroleum ether (seeds) and analyzed by Agilent 6890 GC and 5975B MSD. The chromatographic separation was done on a capillary column of fused silica HP-5ms (0.25 mm × 30 m × 0.25 µm). 1 µl of each extract was injected in the split mode (1:50) by empty baffled liner at 280 ^{0}C (Agilent#5183-2037). The oven was programmed under the same condition as described above (Medini, Marzouki, Chemli, Khouja & Marongiu, 2009; NIST, 2005) [16]. Eluents were detected in EI mode with ionization energy of 70 eV. All the mass spectra of the identified peaks were compared with the spectra from the NIST'05a, WILEY spectral library and F.A.M.E T mix (C_8:C_{24}) in combination with deconvolution reporting software (DRS). The results (table 4) for individual compound those quality matches > 90% is only reported (as their percentage of the total area of peaks in the total ion chromatogram).

2.7 Antimicrobial activity

Antimicrobial activities of the extracts were tested by agar well diffusion method (Igbinosa., Igbinosa., & Aiyegoro, 2009) [17] against three Gram-positive bacteria and one Gram negative bacteria. Extracts were reconstituted to a final concentration of 100 mg ml^{-1}. Nutrient agar was inoculated by spreading 100 µl of the bacterial inoculums. Wells (8 mm diameter) were punched in the agar and 100 µl of extracts were loaded into the wells. The plates were incubated at 37 ^{0}C for 24 h. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition and reported in the scale of millimeter.

3. Results and discussion

3.1 Nutritional composition

The obtained nutritional values (Table 1) show better composition as compared to other exotic underutilized fruits like Lychee, Star fruit (amrak), etc.

Table 1: Nutritional composition of *A. squamosa* pulp

Composition		**Content**
Moisture (g 100g^{-1} FW)		70.8
Ash (g 100 g^{-1} DM)		0.57
Protein (g 100 g^{-1} DM)		5.44
Fat (g 100 g^{-1} DM): (Fresh, dried)		0.067, 0.137
Dietary fiber (g 100 g^{-1} DM)		2.78
Carbohydrates (g 100 g^{-1} DM)		20.41
Food energy (Kcal)		92.90
Mineral elements in *A. squamosa*	**Pulp (ppm)**	**Seeds (ppm)**
Calcium	602.5	382.7
Magnesium	517.6	730
Iron	48.08	23
Copper	9.500	1.92
Manganese	5.569	2.92
Zinc	28.68	9.04
Trace element and heavy metals	**Pulp (ppm)**	**Seeds (ppm)**
Chromium	2.045	1.34
Tin	0.754	0.827
Arsenic	ND	1.55
Cadmium	<0.017	0.008
Selenium	0.089	0.873
Nickel	1.615	0.708
Molybdenum	0.358	0.008

The mineral content (mg 100 g $^{-1}$ of DM) showed the presence of calcium (Ca), magnesium (Mg), Iron (Fe), Zinc (Zn) and manganese (Mn) in highest amounts followed by the other nutrients. A high Ca content (602.5 ppm) can play a crucial role in providing rigidity to the skeleton besides its involvement in neuromuscular function, blood clotting and many other metabolic processes (Campous, Betalleluz, Tauquino, Chirinos, & Pedreschi, 2009) [18]. Past and on-going investigation on the medicinal properties of plant species reported that the mineral compositions have a major role to play in their therapeutic effect. A high content of Mg (517.6 ppm) can play an important role in bone formation and other essential metabolic activities in the body. Also, low content of Pb, Cd, As and Hg showed that the fruit is free from toxic metals. Therefore the collected species can be used in well-balanced diets and can be consumed unreservedly without any health risk. Other vitamins, though present in trace amount, are essential for body metabolism.

3.2 Phytochemical analysis

The quantitative estimates of the crude phytochemicals of *A. squamosa* pulp DM were obtained as: alkaloids, 1.25 (pulp), 0.97 (seeds) in g 100 g $^{-1}$ and Saponins, 0.57 (pulp), 1.09 (seeds) in g 100 g^{-1} (Table 2). Alkaloids are good spasmolytic and anesthetic agents while saponins help in boosting the immunity system, in lowering cholesterol levels in the blood and reducing the risk of getting intestinal cancer. Also, alkaloids are the most efficient therapeutically significant plant substance and saponins are known as anti-nutritional factors that can reduce the uptake of certain nutrients including cholesterol and glucose at the gut through intra lumenal physicochemical interaction or other yet unidentified activity (Okwu & Okwu, 2004) [19].

Phenolic content (TPC) contributes directly to anti-oxidative action. The highest phenolic content was found to be in fresh pulp followed by dried pulp and then seeds. Highest flavonoid content (TFC) was found to be present in seeds followed by dried and fresh pulp. The seeds were found to have a high flavonol content followed by dried and fresh pulp. (Table 3). A similar qualitative analysis of the fruit peel gave positive tests for alkaloids, saponins, flavonoids, glycosides, carbohydrates, proteins and tannins.(Sharma and Chand, 2013) [4] .

Table 2

Parameter	Pulp (g 100^{-1})	Seeds (g 100^{-1})
Alkaloids	1.25	0.97
Saponins	0.57	1.09

Table 3

Solvent	TPC (µg GAE) 100 mg $^{-1}$ EY	TFC (µg CE) 100 mg $^{-1}$ EY	Flavanol (µg BHTE) 100 mg $^{-1}$ EY
Fresh pulp	546.25	88	156.190
Dried pulp	536.87	205	349.50
Seeds	250.625	339	605.71

3.3 Antioxidant activities

3.3.1 ABTS radical scavenging assay

The results are based on the ability of antioxidant to decolorize the $ABTS^{+}$ cation radical. The highest antioxidant potential was found in *A. squamosa* pulp (5 mg/ml); followed by seeds (9.06 mg/ml). This suggests that the fruit pulp has good antioxidant and free radical scavenging activity.

3.3.2 FRAP assay

The ability of the plant extracts to reduce ferric ions into ferrous ions under low pH was determined using the FRAP reagent. The highest antioxidant activity was found to be in dried pulp (62.88 µg 100 mg $^{-1}$), followed by fresh pulp (45.58 µg 100 mg $^{-1}$) and seeds (23.07 µg 100 mg^{-1}).

3.4 GC/MS analysis

The details of all identified compounds in methanolic extracts are grouped by their chemical nature (Table 4a, 4b and 4c).

Furanmethanol is identified in dried as well as fresh pulp extracts, which can serves pharmaceutical industry. The reported saturated fatty acids such as oleic acid, linoleic acids are found in animals and plants and are primarily used to produce hormone-like substances that regulate a wide range of functions, including blood pressure, blood clotting, blood lipid levels, the immune response, and the inflammation response to injury infection (Meechaona, Sengpracha, Banditpuritat, Kawaree & Phutdhawong, 2007) [20]. The presence of phytosterols in seed extract may be contributing towards antimicrobial and antioxidant activity. They are well known towards their medical, cosmetic, functional food applications and also known for their saturated fat reducing and cholesterol lowering activity; thus they may reduce risk of heart disease (Gabay *et al*., 2010) [21]. Hexadecanoic acid (used in cosmetics, soaps, antioxidant), Dodecanoic acid (acne treatment, increases HDL cholesterol) and Pentadecanoic acid were also found. 2-octanol methyl esters might be used in food and cosmeceutical industries pertaining to its flavor and essence.

Table 4a: GCMS profiling of *A. squamosa* fresh pulp extract

RT (min)	Compounds	CAS#	% Area
3.524	Methylglyoxal	000078-98-8	3.46
3.883	2-oxo-, Propionic acid, methyl ester	000600-22-6	2.56
4.825	3-Furanmethanol	004412-91-3	2.16
4.825	2-Furanmethanol	000098-00-0	2.16
5.341	2-Cyclopentene-1,4-dione	000930-60-9	3.89
	4,5-dihydro-2-methyl, 1H-Imidazole		
	N-methyl-1,3-Propanediamine		
5.812	l-Guanidinosuccinimide	000534-26-9	1.43
9.132	2,3-dihydro-3,5-dihydroxy-6-methyl-	006291-84-5	3.14
9.132	4H- Pyran-4-one	1000130-20-8	3.14
10.085	Pentanoic acid, 2,2-dimethyl-, ethenyl	028564-83-2	5.21
	ester		
11.050	5-(hydroxymethyl)- 2-	044970-05-0	5.38
11.521	Furancarboxaldehyde	000067-47-0	8.74
11.700	2-octanol	000123-96-6	1.39
11.925	3-methyl-1,2-Benzenediol	00048-17-5	1.80
12.126	2,3,5-trimethyl-1,4-Benzenediol	000700-13-0	1.04
12.396	4-methyl-1,2-Benzenediol	000452-86-8	0.66
19.944	14-methyl-, Pentadecanoic acid, methyl	005129-60-2	
	ester		0.97
19.944	Hexadecanoic acid, methyl ester	000112-39-0	0.97
20.325	n-hexadecanoic acid	000057-10-3	3.08
20.325	Tetradecanoic acid	000544-63-8	3.08
21.458	Z-8-Hexadecene	1000130-87-5	0.11
21.458	1-Nonadecanol	001454-84-8	0.11
21.458	Bromoacetic acid, pentadecyl ester	131143-01-6	0.11
21.592	9,12-Octadecadienoic acid (Z,Z)-	000112-63-0	
	methyl		1.01
21.592	ester	056554-62-2	1.01
21.861	10,13-Octadecadienoic acid, methyl	000112-61-8	0.46
22.041	ester	000112-80-1	7.87
22.198	Octadecanoic acid, methyl ester	000057-11-4	1.50
24.452	Oleic acid	0002423-10-1	0.22
24.845	Octadecanoic acid	004727-18-8	1.13
35.634	9-Octadecenal	000083-47-6	0.34
35.634	2-hydroxy- Cyclopentadecanone	1000214-20-7	0.34
	gamma.-Sitosterol		
	22,23-dihydro- Stigmasterol		

Table 4b: GCMS profiling of *A.squamosa* dried pulp extract

RT (min)	Compounds	CAS#	% Area
4.848	3-Furanmethanol	004412-91-3	2.09
4.848	2-Furanmethanol	000098-00-0	2.09
5.341	2-Cyclopentene-1,4-dione	000930-60-9	3.52
5.341	3-Dimethylaminoacrylonitrile	002407-68-3	3.52
5.824	4,5-dihydro-2-methyl,1H-Imidazole	000534-26-9	1.15
6.003	1-Piperidineethanamine	027578-60-5	4.05
7.181	4-methyl- Cyclohexanol	000589-91-3	0.22
9.132	N-methyl-1,3-Propanediamine	006291-84-5	3.06
10.007	2,3-dihydro-3,5-dihydroxy-6-methyl-	028564-83-2	2.71
	4H-Pyran-4-one		
11.566	5- (hydroxymethy)-2-	000067-47-0	8.45
	Furancarboxaldehyde		
11.757	2-Octanol	000123-96-6	1.11
11.757	2-Pentadecanol	001653-34-5	1.11

11.936	3-methyl-1,2- Benzenediol	000488-17-5	1.42
12.138	3-Isopropylthiophenol	178959-93-8	0.82
12.138	o-isopropyl-Benzenethiol	006262-87-9	0.82
12.407	4-methyl-1,2-Benzenediol	000452-86-8	0.54
13.046	1,4-Butanediamine	000110-60-1	0.42
16.332	Decahydro-1,1,7-trimethyl-4-methylene-,[1ar (1a.alpha.,4a.alpha.,7.beta.,	006750-60-3	0.35
16.332	7a.beta.,7b.alpha.)]- 1H-Cycloprop[e]azulen-7-ol	0077171-55-2	0.35
19.944	Spathulenol	005129-60-2	0.79
19.944	14-methyl-Pentadecanoic acid, methyl ester	000112-39-0	0.79
20.325	Hexadecanoic acid, methyl ester	000057-10-3	2.70
21.592	n-hexadecanoic acid	056554-62-2	0.89
21.592	10,13-Octadecadienoic acid, methyl Ester	000112-63-0	0.89
21.648	9,12-Octadecadienoic acid (Z,Z) methyl ester	000112-62-9	1.72
21.861	9-Octadecenoic acid (Z)-, methyl ester	005129-61-3	0.39
22.052	16-methyl-,Heptadecanoic acid, methyl ester	000112-80-1	8.63
22.052	7-Pentadecyne	000593-39-5	8.63
22.714	2,3-Dihydroxypropyl elaidate	1000131-11-1	0.26
24.845	22,23-dihydro- Stigmasterol	004727-18-8	0.79
25.708	gamma- sitosterol	022089-89-0	0.12
26.594		002716-53-2	0.62
35.623		1000214-20-7	0.47
35.623		000083-47-6	0.47

Table 4c: GCMS profiling of *A.squamosa* seed extract

RT (min)	**Compounds**	**CAS#**	**% Area**
12.676	2,4-Decadienal	025152-84-5	1.52
14.268	Caryophyllene	000087-44-5	2.87
14.268	4,11,11-trimethyl-8-methylene-,[1R-(1R*,4 Z,9S*)]-Bicyclo[7.2.0]undec-4-ene	000118-65-098	2.87
20.381	Dibutyl phthalate	000084-74-2	1.85
20.381	1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester	000084-64-0	1.85
20.605	Hexadecanoic acid, ethyl ester	000628-97-7	2.97
21.592	9,12-Octadecadienoic acid	000112-63-0	1.98
21.760	Phytol	000150-86-7	1.64
22.198	Linoleic acid ethyl ester	000544-35-4	3.97
22.243	Ethyl Oleate	000111-62-6	8.22
22.243	9-Octadecenoic acid	006512-99-8	8.22
24.845	7-Pentadecyne	022089-89-0	1.26
24.845	13-Octadecenal	058594-45-9	1.26
25.787	22,23-dihydro- Stigmasterol	1000214-20-7	17.40
25.787	gamma.-Sitosterol	000083-47-6	17.40
25.787	beta.-Sitosterol	000083-46-5	17.40

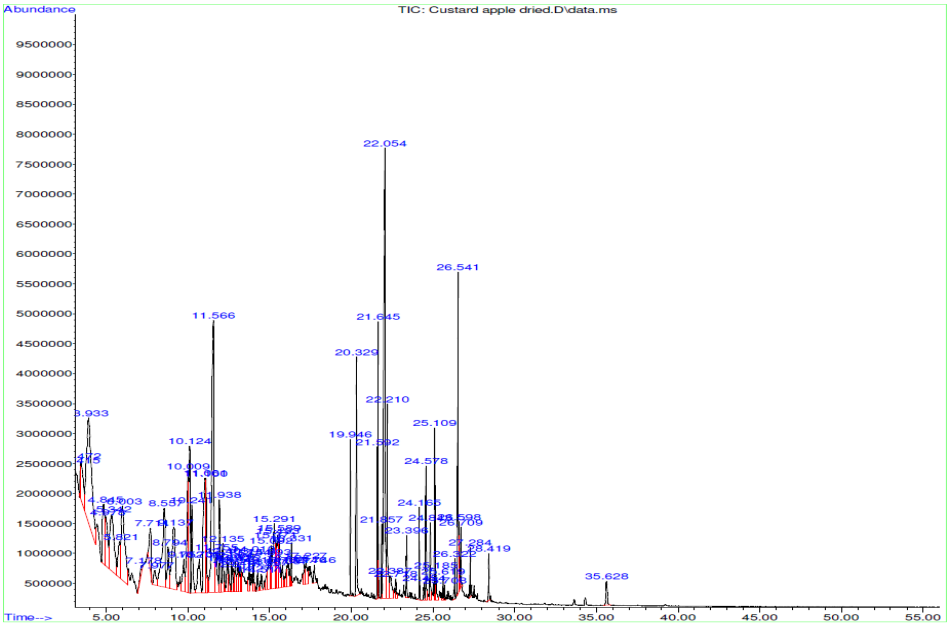

Fig 1(A): GCMS chromatogram of methanolic extract of dried pulp of *A. squamosa.*

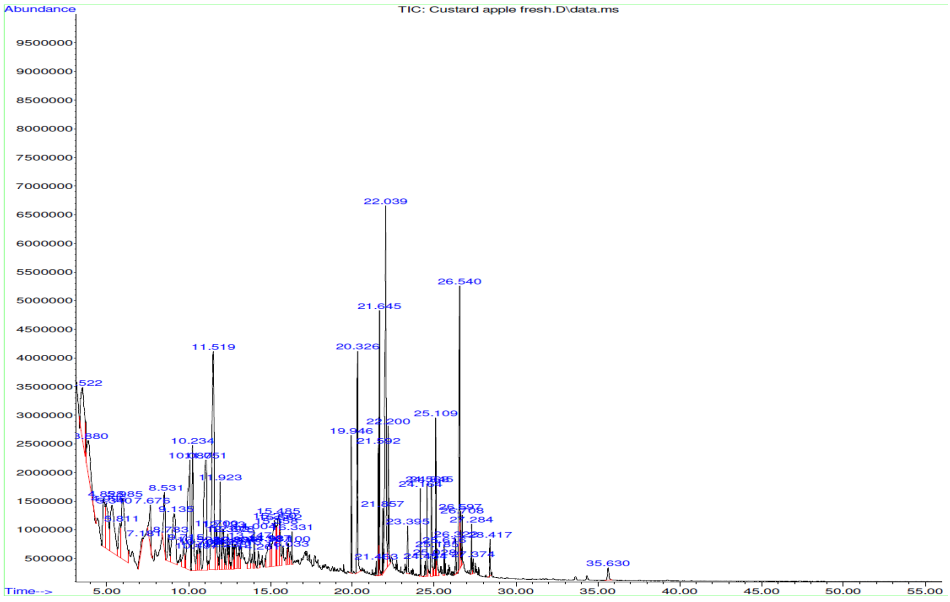

Fig 1(B): GCMS chromatogram of methanolic extract of fresh pulp of *A. squamosa*.

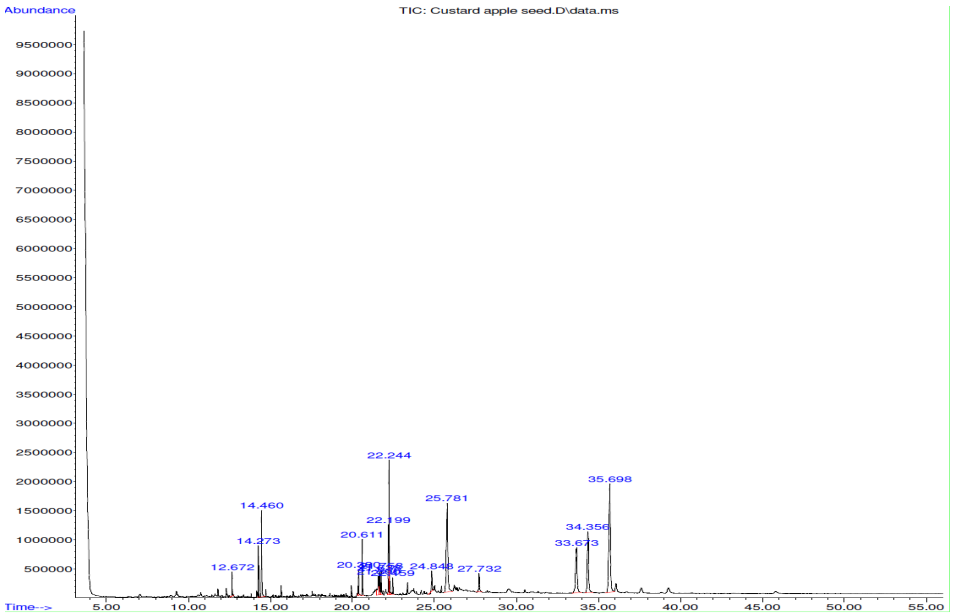

Fig 1(C): GCMS chromatogram of methanolic extract of seeds of *A. squamosa.*

* % Matching with NIST library; RT Retention time of the compound, in minute; "area (%)" the percentages of the area of the total ion chromatogram represented by the peaks of each of the compounds identified; ; a, b, c Identified by NIST and WILAY spectral library, mass fragmentation and co-injection with authentic material

3.5 Antibacterial assay

Screening of the dried pulp, fresh pulp and seeds extracts in methanol was done for antibacterial activities by disc diffusion method against gram positive *B. subtilis, S. aureus, S. epidermidis* and gram negative *P. mirabilis.* A zone of inhibition was observed only for *P. mirabilis* (5 mm diam.) in 500 mg/ml and *S. epidermidis* (4.7 mm dia) in 500 mg/ml in pulp extract indicating that antibacterial activity is present. No zone of inhibition was observed in any of the seeds extract (as high as 700 mg/ml), indicating that they do not possess antibacterial activity for them.

Table 5: Antibacterial activity of *A. squamosa* pulp and seeds (500mg/ml)

Bacterial strains	**Diameter of zone of inhibition(mm)**	
Gram positive	**Pulp**	Seed
Staphylococcus aureus	-	-
Staphylococcus epidermidis	4.7	-
Bacillus subtilis	-	--
Gram negative		
Proteus mirabilis	5	--

4. Conclusion

The chemical composition of the exotic Indian *A. squamosa* shows it can be a potential source of useful source of nutraceutical and flavoring agents. Evidently, the fruit is a rich source of bioactive compounds and may be used to develop value added products and other food applications to enhance the health benefits. In addition, it can also be used as food additive because of its typical flavor and nutrient contents. These results are useful to provide more value addition and usefulness from this fruit. The obtained compounds have potent antimicrobial along with antioxidant properties and may play an important role in drug development, health supplement and spa. Furthermore, the high correlation observed between the various assays employed and phenolic contents is a strong indication that these phenolics are among the predominant source of antioxidant activity in *A. squamosa.* Thus, there is enormous scope for future research and further pharmacological investigation on *A. squamosa.*

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