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Screening of nutritional, phytochemical, antioxidant and antibacterial activity of underutilized seeds of *Scirpus articulatus:* the basis of Khubahi Ramdana industry.

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Abstract
The *Scirpus articulatus* (Family: Cyperaceae) is widely distributed in India, especially in districts of central and north Bihar as well in some other parts of the country and pops of its seeds are eaten as granular sweets. These underutilized seeds were investigated for their nutritional, phytochemical, antioxidant and antibacterial potential. All assays were carried out in three solvents of increasing polarity. Their nutritional profiling demonstrated them to be an excellent source of phenolic compounds and natural antioxidants in methanolic fraction. They showed 0.0265 g and 0.808 g 100 g $^{-1}$ DM alkaloids and saponins respectively. Highest phenolic, flavonoid and flavonol contents were obtained as 26.6733 mg GAE 100 mg $^{-1}$, 9.568 µg CE 100 mg $^{-1}$ and 5.851 mg RE 100 g^{-1} respectively. No significant quantities of antioxidants were obtained in either of the hexane or chloroform fractions. The methanolic extract of seeds obtained a good antioxidant potential of ABTS (IC_{50} = 0.75 mg ml $^{-1}$ and FRAP (970.90 µg BHTE 100 mg $^{-1}$). Therefore, it seems reasonable to consider *S. articulatus* seeds as new valuable ingredient for food and nutraceutical applications.

Fig 1: *Scirpus articulatus* seeds

Fig 2: Granular sweets of Khubahi

Keywords: Nutritional, Phytochemicals, antioxidants, antimicrobial, GC/MS, Flavour and fragrance profile, MP-AES.

1. Introduction
Scirpus articulatus is a perennial glabrous plant with densely tufted robust stems which are light green and spongy, 15 to 150 cms high. The inflorescence arises below the middle of the terete, transversely septate stem and the wide septa are visible externally at intervals. They are ovoid to cylindrical oblong, 0.5 to 1.8 cm long. The glums are broadly ovate, concave, narrowed with an acute or sub-acute apex. Bristles are absent and the 3 stamens have linear yellow anthers [1]. The plants of *Scirpus articulatus* yield black colored seeds that are consumed in the form of sweets in local districts of Bihar. The seeds are popped by roasting them on sand and their pops are processed into granular sweets after passing them in hot concentrated sugar solutions. It provides livelihood to the poor and backward classes of

Bihar. More tasty sweets of khubahi are also prepared by mixing khoa (condensed milk) and are sold at a commensurate price [1].

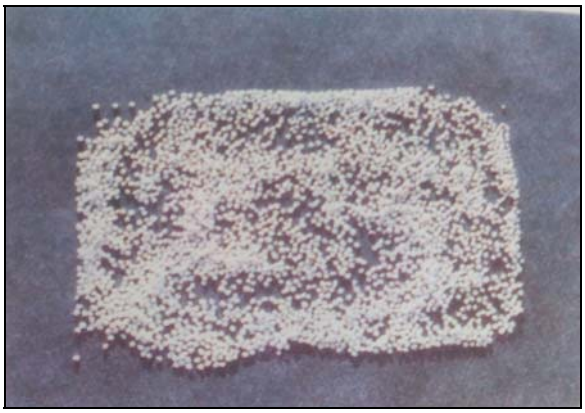

Fig 3: Popped seeds of *Scirpus articulates*

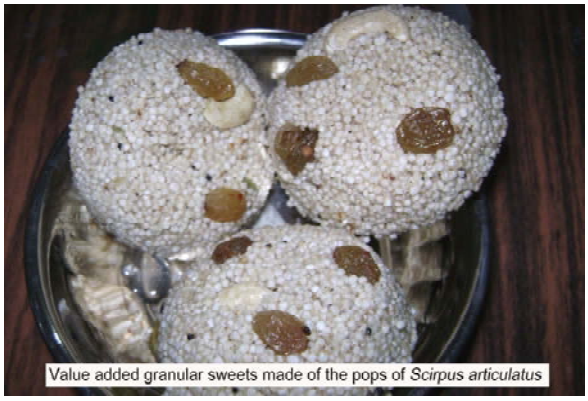

Fig 4: Sweets made from khubahi

Khubahi pops are the only food item prescribed to the patients of smallpox because of their easy digestion without involving the movement of jaws. The patients suffering from this disease find it difficult to articulate their mouth parts with sores inside. But there are some occupational hazards associated with the dehusking of these seeds like asthma to those who perform this operation. It becomes more acute in persons already suffering from tuberculosis [1].

Because of the growing interest in the nutritional parameters and polyphenolic compounds, there is a need to identify and quantify these important compounds in these seeds to evaluate their potential nutritional as well as health benefits. There is very little literature available on its nutritional or phytochemical analysis. Hence, there is a need to know the composition and find out the bioactive compounds present in them that can lead to a better appreciation of the nutraceutical, pharmaceutical and medicinal benefits.

2. Materials and methods

2.1 Reagents

All analytical grade chemicals and solvents used in the sample preparation were purchased from local suppliers of CDH and SRL. The reference standards (gallic acid, rutin, catechin and 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; ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) and TPTZ (2,4,6- tripyridyl-s-triazine), were procured from Fluka.

2.2 Materials

Seeds were collected from darbhanga district of Bihar, India in January 2014 were collected from Darbhanga authenticated by Dr. Vidyanath Jha, MRM College, Darbhanga. Upon arrival in the laboratory, seed sample was ground and immediately analysed for moisture, ash and other nutritional parameters.

Fig 5: Lush growth of Scirpus articulatus in the campus of M L Academy, Darbhanga

2.3 Extraction

150 grams of seeds were properly crushed and were extracted using three solvent system namely, hexane, chloroform and methanol in round bottom flask and extracted using direct hot extraction for two days with subsequent filtration. Then, the combined supernatants were filtered and their 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 Nutritional constituents determination

Moisture and total ash content were determined by gravimetrical method at 103 ^{0}C to 104 ^{0}C (Ref. 935.29, AOAC, 1995) [2] and at ≤ 525 ^{0}C (Ref. 900.02A, AOAC, 1995) [2] respectively. The total nitrogen content was determined using the Kjeldahl method Ref. 976.05 (AOAC, 1995) [2] 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 crude fibre was found by using Wendee's method [3]. The amount of total carbohydrates [4] 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) [4] and Obadoni & Ochuko (2001) [5] 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) [6]. 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) [7]. 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) [8]. 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) [9]. The antioxidant potential of phenolic compounds was obtained by measuring their radical scavenging potential using ABTS assay or their ability to reduce compounds by donating electrons using the 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 *S. articulatus* seeds were evaluated according to the decolorization of the ABTS radical cation ($ABTS^+$) as percentage inhibition by Re *et al*, (1999) [10]. 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) [11]. 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 extracts of seeds of *S. articulatus*

The three extracts namely hexane, chloroform and methanol were diluted with their respective solvents (100 mg ml^{-1}) 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) [12]. 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 mix (C_8:C_{24}) in combination with deconvolution reporting software (DRS). The results for individual compound those quality matches > 90% is only reported (as their percentage of the total area of peaks in the total ion chromatogram).

2.6.2 Flavour and fragrance profiling of seeds of S. articulatus.

The dried powdered sample of seeds was analysed by the thermal desorption system. The conditions for GC were same as above mentioned except the oven was programmed from 60 °C (0 min) at 3 °C /min to 240 °C (6min) at 5 °C /min to 280 °C (15 min). The compounds were eluted from GC in Total Ion Chromatograph (TIC), which were searched against two databases,

- Agilent NIST'05 library developed by the National Institute of Standards and Technology
- Flavor 2 developed by Agilent.

2.7 Antimicrobial activity

The antibacterial activity of the seed extracts was tested against 3 gram positive, *Staphylococcus aureus, Staphylococcus epidermidis* and *Bacillus subtilis* and 2 gram negative bacterial strains i.e *Escherichia coli* and *Proteus mirabilis* on NA plates by disc diffusion method (Igbinosa., Igbinosa., & Aiyegoro, 2009) [13]. 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 on the scale of a millimeter.

3. Results and discussion

3.1. Nutritional composition

The obtained nutritional values have been shown in Table 1.

The mineral content showed the presence of Potassium (K), Iron (Fe), Zinc (Zn), Copper (Cu) and Manganese (Mn) in good amounts followed by the other nutrients. Past and on-going investigation of the medicinal properties of plant species reported that the mineral compositions have a major role to play in their therapeutic effect. A good content of K (62.55 ppm) can play an important role in lowering blood pressure and reducing risk of kidney stones. Zinc (0.13 ppm) helps to maintain a sense of smell, keeping a healthy immune system, builds proteins, trigger enzymes etc. Manganese (1.21 ppm) acts as a powerful antioxidant, keeps healthy bones and also controls sugar level.

Also, low content of Pb, Ni, as show that they are free from toxic metals. Therefore the collected species can be used in well-balanced diets and can be consumed unreservedly without any health risk.

Table 1: Nutritional composition of *S. articulatus* seeds.

Composition	Content
Moisture (g 100g $^{-1}$ FW)	8.19
Ash (g 100 g $^{-1}$ DM)	4.95
Protein (g 100 g $^{-1}$ DM)	0.15
Fat (g 100 g $^{-1}$ DM): (Fresh, dried)	6.15
Crude fiber (g 100 g $^{-1}$ DM)	20.53
Carbohydrates (g 100 g $^{-1}$ DM)	80.56
Food energy (Kcal)	311.935
Mineral elements in *S. articulatus*	**(ppm)**
Iron	0.85
Copper	0.10
Manganese	1.21
Zinc	0.13
Potassium	62.55
Trace element and heavy metals	**(ppm)**
Arsenic	0.24
Lead	0.17
Nickel	0.02

3.2 Phytochemical analysis

The quantitative estimates of the crude phytochemicals of *S. articulatus* DM were obtained as: alkaloids, 0.0265 g 100 g^{-1} and Saponins, 0.808 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 [14].

Phenolic content (TPC) contributes directly to anti-oxidative action. The highest phenolic content was found to be in methanolic fraction followed by chloroform and hexane fractions. Highest flavonoid content (TFC) and flavanol content were found to be present in a methanolic fraction of seeds. (Table 3).

Table 2: Alkaloids and Saponins content in S. articulatus seeds.

Parameter	Amount (g 100^{-1})
Alkaloids	0.0265
Saponins	0.808

Table 3: Total phenolics content, Total flavonoid content and Total flavonol contents in S. articulatus seeds.

Solvent		Hexane	Chloroform	Methanol
TPC (µg GAE) 100 mg $^{-1}$ EY		0.02151	0.02787	26.6733
TFC (µg CE) 100 mg $^{-1}$ EY		0.1175	0.0804	9.568
Flavanol (µg BHTE) 100 mg $^{-1}$ EY		3.485	5.673	5.851

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 seeds of *S. articulatus* (0.75 mg/ml) in the methanolic extract. This suggests that they have 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 970.90 µg BHTE 100 mg^{-1} in the methanolic fraction of seeds.

3.4 GC/MS analysis

The details of all identified compounds in hexane, chloroform and methanolic extracts are grouped by their chemical nature (Table 4a, 4b and 4c). The reported saturated fatty acids such as oleic acid is 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 [15]. 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 [16]. Hexadecanoic acid (used in cosmetics, soaps), Tetradecanoic acid (cosmetics and medicinal preparations) and Pentadecanoic acid were also found.

Table 4a: List of compounds in hexane fraction detected by GC/MS

S.no	Compounds Detected	CAS#	Area (%)	R.T. (in sec)
1.	1-Pentadecene	013360-61-7	0.27	15.020
2.	1-Tridecene	002437-56-1	0.27	15.020
3.	1-Tetradecanol	000112-72-1	0.27	15.020
4.	Tetradecanoic acid	000544-63-8	0.23	18.194

5.	1-Octadecene	000112-88-9	0.07	18.530
6.	Dichloroacetic acid, heptadecyl ester	1000282-98-2	0.07	18.530
7.	1,2-benzenedicarboxylicacid, bis(2-methylpropyl) ester	000084-69-5	0.17	19.394
8.	Phthalic acid, isobutyl nonyl ester	1000309-04-4	0.17	19.394
9.	Phthalic acid, decyl isobutyl ester	1000308-94-2	0.17	19.394
10.	Nonadecane	000629-92-5	1.83	19.652
11.	Tetracosane	000646-31-1	1.83	19.652
12.	Heneicosane	000629-94-7	1.83	19.652
13.	Hexadecanoic acid, methyl ester	000112-39-0	0.46	19.910
14.	14-methyl-, Pentadecanoic acid, methyl ester	005129-60-2	0.46	19.910
15.	Hexadecenoic acid	002416-20-8	0.47	20.089
16.	Eicosane	000112-95-8	0.53	20.628
17.	Hexadecane	000544-76-3	0.53	20.628
18.	Heptadecanoic acid	000506-12-7	0.17	21.256
19.	Heptafluorobutyric acid, heptadecyl ester	1000282-97-3	0.12	21.334
20.	Trichloroacetic acid, pentadecyl ester	074339-53-0	0.12	21.334
21.	E-15-Heptadecenal	1000130-97-9	1.13	21.401
22.	1-Nonadecene	018435-45-5	1.13	21.401
23.	9-Nonadecene	031035-07-1	1.13	21.401
24.	Phytol	00015086-7	0.10	21.738
25.	Oleic acid	000112-80-1	45.06	22.344
26.	Octadec-9-enoic acid	1000190-13-7	45.06	22.344
27.	Tridecane	000629-50-5	1.94	22.489
28.	Tritetracontane	007098-21-7	1.94	22.489
29.	9-Tricosene	027519-02-4	1.40	23.196
30.	2-Bromo dodecane	013187-99-0	4.22	23.375
31.	2,6,10,14-tetramethyl Hexadecane	000638-36-8	4.22	23.375
32.	Eicosanoic acid	000506-30-9	0.21	23.902
33.	8-hexyl Pentadecane	013475-75-7	0.99	24.968
34.	2-hydroxy-1-(hydroxymethyl) hexadecanoic acid ethyl ester	023470-00-0	0.14	25.091
35.	1,2-Benzenedicarboxylic acid, diis ooctyl ester	027554-26-3	0.20	25.417
36.	Bromoacetic acid, hexadecyl ester	005454-48-8	0.18	26.303
37.	2-Chloropropionic acid, hexadecyl ester	086711-81-1	0.18	26.303
38.	Trans-13-Docosenamide	010436-09-6	0.16	27.245
39.	9-Octadecenamide	000301-02-0	0.16	27.245
40.	Squalene	007683-64-9	0.35	27.693
41.	Campesterol	000474-62-4	1.39	33.604
42.	24-methyl-5-Cholestene-3-ol	1000214-17-4	1.39	33.604
43.	Stigmasterol	000083-48-7	0.37	34.243
44.	4,4-dimethyl Cholesta-6,22,24-triene	1000128-66-9	0.37	34.243
45.	22,23-dihydro- Stigmasterol	1000214-20-7	3.47	35.645
46.	Gamma-sitosterol	000083-46-5	3.47	35.645
47.	Lanosterol	000079-63-0	0.48	35.970
48.	Cycloartanol	004657-58-3	0.91	36.307
49.	3-beta,9,19-Cyclolanost-24-en-3-ol	000469-38-5	0.39	37.417

Table 4b: List of compounds in chloroform fraction detected by GC/MS

S.no	Compounds Detected	CAS#	Area (%)	R.T. (in sec)
1.	Trichloromethane	000067-66-3	7.94	3.491
2.	Dimethyl Sulfoxide	000067-68-5	0.58	4.736
3.	1-Dodecene	000112-41-4	0.22	10.579
4.	(E)-2-Decenal	003913-81-3	0.14	11.779
5.	(E)-2-Tetradecene	035953-53-8	0.39	13.685
6.	1-Pentadecene	013360-61-7	0.17	15.020
7.	2,4-bis(1,1-dimethylethyl) phenol	000096-76-4	0.72	15.334
8.	1-Heptadecene	006765-39-5	0.56	16.276
9.	Tetradecanoic acid	000544-63-8	0.19	18.205
10.	1-Nonadecene	018435-45-5	0.57	18.530
11.	Bis(2-methylpropyl) ester 1,2-benzenedicarboxylic acid	000084-69-5	0.28	19.405
12.	Nonadecane	000629-92-5	1.38	19.652
13.	Hexadecanoic acid, methyl ester	000112-39-0	0.40	19.910
14.	9-Hexadecenoic acid	002091-29-4	0.15	20.101
15.	n-Hexadecanoic acid	000057-10-3	17.01	20.527
16.	(E)-5-Eicosene	074685-30-6	0.87	20.583

17.	Eicosane	000112-95-8	0.55	20.639
18.	Octadecyl ester-2-chloropropionic acid	088104-31-8	0.14	21.345
19.	9-Nonadecene	031035-07-1	0.98	21.402
20.	Heneicosane	000629-94-7	5.25	21.615
21.	Isophytol	000505-32-8	0.22	21.738
22.	Oleic Acid	000112-80-1	36.05	22.321
23.	Octadecanoic acid	000057-11-4	1.89	22.389
24.	Octadecane	000593-45-3	1.92	22.501
25.	(Z)-9-tricosene	027519-02-4	1.12	23.207
26.	Nonadecane	000629-92-5	3.44	23.375
27.	1-(1,5-Dimethylhexyl) -4-(4- methylpentyl)- cyclohexane	056009-20-2	0.54	23.734
28.	Octadecyl 2,2,2-tri chloroethyl ester carbonic acid	1000314-56-3	0.23	24.777
29.	Eicosane	000112-95-8	0.69	24.968
30.	2,3-Dihydroxypropyl ester hexadecanoic acid	000542-44-9	0.30	25.103
31.	Mono (2-ethylhexyl) ester 1,2 benzenedicarboxylic acid	004376-20-9	0.30	25.428
32.	Tetracosane	000646-31-1	0.37	25.731
33.	1,2-Diethyl-cyclohexadecane	1000155-85-3	0.25	26.314
34.	(Z)-9,17-octadecadienal	056554-35-9	2.21	26.505
35.	(all-E)- 2,6,10,15,19,23- hexamethyl-2,6,10,14,18,22-tetracosahexaene	000111-02-4	0.26	27.693
36.	Eicosane	000112-95-8	0.42	28.344
37.	Octadecyl ester 17-pentatriacontene bromoacetic acid	018992-03-5	0.16	30.643
38.	Campesterol	000474-62-4	1.56	33.637
39.	Stigmasterol	000083-48-7	0.42	34.277
40.	22,23-Dihydro- stigmasterol	1000214-20-7	3.83	35.701
41.	24-Cholestadien-3.beta 24-isopropyl-5-ol	077643-24-4	0.31	36.015
42.	Cycloartanol	004657-58-3	0.77	36.352

Table 4c: List of compounds in methanol fraction detected by GC/MS

S.no	Compounds Detected	CAS#	Area (%)	R.T.(in sec)
1.	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one	028564-83-2	0.48	9.794
2.	1,2-Benzenediol	000120-80-9	0.86	10.781
3.	2,3-dihydro Benzofuran	000496-16-2	0.99	11.061
4.	1,2,4-Triazolo(4,3-a) pyrimidine	000274-98-6	0.99	11.061
5.	2-Methoxy-4-vinylphenol	007786-61-0	1.13	12.631
6.	1-(3-methoxyphenyl) Ethanone	000586-37-8	1.13	12.631
7.	2,3,4,6-tetramethyl Phenol	003238-38-8	1.13	2.631
8.	1-Hexadecene	000629-73-2	0.52	16.265
9.	1-Octadecene	000112-88-9	0.52	16.265
10.	(E)2-Tetradecene	035953-53-8	0.52	16.265
11.	8-Pentadecanone	000818-23-5	0.28	17.229
12.	8-Octadecanone	079246-41-6	0.28	17.229
13.	1-Nonadecene	018435-45-5	0.44	18.530
14.	3-Eicosene	074685-33-9	0.44	18.530
15.	Chloro Acetic acid-hexadecyl ester	052132-58-8	0.44	18.530
16.	Phthalic acid, isobutyl nonyl ester	1000309-04-4	1.50	19.405
17.	Hexadecanoic acid, methyl ester	000112-39-0	1.25	19.910
18.	14-methyl Pentadecanoic acid, methyl ester	005129-60-2	01.25	19.910
19.	n-Hexadecanoic acid	000057-10-3	24.36	20.415
20.	Tetradecanoic acid	000544-63-8	24.36	20.415
21.	Tridecanoic acid	000638-53-9	24.36	20.415
22.	Heptafluorobutyric acid, n-octadecyl ester	000400-57-7	0.45	20.560
23.	Trichloroacetic acid, pentadecyl ester	074339-53-0	0.45	20.560
24.	9,12-Octadecadienoic acid (Z,Z)-,methyl ester	000112-63-0	1.80	21.558
25.	8-Octadecenoic acid, methyl ester	002345-29-1	3.09	21.615
26.	Oleic acid	000112-80-1	55.49	22.164
27.	Tricosane	000638-67-5	0.21	23.331
28.	2-hydroxy-1-(hydroxymethyl) Hexadecanoic acid, ethyl ester	023470-00-0	0.63	25.080
29.	2,3-dihydro 19-Octadecenoic acid propyl ester	000111-03-5	1.33	26.493
30.	Campesterol	000474-62-4	1.06	33.559
31.	24-methyl 5-Cholestene-3-ol	1000214-17-4	1.06	33.559
32.	22,23-dihydro-Stigmasterol	1000214-20-7	2.17	35.533
33.	gamma.-Sitosterol	000083-47-6	2.17	35.533

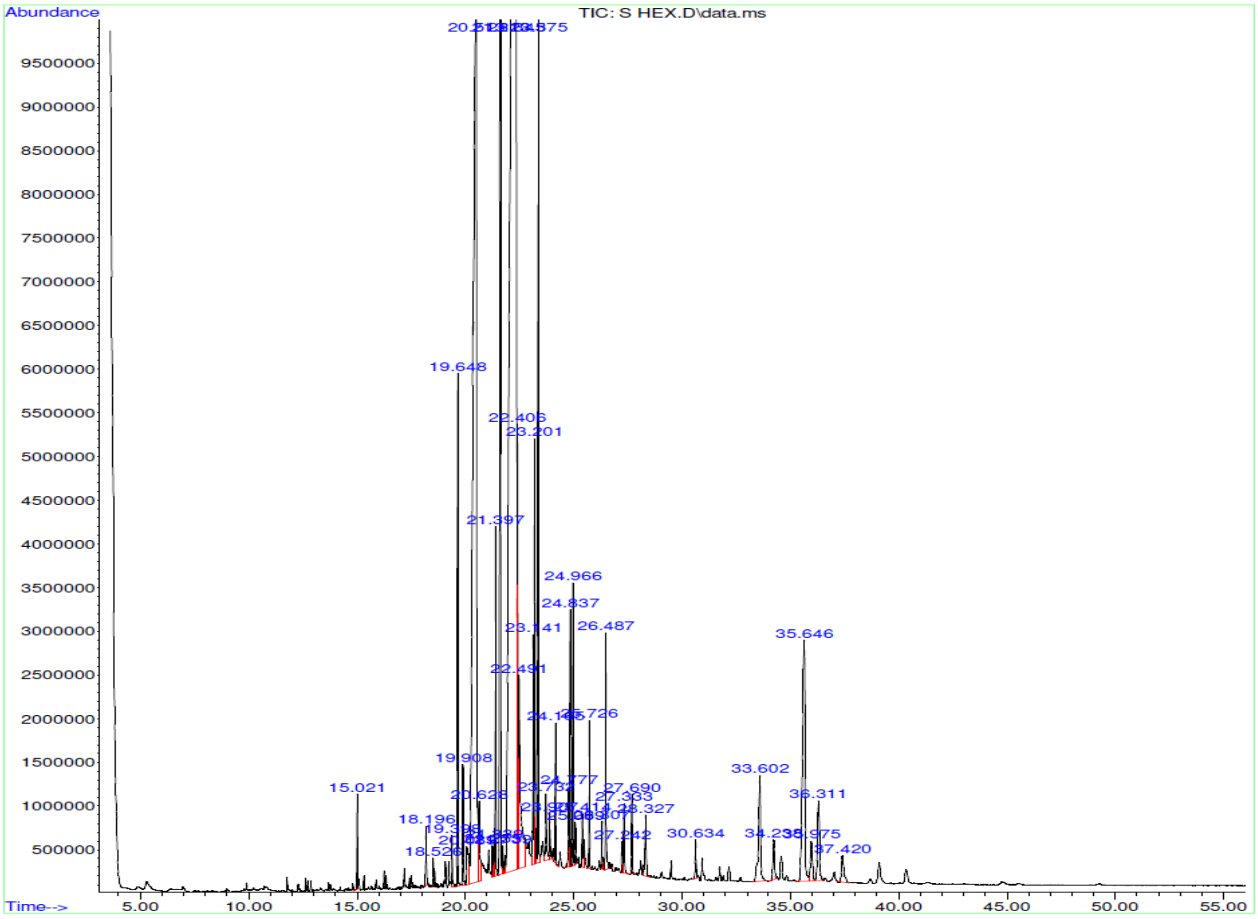

Fig 1(A): GCMS chromatogram of hexane extract of seeds of *S. articulatus.*

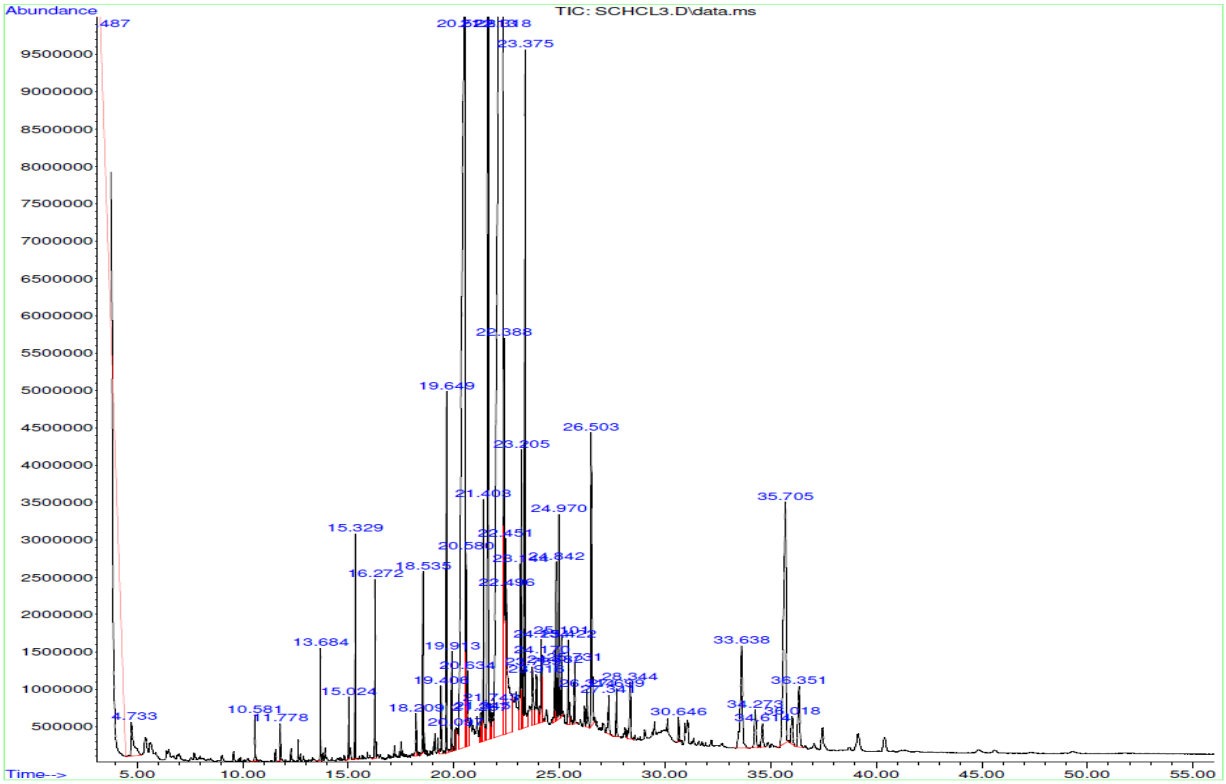

Fig 1(B): GCMS chromatogram of chloroform extract of seeds of *S. articulatus.*

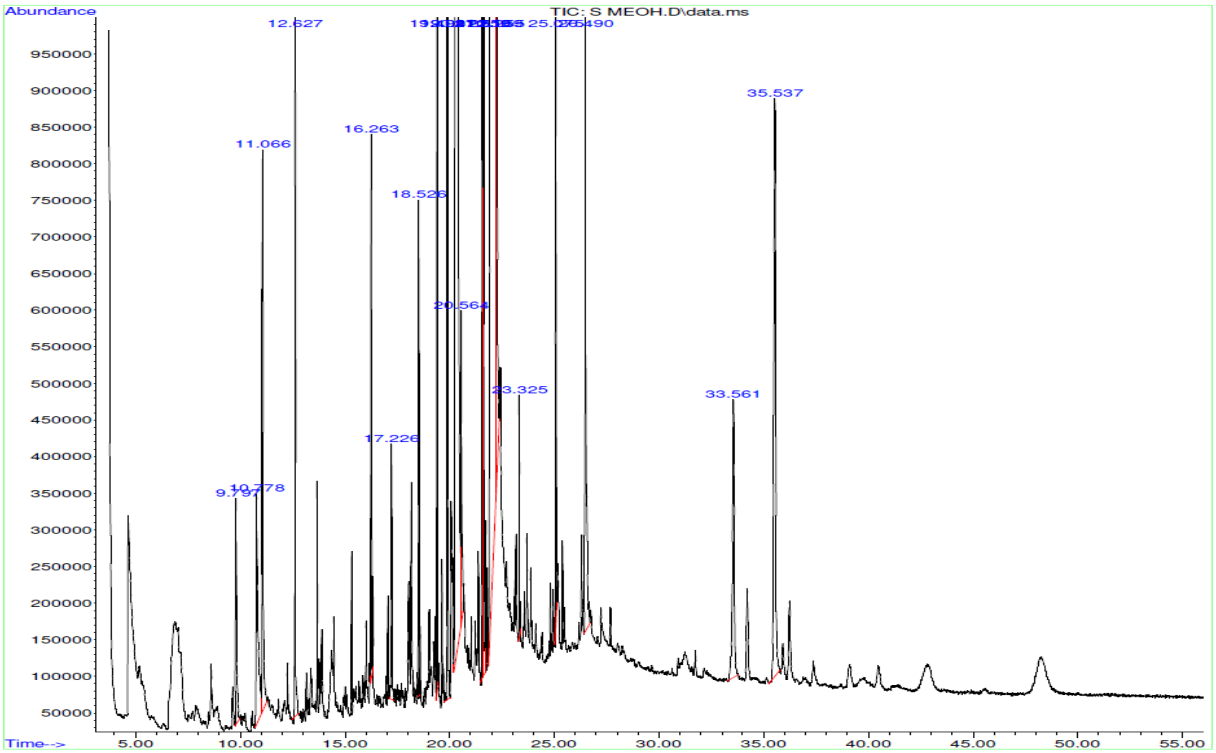

Fig 1(C): GCMS chromatogram of methanolic extract of seeds of *S. articulatus.*

3.5. Flavour and Fragrance profiling of seeds of *S. articulatus.*

Thermal Desorption System (TDS) was used for profiling of volatile and semi-volatile compounds which are responsible for flavour and fragrance in the sample. Diosgenin is a food saponin which is cancer chemopreventive and has therapeutic effect; Vanillin which is used as flavouring agent in foods and beverages; Anthranilic acid, used in perfumes to imitate orange and jasmine ; carbonic acid is a fuzzing agent which is used in carbonated beverages.

Table 5: List of oleo/aromatic compounds in seeds of *S. articulatus.*

S.no.	Compound name	CAS#	Area %	RT (in min)
1.	p-xylene	000106-42-3	0.02	3.787
2.	1,3-dimethyl Benzene	000108-38-3	0.02	3.787
3.	2-Propenoic acid, butyl ester	000141-32-2	0.05	4.131
4.	1,1,2,2-tetrachloro- Ethane	000079-34-5	0.05	4.398
5.	1,2,3,3-tetrachloro-1-Propene	020589-85-9	0.02	4.642
6.	2-chloro-2-nitro Propane	000594-71-8	0.03	5.209
7.	1,1,2,3,3-pentachloro Propane,	015104-61-7	0.01	8.230
8.	2-Dodecene	007206-26-0	0.02	9.275
9.	2-Decenal	003913-81-3	0.02	10.485
10.	Cyclododecane	000294-62-2	0.05	12.407
11.	Vanillin	000121-33-5	0.04	12.618
12.	Z-1,6-Tridecadiene	1000130-98-3	0.13	13.540
13.	Chloroacetic acid, tridecyl ester	018277-85-5	0.07	13.751
14.	Cyclotridecane	000295-02-3	0.19	14.740
15.	Oleic Acid	000112-80-1	0.19	14.740
16.	Z,Z-10,12-Hexadecadienal	1000130-86-6	0.33	15.929
17.	1-chloro- Tetradecane	002425-54-9	0.14	16.251
18.	Tetradecanoic acid	000544-63-8	0.17	17.017
19.	Bromoacetic acid, hexadecyl ester	005454-48-8	0.21	17.273
20.	Carbonic acid, tetradecyl 2,2,2-trichloroethyl ester	1000314-56-0	0.35	17.895
21.	Phthalic acid, isobutyl octadecyl Ester	1000309-06-1	0.37	18.139
22.	Nonadecane	000629-92-5	0.21	18.373
23.	n-Hexadecanoic acid	000057-10-3	11.63	19.061
24.	Tetradecanoic acid	000544-63-8	11.63	19.061

25.	Heneicosane	000629-94-7	1.00	20.305
26.	Eicosane	000112-95-8	1.00	20.305
27.	Tetracosane	000646-31-1	1.00	20.305
28.	Octadecanoic acid	000057-11-4	6.77	21.039
29.	2-Chloroethyl linoleate	025525-76-2	2.94	21.516
30.	Eicosanoic acid	000506-30-9	1.20	22.649
31.	Tricosane	000638-67-5	0.84	22.872
32.	Erucic acid	000112-86-7	0.84	22.872
33.	tert-Hexadecanethiol	025360-09-2	0.84	22.872
34.	Palmitic anhydride	000623-65-4	1.59	23.827
35.	gamma-Sitosterol	000083-47-6	2.92	24.438
36.	Isobutyric acid, tetradecyl ester	167871-30-9	1.30	26.860
37.	Cholesta-3,5-diene	000747-90-0	0.74	28.437
38.	Stigmastan-3,5-diene	1000214-16-4	4.05	29.726
40.	beta.-Sitosterol acetate	000915-05-9	4.05	29.726
41.	Vitamin E	010191-41-0	0.18	30.114
42.	Silicic acid, diethyl bis (trimethylsilyl ester)	003555-45-1	0.22	31.414
43.	Campesterol	000474-62-4	1.90	31.847
44.	Stigmasterol	000083-48-7	0.89	32.458
45.	Diosgenin	000512-04-9	0.46	33.025
46.	gamma.-Sitosterol	000083-47-6	10.96	33.925
47.	Lanosterol	000079-63-0	0.40	35.424
48.	Anthranilic acid	015236-34-7	0.14	36.557

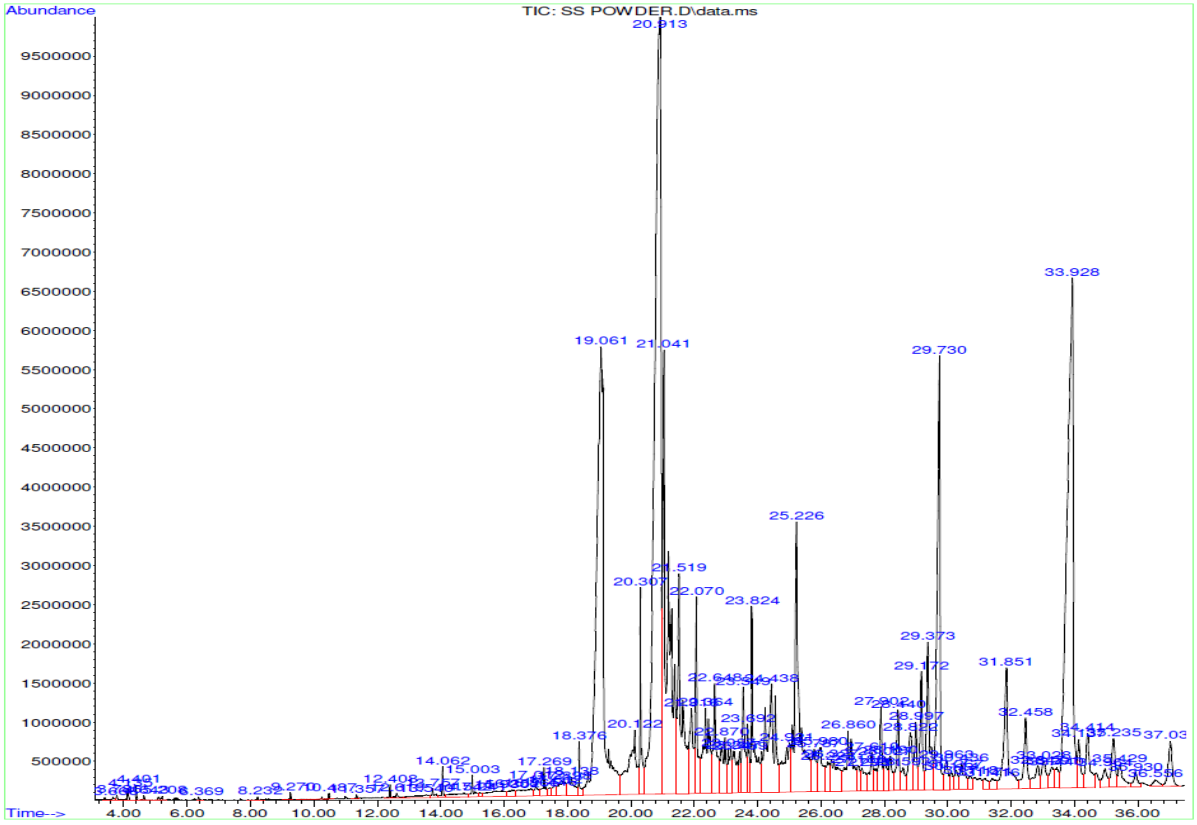

Fig 1(D): Chromatogram of Flavour and Fragrance profile of seeds of *S. articulatus.*

* % 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

3.6 Antibacterial assay

Screening of the three seed extracts namely, hexane, chloroform and methanol were done for antibacterial activities by agar well diffusion method against gram positive *B. subtilis*, *S. aureus, S. epidermidis* and gram negative *P. mirabilis* and *E. coli.* A zone of inhibition was observed for all the methanolic fractions at 700 mg/ml indicating antibacterial activity. No zone of inhibition was observed in any of the seed extract for hexane or chloroform fractions (as high as 700 mg/ml), indicating that they do not possess antibacterial activity for them.

Table 6: Antibacterial activity of *S. articulatus* seeds methanolic extracts.

Bacterial strains	Diameter of zone of inhibition(mm): Methanol fraction	
Staphylococcus aureus		1.9
Staphylococcus epidermidis		1.5
Bacillus subtilis		1.65
Gram negative		
Proteus mirabilis		1.55
Escherichia coli		1.55

4. Conclusion

The chemical composition of the underutilized seeds of *Scirpus articulatus* show that they can be a potential source of nutraceuticals as well as flavoring agents. Evidently, these seeds are a rich source of bioactive compounds and may be used in developing value added products and other food applications to extract out their health benefits. In addition, they can also be used as food additives because of their flavor and nutritional contents. The obtained compounds have antimicrobial potential along with antioxidant properties and may play an important role in drug development, health supplements and spa. Moreover, the good correlation obtained between the various assays employed and phenolic contents strongly indicates that these phenolics are among the predominant source of antioxidant activity in *S. articulatus.* Also, the crop has a potential to be used as an export item through proper phytochemical investigation and nutritional analysis. Thus, there is enormous scope for future research and further pharmacological investigation on *S. articulatus.*

5. Acknowledgement

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