



Research Article

Phytochemical and GC-MS Evaluation of Bioactive Principle of *Vitis vinifera* Peels

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Abstract

Background and Objectives: *Vitis vinifera* is small round or oval berry that feature semi-translucent flesh encased by a smooth skin. It is grown for its juice and the peels discarded as waste. This is done due to lack of awareness of the health benefits of its peels. This study was aimed to determine the phytochemicals and bioactive principle of *V. vinifera* peels. **Materials and methods:** The peels of *V. vinifera* were obtained from the fruits and shade dried at room temperature. The dried peels were pulverized into fine powder. The phytochemicals and bioactive amalgams were done by method of AOAC and Gas Chromatography-Mass Spectrometry (GC-MS), respectively. **Results:** The results of phytochemical studies showed *V. vinifera* peels had high levels of alkaloids, phenol, tannins and oxalate with saponin low while vitamin contents had highest amounts of Vitamin A and B9, moderate amounts of C and E with trace amounts of B1, B2 and B5. Also, the results of proximate contents indicate that carbohydrate content was highest. Other findings are crude fibre, crude protein, fats, moisture and ash. Mineral analysis revealed the order K>Mg>Fe>Na>Ca in the *Vitis vinifera*. The mass spectrum of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. Seven bioactive compounds were identified in methanol peel extract by GC-MS analysis. The result revealed that the peel had high peak areas (%) in the order of octadec-11-enoic acid, methyl ester, 9,12,15-octadecatrien-1-ol, hexadecanoic acid, Methyl 14-methyl pentadecanoate, stearic acid, methyl ester, 5-chloropyridin-2-ol and 8-hydroxyocta-2,4,6-trien-1-ylum. **Conclusion:** The findings therefore, suggest that there is an indication that *V. vinifera* peels contains chief phytochemicals and bioactive compounds that may be linked to its beneficial effects on health.

Key words: *V. vinifera*, phytochemicals, gas chromatography-mass spectrometry, vitamin A and B9, bioactive compounds, methanol peel extract, secondary metabolites

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Even though, human has colossal vigor to devour and acclimatize to an assortment of eating stuff, there are certain things like fruits and vegetables that have become invaluable for human diet. Fruits and their imperative apparatus have a decisive role in supplying priceless nutrients for upholding human health. Fascinatingly, peel, seed and rind of some fruits have higher vitamins, fibers, minerals and other essential nutrients activity than the pulp fractions.

Citrus is of the family Rutaceae and includes some key fruits like oranges, mandarins, limes, lemons, sour orange and grapefruits. Citrus fruits are among the chief horticultural crops, with global agricultural production. Although, the fruits are mainly used for its juice, they have important cost-effective value for their essential oils. Citrus essential oils are obtained as byproducts of the citrus processing and are the most widely used essential oils in the world. In fact, Citrus fruit essential oils and their major components have gained acceptance in the food industry since they have been generally recognized as safe. Citrus essential oils have been reported to possess a wide range of uses¹.

Vitis vinifera (Grape) peels find their place in the waste box of home and it happens because of the lack of awareness about their health benefits. People love to consume the juicy pulp of grape and discard the peel for the fact that it does not taste as the pulp. Research has established that fruit peels contain nutritional and phytochemical values that make it an excellent diet for keeping the body fit and healthy^{2,3}. Hence, the peels have relatively the same nutritional value as the flesh itself. Thus, regular consumption of fruit peel is considered highly beneficial for several health ailments². Studies have bared that grape seed extract (GSE) has antioxidant and free radical scavenging, antidiabetic, cardioprotective, hepatoprotective, anti-carcinogenic, anti-microbial and anti-viral activities^{4,5} etc. Rural inhabitants used the peels as mosquito repellent and in ethnomedicine.

Previous studies on GC-MS analysis of *Vitis vinifera* focused on the plant seed extracts^{4,5} and juice⁶ as well as aqueous grape peels extract⁷. Similarly, Souse *et al.*⁸ worked on Grape pomace (*Vitis vinifera* L.), Benitaka variety, grown in the semiarid region of northeast Brazil. For these reasons, this study was therefore aimed to determine the phytochemicals and bioactive component of methanol extract of grape peel grown in Ebonyi State, Nigeria. This will help to x-ray the bioactive compounds responsible for some of the acclaimed therapeutic potentials. Also, the knowledge derived can be employed to discourage indiscriminate disposal of grape peel as waste and convert/re-utilize them into worth/raw material

for medicinal connotation. Thus, no research has been published on GC/MS evaluation of *V. vinifera* peels from Ebonyi State and the report of this study will fill the gap.

MATERIALS AND METHODS

Collection and identification of plant material: Fresh fruits of *V. vinifera* were purchased at Meat Market in Abakaliki, Ebonyi State, Nigeria in the month of August, 2015. The plant samples were identified and authenticated by Taxonomist, Prof. S.S.C Onyekwelu of the Department of Applied Biology, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria. All chemicals and reagents were of analytical standard.

Methods

Preparation of plant material: The peels of *V. vinifera* were obtained from the fruits and shade dried at room temperature ($28 \pm 3^\circ\text{C}$). The dried peels were pulverized into fine powder using electric blender (CORONA-REF. 121, Landers and Qlink blender, Model No. OBL-15L40). The powdered materials were stored in air tight polythene bags protected from direct sunlight until required for analysis.

Quantitative phytochemical, vitamin, mineral and proximate analysis: The method described by Akubugwo *et al.*⁹ was adopted to assay for the quantitative phytochemical analysis in the peels of *V. vinifera*.

Selected vitamins were determined using atomic absorption spectrophotometer (AAS) based on Association of Official Analytical Chemist AOAC¹⁰.

The standard method of AOAC¹⁰ was used to determine the major components of food.

The selected minerals were determined using Atomic Absorption Spectrophotometer (AAS) based on Association of Official Analytical Chemist AOAC¹⁰.

Plant sample extraction: Twenty grams of the powdered peels were extracted with 50 mL of 40% methanol overnight in a stopped bottle and with occasional stirring at room temperature ($28 \pm 3^\circ\text{C}$). The sample was first sieved using muslin cloth and then filtered using Whatman No.1 filter paper. This process was repeated three times. The filtrate was concentrated under reduced pressure at 40°C for 45 min in a rotary vacuum evaporator and then lyophilized to get a brown aromatic solid extract. The yield of the extract was expressed in terms of the percentage of the dry weight of initial plant material used (yield 35.37% w/w). The dry extract obtained was kept in a refrigerator at 4°C until required for use.

Column fractionation of methanol extract: The dry crude extract was subjected to column chromatography according to standard method. The sample for the column was prepared by adsorbing 20 g of the methanol extract of *V. vinifera* with 60 g of silica gel G (60-120 mesh). The mixture was air dried and carefully layered on top of the packed silica gel in the column (14 cm length) using a glass funnel. The extract in the column was eluted with 100 mL of methanol at the rate of 1 mL min⁻¹. The elutes were concentrated and labeled. The percentage yield of the fraction was recorded. The methanol fraction of *V. vinifera* peels was analyzed by Gas Chromatography-Mass Spectrometry (GC-MS).

Gas chromatography-mass spectrometry (GC-MS): GC-MS analysis was carried out on a GC-MS (Model: QP2010 PLUS Shimadzu, Japan) comprising a AOC-20i auto-sampler and chromatograph interfaced to a mass spectrometer (GC-MS). The instrument was equipped with a VF 5 ms fused silica capillary column of 30 m length, 0.25 mm diameter and 0.25 µm film thickness. The temperatures employed were; column oven temperature 80°C, Injection Temp 250°C at a pressure of 108.0 kPa, with total flow and column flow of 6.20 and 1.58 mL min⁻¹, respectively. The linear velocity was 46.3 cm sec⁻¹ and a purge flow of 3.0 mL min⁻¹. The GC program ion source and interface temperature were 200.00 and 250.00°C respectively with solvent cut time of 2.50 min. The MS program starting time was 3.00 min which ended at 30.00 min. with event time of 0.50 sec, scan speed of 1666 µL sec⁻¹, scan range 40-800 u and an injection volume of 1 µL of the plant extract (split ratio 10:1). The total running time of GC-MS was 30 min. The relative percentage of the extract was expressed as percentage with peak area normalization.

Identification of phytocompounds: Interpretation on the mass spectrum was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The fragmentation pattern spectra of the unknown components were compared with those of known components stored in the NIST V. 3.2 library¹¹. The compound bioactivity prediction was based on Dr. Duke's Phytochemical and Ethnobotanical Databases¹². The relative percentage amount of each phytocomponent was calculated by comparing its average peak area to the total area. The name, molecular weight and structure of the components of the test materials were ascertained.

Statistical analysis: The data obtained in chemical contents was analyzed by one way ANOVA and values were regarded significant at p>0.05. Results expressed as means and standard deviation.

RESULTS AND DISCUSSION

The *V. vinifera* peels were analyzed for phytochemicals and the results were depicted in Table 1. It proved that alkaloids contents were highest in the peels with others less than one. Thus, all the analyzed phytochemicals were significantly different from each other at p<0.05. The report of Ademola *et al.*¹³ on phytochemical constituents and proximate analysis of orange peel (citrus fruit) was in divergence with result of this study. Bupesh *et al.*⁷ in qualitative analysis of grape fruit (*V. vinifera*) skin extract reported that tannins were absent which disagreed with the obtained results.

Table 2 showed the results of vitamin constituents. It posited that vitamin A and B9 were higher in the peels with vitamin C and E in moderate amounts and others less than one. Moreso, all the B vitamins analyzed except B9 were not significant from each other at p>0.05 while vitamin A, C and E were significant at p<0.05. The report of Uraku and Igwenyi¹⁴ on comparative studies of phytochemical and vitamin constituents of *C. sinensis* and *V. vinifera* peels avowed with the obtained outcome.

Proximate contents as shown in Table 3 indicated that carbohydrate, crude protein, moisture, crude fibre, fats and ash were of variable amounts. Furthermore, the proximate

Table 1: Results of phytochemical composition of *V. Vinifera* peels (G/100 g) dry weight

Phytochemicals	<i>Vitis vinifera</i>
Alkaloids	1.58±0.72 ^e
Saponins	0.310±0.06 ^a
Tannins	0.640±0.02 ^c
Oxalate	0.560±0.04 ^b
Phenol	0.710±0.05 ^d

Values are mean±standard deviation of triplicate determination. Values with the same letters are significant to each other at p>0.05 while others with the same showed no significant at p<0.05

Table 2: Results of vitamin composition of *V. vinifera* peels (g/100 g) dry weight

Vitamins	<i>Vitis vinifera</i>
A	210.12±0.56 ^e
B1	0.07±0.01 ^a
B2	0.31±0.02 ^a
B3	0.07±0.01 ^a
B5	0.07±0.02 ^a
B9	63.25±0.21 ^d
C	4.60±0.22 ^c
E	1.16±0.04 ^b

Values are mean±standard deviation of triplicate determination. Values with different letters are significant to each other at p>0.05 while others with the same showed no significant at p<0.05

contents showed significant from each other at $p < 0.05$. The report of Muhammad *et al.*¹⁵ on nutritional and antinutritional composition of *Sclerocarya birrea* peels aligned with the result of this research. Correspondingly, the report of Souse *et al.*⁸ on chemical composition and bioactive compounds of grape pomace (*V. vinifera* L.), Benitaka variety, grown in the semiarid region of northeast Brazil affirmed with our report. However, the protein contents of *C. sinensis* peels as reported by Osarumwse *et al.*¹⁶ denied with the outcome of this work. On the same hand, the work of Ademola *et al.*¹³ contradicted current report. The work of Souse *et al.*⁸ reported high amounts of fibre and carbohydrate with others less than ten and was in disagreement with the outcome of the research.

The result of mineral contents of the peels of *V. vinifera* as presented in Table 4 and demonstrated that K, Mg and Fe had highest quantity with less amounts of Na and Ca. Some minerals viz: Na and Ca had no significant from one another at $p > 0.05$ while K, Mg and Fe were significant at $p < 0.05$. The Phytochemical screening, proximate and elemental analysis of *Citrus sinensis* peels (L.) Osbeck as asserted by Osarumwse *et al.*¹⁶ was in line with result obtained in this work. Also, Roger *et al.*³ made similar submission on assessment of physicochemical and mineral characters of the Orange (*Citrus sinensis*) Peels to this report. Souse *et al.*⁸ reported mineral content of grape pomace in order of $Fe > K > Zn > Mg > Ca$ which divergence with the findings.

Gas chromatogram and mass spectra of methanol peels of *V. vinifera* were presented in Fig. 1 and 7 peaks were shown. This suggested the presence of seven compounds in the peel methanol extract of *V. vinifera*. The proposed compounds, retention time (RT), peak area percentage,

molecular weight and molecular formula were discussed in Table 5. The phytochemicals with highest peak area percentage were Octadec-11-enoic acid, methyl ester with peak area percentage of 40.71, followed by 9,12,15-Octadecatrien-1-ol with peak area percentage of 20.92, followed by hexadecanoic acid with peak area percentage of 10.46 and Methyl 14-methyl pentadecanoate with peak area percentage of 10.35.

Table 6 showed the nature and structures of the phytocompounds with their bioactivities. Some of the compounds act as Catechol-O-Methyltransferase-Inhibitor, Methyl-Guanidine-Inhibitor, Acidifier, Acidulant, Arachidonic

Table 3: Results of proximate contents of *V. vinifera* peels (%) dry weight

Proximate contents	<i>Vitis vinifera</i>
Moisture	6.52 ± 2.44 ^c
Crude fibre	4.96 ± 0.02 ^b
Crude protein	11.35 ± 0.72 ^d
Ash	4.24 ± 0.04 ^b
Fat	1.16 ± 0.01 ^a
Carbohydrate	71.77 ± 1.08 ^e

Values are Mean ± Standard deviation of triplicate determination. Values with the same letters are significant to each other at $p > 0.05$ while others with the same showed no significant at $p < 0.05$

Table 4: Results of minerals contents of *V. vinifera* peels (g/100 g) dry weight

Minerals	<i>Vitis vinifera</i>
Na	12.47 ± 0.88 ^a
K	280.05 ± 5.40 ^d
Ca	8.45 ± 0.45 ^a
Mg	95.84 ± 0.53 ^c
Fe	22.60 ± 0.50 ^b

Values are Mean ± Standard deviation of triplicate determination. Values with the same letters are significant to each other at $p > 0.05$ while others with the same showed no significant at $p < 0.05$

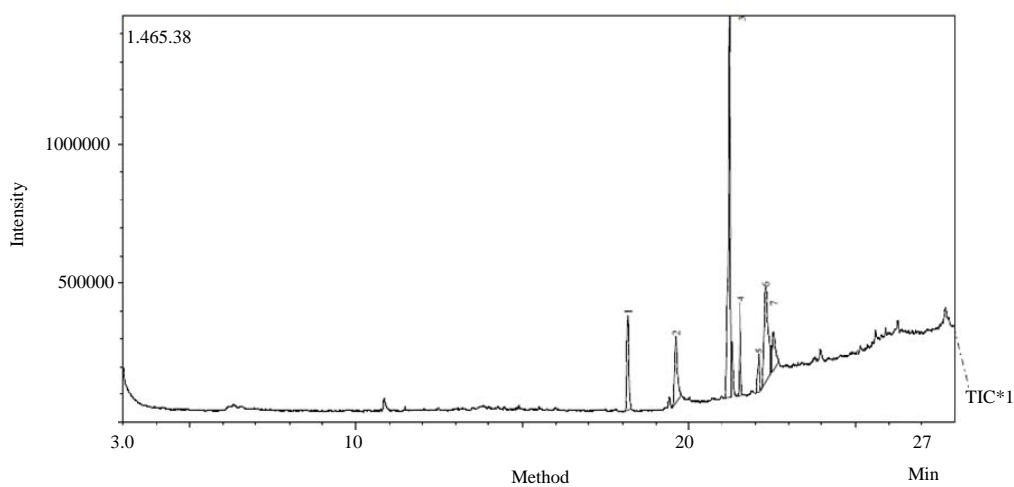


Fig. 1: Gas chromatogram of methanol extract of *Vitis vinifera* peels

Table 5: Proposed phytochemicals of methanol peels of *V. vinifera* with retention time, peak area (%), molecular weight and formular

Peak No.	Name of compound	Retention time	Peak area (%)	Molecular weight	Molecular formular
1	Methyl 14-methyl pentadecanoate	18.180	10.35	270.45	C ₁₇ H ₃₄ O ₂
2	Hexadecanoic acid (Palmitic acid)	19.608	10.46	256.42	C ₁₆ H ₃₂ O ₂
3	Octadec-11-enoic acid, methyl ester	21.225	40.71	296.48	C ₁₉ H ₃₆ O ₂
4	Stearic acid, methyl ester	21.542	7.62	298.50	C ₁₉ H ₃₈ O ₂
5	8-hydroxyocta-2,4,6-trien-1-ylum	22.094	4.03	123.17	C ₈ H ₁₁ O
6	9,12,15-Octadecatrien-1-ol	22.315	20.92	264.44	C ₁₈ H ₃₂ O
7	5-chloropyridin-2-ol	22.540	5.92	129.54	C ₅ H ₄ ClNO

acid-Inhibitor, Increase Aromatic Amino Acid Decarboxylase Activity, Inhibit Production of Uric Acid, Urinary-Acidulant, Urine-Acidifier and Oligosaccharide provider.

A chronic infection like Parkinson's disease is treatable with catechol-O-methyl-transferase inhibitors¹⁷. Since Methyl 14-methyl-pentadecanoate, Octadec-11-enoic acid, Octadecanoic acid and methyl ester are inhibitors of catechol-O-methyl-transferase, they may be effective in the treatment of Parkinson's disease. Catechol-O-methyltransferase is involved in the degradation of neurotransmitters but the inhibitors oppose the degradation of neurotransmitters^{17,18}. Thus, the phytocompounds Octadec-11-enoic acid, methyl ester with the highest concentration of 40.71% in the peel might give an optimistic health benefit.

Hexadecanoic acid with Octadecanoic acid, Octadec-11-enoic acid and methyl ester inclusive had been reported to be an acidifier, acidulant, increase aromatic amino acid Decarboxylase activity, inhibitor of uric acid production and arachidonic acid¹⁷. Acidifiers are chemicals that reduce the pH of the body and are needed for food digestion especially in patients suffering from achlorhydria¹⁷. These patients are not able to secrete HCl for food digestion. These phytocompounds will be beneficial since it increases gastric acid when ingested. Urinary acidifiers help in maintaining a low urine pH when combined with a proper diet which helps in eliminating urinary tract infections, dissolving alkaline bladder stones and promoting kidney health. Acidulant is an additive that gives a sharp taste to foods and also assists in the setting of gels and to act as preservatives¹⁹. Aromatic amino acid decarboxylase activity enhances the production of several neurotransmitters like the serotonin that contributes to the feeling of wellbeing and happiness. Dopamine acts as a vasodilator at normal concentration and in the kidneys increase sodium excretion and urine output²⁰. Also, increase aromatic amino acid Decarboxylase activity is essential for formation of catecholamines, indolamines and trace amines. Furthermore, it is required for converting L-DOPA to dopamine when treating patients with Parkinson's disease (PD). The breakdown of purine nucleotides leads to the formation of uric acid. High concentration of uric acid in the blood can lead

to gout, diabetes and formation of ammonium acid, urate and kidney stones. Hexadecanoic acid, Octadecanoic acid, Octadec-11-enoic acid and methyl ester acknowledged in the peel possibly will help in the inhibition of uric acid.

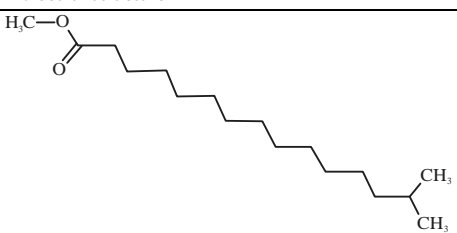
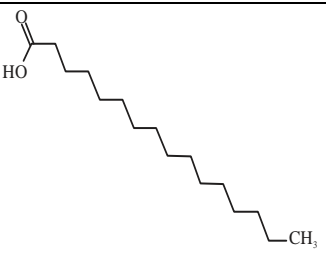
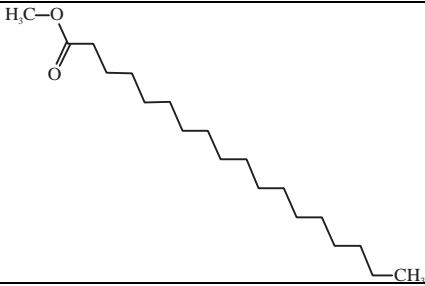
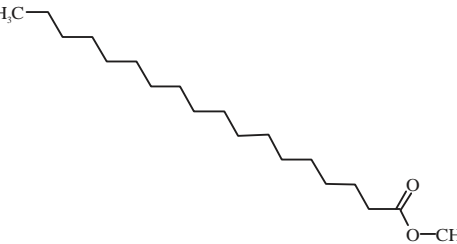
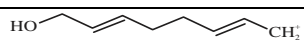
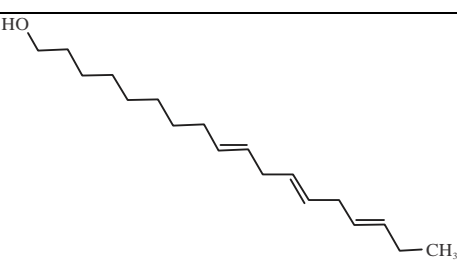
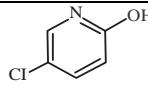
Ghosh and Myers²¹ reported that arachidonic acid, an omega-6 fatty acid stimulates proliferation of prostate cancer cells through production of the 5-lipoxygenase metabolite, 5-HETE (5-hydroxyeicosatetraenoic acid). Thus, 5-HETE is also a potent survival factor for human prostate cancer cells and these cells constitutively produce 5-HETE in serum-free medium with no added stimulus. Perhaps, exogenous arachidonate markedly increases the production of 5-HETE. So, inhibition of arachidonic acid may invariably inhibit 5-lipoxygenase by completely blocks 5-HETE production and induces massive apoptosis in both hormone-responsive (LNCaP) and -nonresponsive (PC3) human prostate cancer cells. Also, arachidonic acid inhibitor is used in treatment of inflammatory conditions and certain types of cardiovascular disease, an arsenal against cancer.

Oligosaccharides in the cells produce specific carbohydrate-binding ligands known as lectins which mediate cell-adhesion with oligosaccharides²². Selectins a family of lectins mediate certain cell to cell adhesion processes, like those of the leukocytes to endothelial cells, thus allowing the white blood cells to help in eliminating the infection or damage¹⁷.

Glycolytic activity enhances the breakdown of glucose to pyruvate for the production of ATP and energy. The 9, 12, 15-Octadecatrien-1-ol and 5-chloropyridin-2-ol are oligosaccharide providers which enhance ATP production²³.

Kadhim *et al.*⁵ and Kumar and Vijayalakshmi⁴, both worked on GC-MS of grape seeds and their results demonstrated 33 and 16 constituents respectively against the outcome of this study where 7 peaks were observed. Also, Bupesh *et al.*⁷ in aqueous skin extract reported 16 compounds. Out of the compounds posited by other researchers only palmitic and stearic acid were in affirmation with the result. The disparity in the number and nature of compounds found in this research could be due to differences in plant parts, solvents of extraction and geographical location.

Table 6: Proposed phytochemicals in *V. vinifera* methanol peel extract nature of compound, nature of compound and bioactivity

Peak No	Name of compound	Nature of compound	Molecular structure	Bioactivity
1	Methyl 14-methyl pentadecanoate	Ester		Catechol-O-Methyl-Transferase-Inhibitor and Methyl-Guanidine-Inhibitor reduce tumour necrosis factor-alpha (TNF-alpha)
2	Hexadecanoic acid	Palmitic acid		Acidifier (inorganic chemicals that either produce or become acid), Acidulant, Arachidonic acid, Arachidonic-Acid-Inhibitor, Increase Aromatic Amino Acid Decarboxylase Activity, Inhibit Production of Uric Acid
3	Octadec-11-enoic acid, methyl ester	Ester		Catechol-O-Methyl-Transferase-Inhibitor, Methyl-Guanidine-Inhibitor, Arachidonic acid-Inhibitor, Increase Aromatic Amino Acid Decarboxylase Activity, Inhibit Production of Uric Acid, Urinary-Acidulant, Urine-Acidifier
4	Octadecanoic acid, methyl ester	Stearic acid		Urine-Acidifier, Urinary-Acidulant, Inhibit Production of Uric Acid, Increase Aromatic Amino Acid Decarboxylase Activity, Arachidonic acid-Inhibitor, Methyl-Guanidine-Inhibitor, Catechol-O-Methyltransferase-Inhibitor
5	8-hydroxyocta-2,4,6-trien-1-ylum	Alkanol		Nf
6	9,12,15-Octadecatrien-1-ol	Alkanol		Oligosaccharide provider
7	5-chloropyridin-2-ol	Alkaloids		Oligosaccharide provider

NF: Not found. Source: Anonymous¹²

CONCLUSION

This is the first report on the GC-MS analysis of *V. vinifera* methanol peel extract. The bioactive compounds identified by GC-MS analysis of the peel have demonstrated to be helpful in

amelioration of many diseases and therefore invaluable in pharmaceuticals as well as in health care services. Further studies are needed to isolate pure active principle of the extract as well as to elucidate their exact mechanism of action in various diseases.

SIGNIFICANCE STATEMENT

This study discovered that *V. vinifera* peel possessed therapeutic potentials through phytochemical and GC/MS characterization of bioactive principle. It could be beneficial for treatment of loads of illnesses. This study will help researchers to convert waste to wealth and uncover the pure active amalgams responsible for counteractive actions. Thus, a new theory on methanol peel extract of *V. vinifera* showed that the peel is enclosed with tannins, Methyl 14-methyl pentadecanoate, Octadec-11-enoic acid, methyl ester, 8-hydroxyocta-2,4,6-trien-1-ylum, 9,12,15-Octadecatrien-1-ol and 5-chloropyridin-2-ol. It is safe and enclosed with health beneficial chemicals.

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