Nutritional and medicinal aspects of *Rumex hastatus* D. Don along with *in vitro* anti-diabetic activity

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**ABSTRACT**

*Rumex hastatus* being used for medicinal and nutritional purposes as a functional food in various countries is hereby evaluated by the gas chromatography-mass spectroscopy, proximate analysis, physicochemical (fluorescence) analysis, quantitative analysis of secondary metabolites and sensory evaluations studies. Various samples of *R. hastatus* were also evaluated for *in vitro* anti-diabetic potential. The investigational study demonstrated that *R. hastatus* is a rich source of carbohydrate, i.e., 432.4 mg/g. Moisture content, protein, fiber, ash content and fats were recorded as 22.8%, 133.9 mg/g, 124.4 mg/g, 54.5 mg/g and 25.6 mg/g, respectively. In the same way, the secondary metabolite displayed a relatively greater amount of flavonoids (84.5 mg/g) followed by saponins (65.5 mg/g) and alkaloids (49.5 mg/g). Similarly, the GC (FID-MS) analysis of *R. hastatus* revealed the detection of 120 compounds. Out of those identified compounds, selected anti-diabetic compounds were sorted out, viz butyl phthalate, phytol, ethylthreonine, dihydrobenzofuran, indoline, guanidine, nerolidol, myristic acid, palmitic acid, caryophyllene, anozol. In physicochemical fluorescence analysis and the sensory evaluation, data were also recorded along with the anti-diabetic with IC\(_{50}\) value of 42.09 µg/ml. The overall investigational analysis of *R. hastatus* obviously demonstrated that this plant was a rich source of primary and secondary metabolites. It may be concluded from the GC (FID-MS) analysis that *R. hastatus* is a potential source of anti-diabetic constituents, which may confer hypoglycemic potential. Based on the recorded data it may also be inferred that *R. hastatus* is among safe and nutritious herbs, which can be used in lieu of green vegetables and functional food with anti-diabetic potential.

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**Introduction**

Globally the advanced researchers are in continuous struggle to combat with various challenging diseases, to fabricate more and more nutritional commodities, to figure out novel resources and to facilitate the mankind.\(^1\)\(^,\)\(^2\) One of the most critical and focused issue is the nutritional crunch throughout the world. A wholesome portion of population goes into the famine, malnutrition and drought calamities each year. In the same way people get various physiological anomalies due to starvation and nutritional deficiency.\(^3\) To cope with such calamities, the agronomists are getting focused on various procedures to enhance the agricultural outcome.\(^4\) Plants are considered as staple by majority of world’s population. The people of those areas wholly and solely depend on herbs, where the access of sophisticated techniques of getting multiple nutritional products is not developed.\(^5\) As reported by several investigators, the plants consist of primary metabolites, secondary metabolites and micronutrients.\(^6\)\(^–\)\(^10\) But the
difference is that each plant species differs in the amount and concentration of these substances. The plants rich in primary metabolites are usually used for nutritional purposes, while secondary metabolites are preferred for medicinal purposes.\textsuperscript{[6,11,12]} The plants may possess fats, protein, carbohydrates, vitamins and minerals in such quantity that are considered sufficient for our daily requirements. One can use specific species of plant as food that possess required amount of primary metabolites and low amount of secondary metabolites. But the plants that possess high amount of secondary metabolites than the primary metabolites can cause serious health complications.\textsuperscript{[13,14]} So this is necessary to figure out the amount of nutritional and medicinal elements in those plants, which are being used as food by people. The nutritional values of various plants have been reported by different investigators.\textsuperscript{[15,16]}

Diabetes mellitus is a metabolic disorder characterized by increase in blood glucose level. It can affect individuals at any stage of life but the frequency of diabetes is considerably high among the obese and aged people.\textsuperscript{[17]} Various therapeutic measures are employed to alleviate the symptoms of this disease. One of the effective therapeutic measures is to decrease the absorption of glucose from the intestine. Therefore, the absorption of glucose from the intestine can be decreased effectively by $\alpha$-glucosidase inhibition. Various plants have been reported to possess the $\alpha$-glucosidase inhibition potential.\textsuperscript{[18]}

*Rumex hastatus* belongs to the family Polygonaceae that consist of 48 genera and 1,200 species.\textsuperscript{[19]} The *Rumex* genus consists of about 200 species, among which a wide variety has been used for medicinal purposes.\textsuperscript{[20]} The *R. dentatus*, *R. maritimus*, *R. bucephalophorus*, *R. nervosus*, *R. abyssinicus* and many more have been used for medicinal purposes including diabetes mellitus.\textsuperscript{[18,21–25]} The *R. vesicarius* has been analyzed for nutritional value and recommended good and safe for nutritional purposes.\textsuperscript{[26]} *R. crispus*, *R. obtusifolius* and *R. acetosa* has been used as vegetable and fodder since long.\textsuperscript{[27–29]} In the same way *R. hastatus* has been used ethnomedicinally for various ailments including GIT ailments, cuts, wounds, bleedings, as appetizer, as anthelmintic, in snake bites, blood pressure, tonsillitis, sore throat, as flavoring agent, carminative and diuretic.\textsuperscript{[30–35]} *R. hastatus* has been reported by several investigators to possess high quantity of flavonoids.\textsuperscript{[36]} Flavonoids consist of wide variety of compounds with excellent anti-diabetic potential in vivo as well as in vitro.\textsuperscript{[37–39]} *R. hastatus* has also been reported to possess anthelmintic, cytotoxic, phytoxic, antibacterial, anticholinesterase and antioxidant potentials.\textsuperscript{[36,40]} The *R. hastatus* has also been used as fodder for animals. In Pakistan *R. hastatus* has been used as vegetable in the northern Himalayan areas.\textsuperscript{[41]}

Going to the detailed literature survey of *R. hastatus*, it is obvious that this plant has been used for multiple purposes. So the current study was arranged to evaluate the *R. hastatus* for its nutritional value and sort out bioactive compounds and provide an implication regarding the use of this plant for nutritional purpose.

**Materials and methods**

**Plant’s collection**

The aerial part of *R. hastatus* was collected from the hilly area of Gorha Gat in the proximity of University of Malakand, Chakdara, Dir (L), KPK, Pakistan. The plant identification was performed by Dr. Ali Hazrat (plant taxonomist) and deposited in the Department of Botany, Shaheed Benazir Bhutto University, Sheringal, Dir upper, KPK, Pakistan, with voucher number (1015SJ). All the extra particles were carefully removed from the plant material and were spread on neat paper in a room in the shade and appropriately dried for two weeks. The paper was changed daily to avoid fungal growth on plant material. After shade drying the plant was pulverized using cutter mill.\textsuperscript{[42]}

**Extraction and fractionation**

The powdered plant sample was soaked in 80% methanol for a period of 8 days followed by filtration. The filtrate obtained was evaporated at low temperature under reduced pressure using rotary evaporator. The evaporation resulted in a semisolid mass, i.e., crude methanolic extract (Rh.Cr).
Some of the Rh.Cr was kept for activities and the remaining was suspended in sufficient amount of water followed by fractionation with various solvents in separating funnel. The fractionation was started with less polar $n$-hexane (500 mL × 3), then chloroform (500 mL × 3), ethyl acetate (500 mL × 3) and final fraction obtained was aqueous.\textsuperscript{[9]} Similarly, the fractions obtained were $n$-hexane (Rh. Hex), ethyl acetate (Rh.EtAc), chloroform (Rh.Cf) and aqueous fraction (Rh.Aq).

**Gas chromatography-flame ionization detector (GC-FID) analysis**

The GC-FID analysis of Rh.Cr was carried out with the help of gas chromatograph Agilent USB-393752 (Agilent Technologies, Palo Alto, CA, USA) via HHP-5MS (5%) phenylmethylsiloxane capillary column (30 m × 0.25 mm × 0.25 μm film thickness; Restek, Bellefonte, PA) attached with FID detector. The oven was allowed to set at temperature of 70°C for a minute and then augmented to 180°C at the rate of 6°C/min for the period of five minutes and lastly to 280°C at the rate of 5°C/min for a period of 20 min. The temperature of detector and injector were maintained at 290°C and 220°C correspondingly. The flow rate of carrier gas (Helium) was maintained as 1 mL/min and the diluted samples (1/1,000 in $n$-pentane, v/v) of 1 μL were injected manually in the split-less mode.

**Gas chromatography–mass spectrometry (GC-MS) analysis**

The -F/MS of Rh.Cr was performed via USB-393752 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with a HHP-5MS 5% phenylmethylsiloxane capillary column (30 m × 0.25 mm × 0.25 μm film thickness; Restek, Bellefonte, PA) outfitted with an Agilent HP-5973 mass selective detector in the electron impact mode (Ionization energy: 70 eV) working under the experimental conditions as those maintained for GC.

**Identification of components**

All the major constituents of Rh.Cr were identified by the comparison of their retention time with the literature of genuine compounds. The identification of compounds was further carried out with the help of the spectral data obtained from the libraries of Wiley and NIST as well as their fragmentation patterns and comparisons of the mass spectra with data reported in literature or with those of mass spectra from literature.\textsuperscript{[43,44]} Each process was carried twice.

**Proximate analysis**

Proximate analysis of powdered plant sample was carried out following the standard procedure of Association of Official Analytical Chemist.\textsuperscript{[45]}

**Moisture content**

Loss on drying (LOD) method was followed for the determination of moisture content of the plant sample. A weighed quantity of powdered plant sample was taken in a suitable container and allowed to dry at 105°C in oven till the achievement of constant weight. Thus the amount of moisture present in the powdered plant sample was figured out from the difference of dried weight of sample and the total weight of the sample.

**Ash content**

Incineration procedure was followed for determination of ash content of powdered plant sample. A weighed amount of sample was put in a crucible and transferred into the muffle furnace and
allowed to incinerate at 550°C for 24 h. Similarly, total ash content was figured out after conversion of dried mass of powdered plant sample into ashes.

**Crude fats**

Soxhlet method was followed for the determination of total fats in the sample. Briefly, 2 g of dried powdered plant sample was transferred into a Soxhlet extractor and petroleum ether was added to the flask of the extractor. The extraction was carried out for 6 h till the exhaustion of sample from fat content. The obtained petroleum ether was filtered and the filtrate obtained was allowed to be evaporated in a weighed beaker. The total fats were calculated as the total increase in weight of the beaker.

**Crude fibers**

The value of crude fiber was figured out from the data of loss in weight after the ignition of dried samples remaining after digestion of fat-free samples with 1.25% each of sulfuric acid and sodium hydroxide solution under specified conditions.

\[
\% \text{ fibre} = \frac{\text{loss of weight on ignition}}{\text{weight of sample used}} \times 100
\]

**Crude protein**

For the determination of crude proteins, the method of micro Kjeldahl nitrogen method was followed. This method involved the digestion of plant sample with concentrated sulfuric acid and catalyst for the conversion of organic nitrogen into ammonium sulfate in the solution. After which the decomposition of ammonium sulfate was carried out via NaOH. The liberated ammonia was distilled into 5% boric acid. After this the titration of trapped ammonia was carried out with 0.05 N HCl for the deduction of nitrogen from ammonia. The indicators used were methylene red and blue both. The percent proteins were calculated from the value of nitrogen obtained multiplied by 6.25.

**Carbohydrate content**

The total crude carbohydrate content was determined by the subtraction formula. In short, the total protein, total fiber, ash content, moisture content and total lipids were subtracted from the dried mass and the total carbohydrates were calculated.

**Secondary metabolites**

**Alkaloids**

The alkaline precipitation gravimetric method was followed to find out alkaloids. A weighed powdered plant sample was taken in a beaker and 10% acetic acid solution in ethanol was transferred into the beaker. The mixture was incubated at 28°C for 4 h. Then it was filtered through Whatman No. 42 filter paper. The filtrate was allowed to be evaporated to one quarter of its original volume followed by the addition of drop-wise NH₄OH for the precipitation of alkaloids. The precipitated alkaloids were received on filter paper and washed with 1% ammonia solution and then dried in an oven at 80°C. So the amount of alkaloids was calculated per gram of the dried powdered sample of *R. hastatus*. 
**Flavonoids**

For the extraction of flavonoids, the procedure of Harborne was followed.\(^{[46]}\) Plant sample (5 g) was taken and boiled in 50 mL of 2 M HCl under reflux for 30 min. It was cooled and filtered using Whatman No. 42 filter paper. The extract was treated with equal volume of ethyl acetate. The flavonoids present in the extract were precipitated which were recovered with the help of weighed filter paper. The amount of flavonoids recovered on filtered paper was calculated.

**Saponins**

For the determination of crude saponins in powdered sample of *R. hastatus*, 20 g of powdered sample was put in a conical flask and 100 mL of 20% ethanol was added to the conical flask. The sample allowed to heat at 55°C in the water bath for 4 h with continuous stirring. After 4 h the sample was filtered and the residue was re-extracted with 200 mL of 20% ethanol. The sample after extraction was allowed to heat until a concentrated volume of 40 mL was obtained. The sample obtained was shifted into a separating funnel and 20 mL of diethyl ether was added to it. After vigorous shaking, the separating funnel was put in a stand to get two layers. The lower aqueous layer was collected while the upper diethyl ether layer was discarded. The aqueous layer obtained was diluted with 60 mL of *n*-butanol and the combined *n*-butanol extract was washed with 10 mL of 5% saline. The final solution obtained was kept in a hot water bath until complete evaporation and the saponins obtained were dried in an oven and the saponins per gram of powdered plant sample was calculated.\(^{[40]}\)

**Fluorescence analysis**

The powdered plant sample was treated with different chemical reagents for the determination of fluorescence characters. The plant sample was put in small quantity on glass slide and treated with various reagents followed by the determination of color under the visible light and ultraviolet light.

**Sensory evaluation**

The sensory evaluation of *R. hastatus* was performed based on the Hedonic Scale following AOAC procedure.\(^{[47]}\) Various parameters were assayed such as color, flavor, taste, general appearance and texture. The evaluation was performed based on the questionnaire to score out of seven points of hedonic scale.

**α-Glucosidase inhibition assay**

α-Glucosidase inhibitory activity was carried out following chromogenic assay.\(^{[48]}\) Enzyme solution was prepared having 0.5 unit/mL and 20 μL of this solution was mixed 120 μL of phosphate buffer having pH 6.9. *p*-nitrophenyl- α-D-glucopyranoside solution (5 mM) was prepared in the same buffer that was employed as substrate. Briefly, 10 μL of test samples having various concentrations (31.25–1,000 μL) were added to them and kept for 15 min at 37°C. After incubation, 20 μL of substrate solution was added to all of them and incubated for further 15 min. Sodium carbonate solution (0.2 M) having volume of 80 μL was added to them to terminate the reaction. Absorption of each sample was measured at 405 nm via double beam spectrophotometer (Thermo electron corporation USA). The reaction mixture with the test sample served as control while acarbose served as positive control. The percent enzyme inhibitory potential was calculated as:

\[
\text{Percent enzyme inhibition} = \frac{\text{Control absorption} - \text{Sample absorption}}{\text{Control absorption}} \times 100
\]
Statistical analysis

The statistical analysis was carried out by Two-way ANOVA followed by Bonferroni post test, in which the positive control was compared with the groups of test samples. $P$ values less than or equal to 0.05 were considered as significant statistically. GraphPad Prism and XL sheet were employed to draw the graphs and IC$_{50}$ values. The standard error mean (SEM) were calculated at 95% confidence intervals.

Results

Proximate composition

In the proximate analysis, the powdered sample of *R. hastatus* exhibited high percentage of carbohydrate as compared to the protein, fats, fibers and minerals. The proximate analysis has been summarized in Table 1. Table 1 represents the percent of protein along with various parameters in the powdered sample of *R. hastatus*. Each of the analysis was performed in triplicates and the percentage of each ingredient is approximately the same in the results. The percent proteins were detected as $13.4 \pm 0.7\%$ (133.9 mg/g), which demonstrate that *R. hastatus* is rich in proteins. Similarly, in Table 1 the percent ash content is summarized. The ash content shows adequate amount of minerals in the plant sample, i.e., 54.5 mg/g. In the same way, the percent fats analysis was also performed, which is summarized in Table 1. The percentage of fats is comparatively less from the other components, i.e., 25.6 mg/g. The powdered sample was also assessed for the percent moisture content, which is summarized in Table 1. Though the powdered sample was dried for 2 weeks prior to Loss on drying procedure, but still the amount of moisture trapped in the tissues of plant was considerably sufficient, i.e., $22.9 \pm 1.4\%$. Moreover, the percent fiber and carbohydrates are summarized in Table 1, which represent a wholesome amount of carbohydrates in the sample of *R. hastatus*. As far as the percent fiber is concerned, it also goes parallel with the percent proteins, i.e., 133.9 mg/g. The proximate analysis shows that the carbohydrate was in high quantity, i.e., more than 40% followed by the moisture content, proteins, fibers, ashes and then fats.

GC (FID-MS) analysis

The GC (FID-MS) analysis of *R. hastatus* revealed the identification of 120 compounds. The literature review of the identified compounds was performed, in which several compounds previously reported to possess anti-diabetic potential were sorted out. The bioactive compounds sorted out were butyl phthalate, phytol, ethylthreonine, dihydrobenzofuran, indoline, guanidine, nerolidol, myristic acid, palmitic acid, caryophyllene, anozol. The anti-diabetic literature of these compounds is summarized in the discussion section.

| S. no. | Sample     | Percent (Mean ± SD) mg/g | Percent (Mean ± SD) mg/g |
|--------|------------|-------------------------|-------------------------|
| 1.     | Protein    | $13.4 \pm 0.7$          | 133.9                   |
| 2.     | Carbohydrates | $43.2 \pm 1.8$          | 432.4                   |
| 3.     | Fats       | $2.6 \pm 0.1$           | 25.6                    |
| 4.     | Fiber      | $12.4 \pm 0.9$          | 124.4                   |
| 5.     | Moisture   | $22.9 \pm 1.4$          | –                       |
| 6.     | Ash        | $5.5 \pm 0.6$           | 54.5                    |
| 7.     | Saponins   | $6.5 \pm 0.2$           | 65.5                    |
| 8.     | Flavonoids | $8.5 \pm 0.3$           | 84.5                    |
| 9.     | Alkaloids  | $4.9 \pm 0.2$           | 49.5                    |
Saponins, flavonoids and alkaloids

The total saponins, flavonoids and alkaloids have been summarized in Table 2. The data is represented in the terms of percentage and mg/g of the powdered sample. The table shows that the flavonoids are comparatively in greater amount, i.e., 84.3912 (8.4391%), 81.8865 (8.1886%) and 87.3657 (8.7365%) mg/g in test 1, 2 and 3, respectively. Similarly, the saponins were 6.57321%, 6.31794% and 6.75507% in test 1, 2, and 3, respectively. As far as the alkaloids are concerned, this plant possess normal amount of them, i.e., 4.7973%, 5.1443% and 4.9001% in test 1, 2 and 3, respectively. The percent of secondary metabolites present in *R. hastatus* is highest for flavonoids following saponins and alkaloids.

Fluorescence analysis

The fluorescence characteristics of powdered sample of *R. hastatus* were studied under ordinary visible light and ultraviolet light. The powdered sample was treated with various reagents in on a slide on bench top and studied for the color under UV and visible lamps. The powdered sample upon treatment with any reagent gave grayish brown color in visible light and brown color under UV lamp. The powdered sample treated with FeCl₃ exhibited grayish black color in visible light and yellowish gray color under UV lamp. Similarly, the plant sample was treated with variety of chemical reagents, i.e., HCl, HNO₃, K₂Cr₂O₇, H₂SO₄, Br₂, H₂O₂, CCl₄, CH₃OH, CH₃COOH, xylene, NH₃ and I₂. All the reagents displayed different colors in different environments. All the results obtained have been summarized in Table 3.

### Table 2. Fluorescence analysis of powdered sample of *Rumex hastatus* under visible and UV lights.

| S. no. | Treatments          | Visible light    | Ultraviolet light |
|--------|---------------------|------------------|-------------------|
| 1      | Only Powder         | Grayish brown    | Brown             |
| 2      | Powder + FeCl₃      | Grayish black    | Yellowish gray    |
| 3      | Powder + Conc. HCl  | Yellowish red     | Yellowish gray    |
| 4      | Powder + 10% HNO₃   | Pinkish yellow   | Greenish yellow   |
| 5      | Powder + 10% K₂Cr₂O₇| Yellowish brown  | Greenish brown    |
| 6      | Powder + 1 M NaOH   | Brownish black   | Grayish brown     |
| 7      | Powder + AgNO₃      | Pinkish red      | Bluish green      |
| 8      | Powder + Conc. HNO₃| Blackish brown   | Grayish brown     |
| 9      | Powder + Conc. H₂SO₄| Brown            | Grayish brown     |
| 10     | Powder + Br₂ water  | Light brown      | Yellowish brown   |
| 11     | Powder + 5% H₂O₂    | Blackish brown   | Greenish brown    |
| 12     | Powder + CCl₄       | Brownish black   | Greenish black    |
| 13     | Powder + CH₃OH      | Blackish brown   | Yellowish brown   |
| 14     | Powder + CH₃COOH    | Pinkish brown    | Blackish green    |
| 15     | Powder + Xylene     | Grayish brown    | Reddish green     |
| 16     | Powder + NH₃        | Grayish brown    | Yellowish brown   |
| 17     | Powder + I₂         | Deep black       | Grayish black     |

### Table 3. α-Glucosidase inhibitory potential of various samples of *Rumex hastatus*.

| Samples  | Conc. 31.25 µg/mL | Conc. 62.5 µg/mL | Conc. 125 µg/mL | Conc. 250 µg/mL | Conc. 500 µg/mL | Conc. 1,000 µg/mL | IC₅₀ µg/mL |
|----------|-------------------|------------------|-----------------|-----------------|----------------|------------------|-----------|
| Rh.Cr    | 32.33 ± 0.49***   | 41.33 ± 0.33***  | 51.00 ± 1.15*** | 52.43 ± 0.97*** | 62.17 ± 1.40*** | 71.34 ± 1.30***  | 108.83    |
| Rh.Hex   | 29.67 ± 0.89***   | 35.61 ± 1.70***  | 38.00 ± 0.58*** | 44.90 ± 0.52*** | 47.96 ± 1.01*** | 51.33 ± 1.20***  | 978.35    |
| Rh.Chf   | 49.61 ± 1.70***   | 55.17 ± 1.40***  | 61.90 ± 0.52*** | 64.00 ± 1.15*** | 71.43 ± 0.97*** | 77.17 ± 1.40***  | 42.09     |
| Rh.EtAc  | 39.30 ± 0.43***   | 46.33 ± 1.20***  | 51.73 ± 0.78*** | 57.87 ± 0.26*** | 64.13 ± 0.20*** | 69.50 ± 0.58***  | 111.84    |
| Rh.Aq    | 28.33 ± 0.68***   | 36.61 ± 1.70***  | 41.40 ± 0.33*** | 46.34 ± 1.30*** | 49.86 ± 1.04*** | 56.20 ± 1.11***  | 534.61    |
| Rh.Sp    | 42.53 ± 1.07***   | 43.86 ± 1.39***  | 58.66 ± 1.20*** | 61.00 ± 1.73*** | 65.16 ± 1.58*** | 70.66 ± 2.02***  | 104.96    |
| Rh.Fl    | 39.17 ± 1.33***   | 46.73 ± 0.78***  | 53.83 ± 1.20*** | 63.90 ± 0.52*** | 67.96 ± 1.01*** | 73.87 ± 0.26***  | 88.73     |
| P.cont   | 57.90 ± 0.52      | 65.96 ± 1.01     | 73.87 ± 0.26    | 79.56 ± 1.27    | 86.23 ± 0.39    | 81.09 ± 0.26     | 13.92     |

Key: Rh.Cr: Crude methanolic extract, Rh.Hex: n-hexane fraction, Rh.Chf: chloroform fraction, Rh.EtAc: ethyl acetate fraction, Rh.Aq: aqueous fraction, Rh.Sp: saponins, Rh.Fl: Flavonoids, P.cont: Acarbose
**Sensory evaluation**

The results of sensory evaluations have been summarized in Figure 1. The highest value was shown by the color, i.e., 6.5 followed by the 5.8 that of flavor and the least value has been shown by texture, i.e., 5.3. The overall sensory evaluation reveals the mean value of 5.74 ± 0.211.

**α-Glucosidase inhibitory effect**

The α-glucosidase inhibition assay revealed marked activity of various samples of *R. hastatus* (Table 4). Among the test samples Rh.Chf exhibited highest activity exhibiting 49.61 ± 1.70, 55.17 ± 1.40, 61.90 ± 0.52, 64.00 ± 1.15, 71.43 ± 0.90 and 77.17 ± 1.40 at the concentrations of 31.25, 62.5, 125, 250, 500 and 1,000 µg/mL, respectively with IC\(_{50}\) value of 42.09 µg/mL. Similarly, flavonoids and saponins also demonstrated significant enzyme inhibition with IC\(_{50}\) values of 88.74 and 104.96 µg/mL, respectively. The rest of sample also demonstrated enzyme inhibition potential up to considerable extent. The overall activity was recorded as dose dependent response.

**Discussions**

The GC (FID-MS) analysis of methanolic extract of *R. hastatus* revealed the presence of 120 compounds summarized in Table 4 and the chromatogram in Figure 2. The literature survey of these compounds showed the presence of several reported anti-diabetic compounds given in Figure 3. The bioactive compounds sorted out were butyl phthalate, phytol, ethylthreonine, dihydrobenzofuran, indoline, guanidine, nerolidol, myristic acid, palmitic acid, caryophyllene, anozol. Butyl phthalate is one of the constituent identified in the sample of *R. hastatus*, and one of the closely similar structure, i.e., butyl iso-butyl phthalate has previously been published with significant α-glucosidase inhibition potential.\(^\text{49}\) *R. hastatus* also reveals the presence of medicinally important compound, i.e., phytol, which possess antidiabetic effect due activation of nuclear receptors and heterodimerization with PPAR\(\gamma\).\(^\text{50}\) This plant also possess guanidine derivatives that are potentially antidiabetic compounds.\(^\text{51}\) Insulinotropic polypeptides contains threonine and thylthreonine is also an identified constituent in the GC (FID-MS) analysis of *R. hastatus*.\(^\text{52}\) Benzoic acid derivatives have been reported with antidiabetic potential and benzamides are getting fame with good results.\(^\text{53}\) Dihydrobenzofuran is also one of the constituent of *R. hastatus* that possess antidiabetic properties.\(^\text{54}\) In the same way, indoline derivatives are also effective antidiabetic compounds, which have been identified in the sample of *R. hastatus*.\(^\text{55}\) The *Momordica charantia* (bitter gourd) has been demonstrated with significant antidiabetic activity and it has been reported with high percentage of Nerolidol.\(^\text{56}\) The GC (FID-MS) analysis of *R. hastatus* also reveals the presence of fatty acids, i.e., Myristic acid and palmitic acid and GPR-40 “fatty acid receptors” have been identified on the B-cells of pancreas that

![Figure 1. Sensory evaluations of Rumex hastatus on hedonic scale.](image-url)
| S.No | Compound | RT | Common name | Formula | Hits (DB) |
|------|----------|----|-------------|---------|-----------|
| 1    | GUANIDINE (CARBONATE) | 6.011 | Guanidine | CH5N3 | 10 |
| 2    | (2S*,3R*)-2-tert-Butyl-3-ethyloxtane | 6.1 | NF | C8H16O | 1 |
| 3    | Benzene, 1-methyl-4-(1-methylthyl) | 6.28 | p-Cymol | C10H14 | 10 |
| 4    | Thiophene, 2-methoxy-5-methyl- | 6.708 | NF | C6H8OS | 8 |
| 5    | 3-Methyl-2-cyclohexen-1-one | 6.946 | Seudenone | C7H10O | 10 |
| 6    | (E)-1-Ethoxyhex-1-ene | 7.154 | NF | C8H16O | 2 |
| 7    | 8-Hydroxy-2-octanone | 7.291 | NF | C8H16O2 | 10 |
| 8    | r-1-Fuorot-2-iodo-1-methylcyclohexane | 7.554 | NF | C7H12I | 9 |
| 9    | Benzene, (2-methyl-1-propenyl) | 7.639 | NF | C10H12 | 10 |
| 10   | 1,2,4,4-Tetramethylcyclopentene | 7.936 | NF | C9H16 | 10 |
| 11   | 1-methyl-3-hepten-2-one | 8.412 | NF | C8H14O | 4 |
| 12   | 5,6-Dimethylundecane | 8.486 | NF | C13H28 | 8 |
| 13   | (25,3R)-2-ethylthreonic hydrate | 8.859 | 4-Azidohept-1-ene | C7H13N3 | 2 |
| 14   | 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one | 9.038 | NF | C6H8O4 | 10 |
| 15   | Cpd 18: 4-Azidohept-1-ene | 9.236 | 4-Azidohept-1-ene | C7H13N3 | 2 |
| 16   | 1,2,3-Trimethylcyclopentene | 9.408 | NF | C8H14 | 10 |
| 17   | omega.-Isonitrosoacetophenone | 9.522 | Oximinoacetophenone | C8H7NO2 | 10 |
| 18   | 3-Methylbenzamide | 9.763 | m-Toluamide | C8H9NO | 10 |
| 19   | 2-(2-Butoxyethoxy)ethanol | 9.791 | BuCb | C8H18O3 | 10 |
| 20   | iso-Butylamine | 10.028 | Valamine | C4H11N | 10 |
| 21   | Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)- | 10.187 | alpha-Thujene | C10H16 | 10 |
| 22   | Dihydrobenzofuran | 10.579 | Dihydrocoumarone | C8H8O | 10 |
| 23   | 5-Hexyne-2,5-diol, 2,5-dimethyl | 10.695 | NF | C8H14O2 | 10 |
| 24   | 2-Furancarboxaldehyde, 5-(hydroxymethyl) | 11.315 | NF | C6H6O3 | 10 |
| 25   | Geranyl acetate, 2,3-epoxy- | 11.568 | NF | C12H20O3 | 10 |
| 26   | Nonanoic acid | 11.732 | Pelargic acid | C9H18O2 | 10 |
| 27   | R-(+)-METHYL-3-ISOPROPYL-6-OXOHEPTANOATE | 11.798 | NF | C11H20O3 | 6 |
| 28   | Cyclohexene, 1-acetyl-2-(1-hydroxyethyl)- | 11.883 | NF | C10H16O2 | 10 |
| 29   | 3-Buten-2-one, 4-(2,6,6-trimethyl-2-cyclohexen-1-yl) | 11.943 | NF | C13H20O3 | 10 |
| 30   | 2,3,5-Trimethylanizole | 12.153 | NF | C10H14O | 10 |
| 31   | Phenol, 5-methyl-2-(1-methylethyl) | 12.378 | Thymol | C10H14O | 10 |
| 32   | 2,4-Pentadien-1-ol, 3-propyl-, (2Z)- | 12.476 | NF | C8H14O | 10 |
| 33   | 2-Methoxy-4-vinylphenol | 12.655 | p-Vinylguaiacol | C9H10O2 | 10 |
| 34   | trans-3-methyl-4-hexenal | 12.861 | NF | C7H12O | 10 |
| 35   | Tricyclo[4.2.1(4,7).0(3,8)]nona-5-en-2-one | 12.973 | NF | C9H10O | 10 |
| 36   | 3-Methyl-4-methylamino-1,2,4-triazole-5-thiol | 13.05 NF | C4H8N4S | 2 |
| 37   | 3-Oxatricyclo[3.2.1.0(2,4)]octane, (1.alpha.,2.beta.,4.beta.,5.alpha.)- | 13.289 | NF | C7H10O | 10 |
| 38   | Phenol, 2,6-dimethoxy- | 13.446 | Syringol | C9H18O3 | 10 |
| 39   | 5-Allyl-2-Methoxyphenol | 13.582 | Chavibetol | C10H12O2 | 10 |
| 40   | 1,5,5-Trimethyl-6-[3-acetoxybutyl]-3,6-epidioxycyclohexene | 13.738 | NF | C15H20O2 | 10 |
| 41   | 4-(4-Methylphenyl)pentanal | 13.916 | NF | C12H16O | 10 |
| 42   | n-Octoic acid | 14.106 | OCTANOIC ACID | C8H16O2 | 10 |
| 43   | 2,6 - di - methoxy - 4 - vinyl - phenol | 14.187 | NF | C8H14O2 | 10 |
| 44   | 4-(4-Methylphenyl)pentanal | 14.416 | NF | C12H16O | 10 |
| 45   | 1,2-Benzenedicarboxylic acid, diethyl ester | 14.602 | NF | C8H12O | 10 |
| 46   | 1,5,5-Trimethyl-6-[3-acetoxybutyl]-3,6-epidioxycyclohexene | 14.893 | NF | C15H20O2 | 10 |
| 47   | 7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl | 15.081 | NF | C13H22O3 | 10 |
| 48   | 4-(4-Methylphenyl)pentanal | 15.278 | NF | C12H16O | 10 |
| 49   | Benzene, 1-(bromomethyl)-4-hydroxy- | 15.689 | NF | C10H12O2 | 10 |
| 50   | Hydroxy-terpenyl acetate | 15.916 | NF | C12H20O3 | 10 |
| 51   | 1,2-Benzenedicarboxylic acid, diethyl ester, exo- | 16.106 | NF | C12H18O2 | 9 |
| 52   | 4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3-en-2-one | 16.238 | NF | C13H18O2 | 10 |
| 53   | 4-(4-Methylphenyl)pentanal | 16.251 | NF | C10H14O2 | 10 |
| 54   | 4-(4-Methylphenyl)pentanal | 16.567 | NF | C13H20O2 | 10 |
| 55   | 1,5-Trimethyl-6-[3-acetoxybutyl]-3,6-epidioxycyclohexene | 16.674 | NF | C15H24O2 | 10 |
| 56   | 7-Oxacyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl | 16.962 | NF | C13H22O3 | 10 |
| S.No | Compound | RT (s) | Common name | Formula | Hits (DB) |
|------|----------|-------|-------------|---------|-----------|
| 63   | 2-(1-Cyclohexen-1-Yl)Cyclohexyl Acetate | 18.862 | NF | C14H22O2 | 10 |
| 64   | o-Toluic acid, 3-chloroprop-2-enyl ester | 18.919 | NF | C11H11ClO2 | 4 |
| 65   | 5-endo-(Phenylsulfonyl)-5-exo-methylbicyclo[2.2.2]oct-2-ene | 18.963 | NF | C15H18O2S | 2 |
| 66   | 3,5,7-trimethyl-2E,4E,6E,8E-undecatetraene | 19.11 | NF | C14H24 | 10 |
| 67   | Bisabolol oxide A | 19.203 | Bisabolol oxide A | C15H26O2 | 8 |
| 68   | N-(2-methylpropyl)indoline | 19.283 | NF | C12H17N | 2 |
| 69   | 2-Hexanol, 3,3,5-trimethyl-2-(3-methylphenyl)- | 19.348 | NF | C16H26O | 10 |
| 70   | 1-Phenylcyclohexylamine | 19.532 | NF | C12H17N | 10 |
| 71   | Tridecanedial | 19.632 | Tridecanedial | C13H24O2 | 10 |
| 72   | endo-(2R/S,4R/S,4'S)-2,4-Diethoxy-6-(carbonyl-4'-tert-butyloxadiazol-2'-one) | 19.755 | NF | C17H27NO6 | 10 |
| 73   | Nerolidoloxide | 19.833 | Nerolidoloxide | C15H26O3 | 10 |
| 74   | Bisabolol oxide A | 19.203 | Bisabolol oxide A | C15H26O2 | 8 |
| 75   | 2-(1-Cyclohexen-1-Yl)Cyclohexyl Acetate | 18.862 | NF | C14H22O2 | 10 |
| 76   | Acetyl bromide | 20.472 | Acetyl bromide | C2H3BrO | 10 |
| 77   | 2-Cyclopentene, 4-(hydroxymethyl)-1,1,2,3-tetramethyl- | 20.298 | NF | C10H18O | 10 |
| 78   | (2S*,6R*)-6-Allyl-2-hydroxy-2-vinyl-1-cyclohexanone | 20.979 | NF | C11H17O2 | 1 |
| 79   | (Z)-4-Methoxy-2-(N-methylanilino)penta-2,4-dienenitrile | 21.132 | NF | C13H14N2O | 1 |
| 80   | 2-Methoxy-5-(acetoxymethyl)phenol | 21.391 | NF | C10H12O4 | 4 |
| 81   | Tetradecanoic acid | 21.925 | Myristic acid | C14H28O2 | 10 |
| 82   | 3-Cyclohexene-1-acetaldehyde, α,4-dimethyl- | 22.008 | NF | C10H16O | 10 |
| 83   | 3,7-Cyclodecadien-1-one, 3,7-dimethyl-10-(1-methylethylidene)-, (E,E)- | 22.337 | NF | C15H22O | 10 |
| 84   | 4,4-Dimethyladamantan-2-ol | 23.363 | NF | C12H20O | 10 |
| 85   | 7,11-Hexadecadien-1-ol | 23.555 | NF | C16H30O | 10 |
| 86   | cis-9-Hexadecenoic acid | 27.3 | NF | C16H30O2 | 6 |
| 87   | Hexadecanoic acid, ethyl ester | 28.44 | NF | C16H32O2 | 10 |
| 88   | 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- | 31.04 | NF | C20H40O | 10 |
| 89   | 1,2-Benzenedicarboxylic acid, dibutyl ester | 31.552 | NF | C12H20O | 10 |
| 90   | 3-Eicosyne | 31.947 | NF | C20H36O2 | 10 |
| 91   | cis-11-Hexadecenoic acid | 32.057 | NF | C16H30O2 | 6 |
| 92   | 4,6-di-tert-Butyl-m cresol | 32.995 | NF | C13H17FN2O2 | 1 |
| 93   | N,N,N',N'-Tetramethyl-1,2-di-p-tolyl-ethane-1,2-diamine | 38.001 | NF | C24H38O4 | 10 |
| 94   | trans-Caryophyllene | 39.5 | NF | C15H24O2 | 10 |
| 95   | 2-Methylbut-2-enol, 3-hydroxy-3-isopropyl-6,8a-dimethyl-8-oxo-1,2,3,3a... | 34.324 | NF | C20H30O4 | 10 |
| 96   | 1,2-benzenedicarboxylic acid, bis(2-ethylhexyl) ester | 38.001 | NF | C19H30O4 | 10 |
| 97   | 4-H-1-Benzopyran-4-one, 5-hydroxy-7-methoxy-2-(4-methoxyphenyl)- | 43.277 | NF | C17H14O5 | 10 |
| 98   | Hexadecanoic acid, ethyl ester | 43.698 | NF | C16H30O2 | 10 |
| 99   | 1-methylene-3-propylcyclobutane | 46.393 | NF | C9H17NO | 10 |
| 100  | Hexadecanoic acid, 2-methylpropyl ester | 47.013 | NF | C10H20O2 | 10 |
| 101  | 9,12,15-Octadecatrienonic acid, methyl ester | 47.914 | NF | C19H30O2 | 10 |
| 102  | Linoleic acid ethyl ester | 51.971 | NF | C18H32O2 | 10 |
| 103  | 9-Octadecenonic acid (Z) | 52.971 | NF | C18H32O2 | 10 |
| 104  | cis-8-methyl-exo-tricyclo[5.2.1.0(2.6)]decane | 53.971 | NF | C18H32O2 | 10 |
| 105  | Undecanoic acid, ethyl ester | 56.971 | NF | C12H26O2 | 10 |
| 106  | Propanamide, N-(2-fluorophenyl)-3-(4-morpholyl)- | 58.971 | NF | C13H17FN2O2 | 1 |
| 107  | trans-Caryophyllene | 59.971 | NF | C15H24O2 | 10 |
| 108  | 2-methylbut-2-enolic acid, 3-hydroxy-3-isopropyl-6,8a-dimethyl-8-oxo-1,2,3,3a... | 60.971 | NF | C20H30O4 | 10 |
| 109  | 1,2-benzenedicarboxylic acid, bis(2-ethylhexyl) ester | 61.971 | NF | C20H40O4 | 10 |
| 110  | (E)-3,3'-Dimethoxy-4,4'-dihydroxystilbene | 62.971 | NF | C16H32O2 | 10 |
| 111  | n-Hexadecane | 63.971 | NF | C16H32O2 | 10 |
| 112  | Xanthanin | 64.971 | NF | C18H20O5 | 4 |
| 113  | 13-Docosanamide | 65.971 | NF | C22H43N2O | 10 |
| 114  | Octadecamethyl-cyclononasiloxane | 66.971 | NF | C18H40O5Si9 | 10 |
| 115  | n-Tetracosane | 67.971 | NF | C24H50O2 | 10 |
| 116  | 4-H-1-Benzopyran-4-one, 5-hydroxy-7-methoxy-2-(4-methoxyphenyl)- | 68.971 | NF | C19H30O4 | 10 |
| 117  | Hexadecamethyheptasiloxane | 69.971 | NF | C20H40O4 | 10 |
| 118  | 4-H-1-Benzopyran-4-one, 5-hydroxy-6,7-dimethoxy-2-(4-methoxyphenyl)- | 70.971 | NF | C21H36O6 | 10 |
| 119  | (-)-α-Selinene | 71.971 | NF | C15H24O2 | 10 |
| 120  | N-Butyl-N-(2-(9H-9-carbazolyl)propyl)amine | 72.971 | NF | C19H24N2 | 10 |
are responsible for insulin secretion.\textsuperscript{57} Caryophyllene has also been reported with notable antidiabetic activity.\textsuperscript{58} Benzoic acid derivatives have also been reported with significant hypoglycemic effect and the GC (FID-MS) of \textit{R. hastatus} also contains Anozol, which is benzoic acid ester. The MS spectra of bioactive compounds of \textit{R. hastatus} have been given in Figure 4.

To evaluate the nutritional value of certain green vegetables, the sensory evaluation of that specific food should be performed on priority basis. The sensory evaluation can give a specific value from hedonic scale, which represents the mean acceptance and nutritional esthetics of certain vegetables. Going to the results of hedonic scale evaluation, the mean value calculated for \textit{R. hastatus} was 5.74 ± 0.211 out of 7.00, which represents that this plant possesses an acceptable place in the group of green vegetables. Similarly, the proximate value of certain species can decide their use as food based on their nutritional value. Proximate analysis is performed for dietary fiber, carbohydrates, fats, protein, moisture content and ash content. Each of the parameter has its one vital function and nutritional benefits for human beings and other animals. Dietary fiber can absorb huge
amount of water and make the stool soft to get easily out of alimentary canal, so in this way dietary fiber can relieve hemorrhoids and constipation. Fiber has also been reported to alleviate obesity, diabetes and cancer.59-62 As far as the total fats are concerned, this plant contains 2.5% of fats and can be considered as balanced when taken in lieu of milk that contain 3.5–5% fats sufficient for daily requirements as excess cause atherosclerosis other complications.63-65 In the same way, the deficiency of protein intake can cause various pathological disorders, including lung diseases, cardiac disorders, neurological disorders and cancer.66-68 The carbohydrates, being the major portion of plant and the most abundant biomolecule in the universe help in the energy production and some percent of our body mass. It is obvious from the results that R. hastatus is a good source of carbohydrates, i.e., > 40% which meet the daily body requirement. In the same way, though the moisture content is high, i.e., above 20% and high moisture content degrade the bioactive compounds by increasing the microbial growth but still this plant can be protected from microbial growth by various other factors like viscosity, micronutrients, etc.69 Nonetheless, these secondary metabolites have also been reported to possess

Figure 4. (a–h) Mass spectra of bioactive compounds of Rumex hastatus.
antimicrobial, antioxidant and other beneficial effects.\textsuperscript{[36,73–76]} Moreover, the fluorescence analysis of the samples of \textit{R. hastatus} revealed various colors under different wavelengths. The fluorescence analysis is a significant system of identification of powdered drugs with their particular references.\textsuperscript{[77]}

\(\alpha\)-Glucosidase enzyme is responsible for the breakdown of large molecules of carbohydrate to glucose units. To target this enzyme may alleviate the blood glucose level in diabetic patients by decreasing the absorption of glucose from the intestine. Plethora of plants have been reported to possess multiple compounds responsible for \(\alpha\)-glucosidase inhibition, which may be used to alleviate the symptoms of diabetes mellitus.\textsuperscript{[39]} As discussed earlier, the \textit{R. hastatus} has been used by multiple communities for variety of ailments and various species of this genus has been reported to possess anti-diabetic potential.\textsuperscript{[18,25]} The current investigational study reveals that \textit{R. hastatus} contains variety of compounds responsible for \textit{in vitro} inhibition of \(\alpha\)-glucosidase.

**Conclusion**

It may be inferred from the current investigational studies that \textit{Rumex hastatus} is a good source of basic nutritional components along with secondary metabolites. It can meet the basic needs of human body for energy production, growth and other vital functions. Furthermore, this plant possesses marked \textit{in vitro} anti-diabetic potential, so it may be a possible remedy for the management of diabetes mellitus. \textit{R. hastatus} can also be used as vegetable when conventional vegetables are scarce, unavailable or expensive.

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