Evaluation of *Rumex hastatus* D. Don for cytotoxic potential against HeLa and NIH/3T3 cell lines: chemical characterization of chloroform fraction and identification of bioactive compounds

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**Abstract**

**Background:** The importance of *Rumex* genus and the renowned ethnopharmacological and biological potentials of *Rumex hastatus* is evident from the previous reports. Recently the *R. hastatus* has been evaluated for anticancer potential against HepG2, MCF7 or LNCaP cell lines with considerable cytotoxicity. We also reported the anti-tumor and anti-angiogenic potentials of *R. hastatus*. The current study has been arranged to evaluate cytotoxic potential of this plant against HeLa and NIH/3T3 cell lines and sort out the most active fraction of *R. hastatus* along with the identification of bioactive compounds responsible for cytotoxicity.

**Methods:** The cytotoxic potential of methanolic extract and sub-fractions of *R. hastatus* was performed following (3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide) MTT calorimetric assay. Four concentrations (500, 250, 125 and 62.5 μg/ml) of each sample were used against both cell lines. Two cell lines i.e. HeLa and NIH/3T3 were used in the assay. Furthermore, chemical characterization of chloroform fraction was performed by GC-MS analysis.

**Results:** The current investigational study demonstrates that all the solvent fractions of *R. hastatus* were active against HeLa and NIH/3T3 cell lines. Among all the fractions, chloroform fraction was dominant in activity against both cell lines. The observed IC₅₀ values of chloroform fraction were 151.52 and 53.37 μg/ml against HeLa and NIH/3T3 respectively. The GC-MS analysis of chloroform fraction revealed the identification of 78 compounds with the identification of bioactive ones like ar-tumerone, phytol, dihydrojasmon, sitostenone etc.

**Conclusion:** It can be concluded from our results that *Rumex hastatus* D. Don possess strong cytotoxic potential. Moreover, the observed IC₅₀ values and GC-MS analysis of chloroform fraction reveal that most of the bioactive compounds are in chloroform fraction. It can be further deduce that the chloroform fraction is a suitable target for the isolation of compounds having potential role in cancer therapy.

**Keywords:** *Rumex hastatus*, Cytotoxicity, Anticancer, HeLa, NIH/3T3, GC-MS

**Abbreviations:** eV, Electron volt; FBS, Fetal bovine serum; FID, Flame ionization detector; GC-MS, Gas chromatography-mass spectrometry; HeLa, Human cervical carcinoma cell line or Henrietta Lacks cell line; HepG2, Human liver cancer cell line/Hepatoblastoma G2 cell line; IC₅₀, Median inhibitory concentration; LNCaP, Lymph node carcinoma of the prostate; MTT, 3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide; MCF7, Breast cancer cell line/Michigan (Continued on next page)
Background
The leading research teams around the world are in continuous struggle to explore novel aspects to facilitate life. The facilitation of life also encompasses decreased morbidity and mortality [1]. One of the leading causes of mortality is cancer worldwide which is considered as the most challenging disease. Several factors have been reported which cause cancer and hyper proliferative conditions [2]. The free radicals induced lesions have been considered as one of the leading causes of cancer [3]. Attention of the advanced clinical investigators has been focused on the therapeutic measures of this disease. Various therapeutic strategies are followed for the treatment of cancer and chemotherapy has been considered as the most acceptable and positive prognostic therapeutic approach [4]. The drugs from natural sources being bio-degradable are preferred over the synthetic ones due to their comparative safe and efficacious nature [5]. Several natural anticancer drugs are available in the market like etoposide, docetaxel, irinotecan, paclitaxel, topotecan, vincristine and vinblastine [6]. Various derivatives of natural anticancer drugs are also being synthesized and exploited against cancer [7]. The exploration of anticancer agent is not confined to the laboratory rather their availability is also evidenced in plants, marine animals, bacteria, algae, fungi, reptiles etc [8, 9]. The most feasible and economic source of anticancer agents is plants. Numerous anticancer compounds have been isolated from plants and various investigators have reported plethora of plants’ secondary metabolites with strong anticancer potentials [10]. Several families of plants have been reported to possess anticancer compounds. One of the plants’ families i.e., Polygonaceae is also famous for anticancer activities [11]. Rumex is one of the most important genera of this family and several species of this genus have been reported to possess strong anticancer potentials [12]. Several antitumor compounds have also been isolated from different species of this genus, for example, Rumex hynemo-sepalus has been reported with the isolation of antitumor compounds, i.e. leucodelphinidin and leucopelargonidin [13]. Several species of Rumex have been employed ethnomedicinally in the treatment of inflammation, swelling, hyper proliferative skin diseases [14].

Rumex hastatus is one of the most important species which has been used traditionally for the treatment of various ailments like rheumatism, tonsillitis, piles etc [15–17]. Previously, the R. hastatus has been evaluated for anticancer potential against HepG2, MCF7 or LNCaP cell lines with considerable cytotoxicity [18]. Previously, R. hastatus has been evaluated for anticholinesterase, antioxidant, anti-tumor, anti-angiogenic, phytotoxic and antibacterial potentials [19–22]. Based on the ethnomedicinal uses and literature review of R. hastatus, the current study was designed to explore cytotoxic potential of this plant against cell lines and to find out the bioactive phytoconstituents responsible for anticancer activity using GC-MS analysis.

Methods
Plant collection, extraction and fractionation
The aerial parts of mature plant of R. hastatus were collected from the surrounding area of University of Malakand, Pakistan. The plant’s name was confirmed by Dr. Ali Hazrat, Plant Taxonomist, Department of Botany, Shaheed Benazir Bhutto University, Sheringal Dir (U), KPK, Pakistan, and deposited with voucher specimen No. 1015SA. The plant’s material was shade dried, powdered and subjected to maceration process. Afterwards, it was filtered and the filtrate was evaporated under reduced pressure using rotary evaporator at 40 °C [23, 24]. Similarly, the crude methanolic extract (Rh.Cr) was obtained weighing 400 g (5.7 %). The suspension of Rh.Cr weighing 300 g was subjected to fractionation process with the order of increasing polarity. In this way, the fractions obtained were 19 (6.3 %), 21 (7 %), 29 (9.6 %) and 120 (40 %) g of n-hexane (Rh.Hex), chloroform (Rh.Chf), ethyl acetate (Rh.EtAc) and aqueous fraction (Rh.Aq) respectively [25, 26].

Gas Chromatography (GC) analysis
Samples were subjected to GC analysis using an Agilent USB-393752 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with HHP-5MS 5 % phenethylsilsiloxane capillary column (30 m × 0.25 mm × 0.25 μm film thickness; Restek, Bellefonte, PA) equipped with an FID detector. The initial temperature of the oven was retain at 70 °C for 1 min, followed by increase at the rate of 6 °C/min to 180 °C for 5 min and finally at the rate of 5 °C/min to 280 °C for 20 min. The temperature of injector and detector were set at 220 and 290 °C, correspondingly. Helium was used as carrier gas at a flow rate of 1 ml/min, and diluted samples (1/1000 in n-pentane, v/v) of 1.0 μl were injected manually in the splitless mode.
Gas Chromatography–Mass Spectrometry (GC/MS) analysis
GC/MS analysis of samples were processed using an Agilent 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 same experimental conditions as described for GC.

Identification of components
Compounds were recognized by comparison of their retention times with those of authentic compounds in the literature under the same set of conditions. Further identification were done through the spectral data obtained from the Wiley and NIST libraries and further confirmed by comparisons of the fragmentation pattern of the mass spectra with data published in the literature [27, 28].

MTT assay on HeLa and NIH/3T3 cell lines
Cytotoxic activity of various samples of R. hastatus was assayed in 96-well flat-bottomed micro plates following the standard MTT (3-[4, 5-dimethylthiazole-2-y]-2, 5-diphenyl-tetrazolium bromide) colorimetric assay [29]. Briefly, HeLa cells (Cervical Cancer) and Mouse embryonic fibroblast NIH/3T3 cell lines were cultured in Minimum Essential Medium Eagle. The media was supplemented with 5 % of fetal bovine serum (FBS), 100 μg/ml of streptomycin and 100 IU/ml of penicillin in 75 cm² flasks and incubated in 5 % CO₂ incubator at 37 °C. Growing cells were harvested exponentially and counted with haemocytometer followed by dilution with a particular medium. Cell culture was prepared having the concentration of 6 × 10⁴ cells/ml and transferred (100 μl/well) into 96-well plates. After overnight incubation, medium was discarded and 200 μl of fresh medium was added with various concentrations of plant samples (1–30 μM). After 48 h, 200 μl MTT (0.5 mg/ml) was added to each well and incubated additionally for 4 h. Afterward, 100 μL of DMSO was added to each well. The extent of MTT reduction to formazan within cells was figured out by measuring the absorbance at 570 nm, employing a micro plate reader (Spectra Max plus, Molecular Devices, CA, USA). The samples causing 50 % growth inhibition for both cell lines were recorded as IC₅₀. The percent inhibition was calculated by the formula given below;

\[
\% \text{ Inhibition} = \frac{\text{Mean OD of test sample} - \text{Mean OD of negative control}}{\text{Mean OD of positive control} - \text{Mean OD of negative control}} \times 100
\]

The results i.e., Percent inhibition were processed via Soft- Max Pro software (Molecular Device, USA).

Statistical analysis
All the tests were performed in triplicate and values were expressed as means ± S.E.M. Multiple group comparison was performed by Two way ANOVA followed by Bonferroni post test in which the P < 0.05 were considered significant.

Results
MTT assays
The MTT assay was carried out against two types of cell lines, i.e., HeLa and NIH/3T3. The crude methanolic extract and sub-fractions of R. hastatus were assay against both cell lines. All the samples were found active against both cell lines with chloroform fraction more dominant as shown in Table 1. In HeLa cell line cytotoxicity assay, the chloroform fraction revealed significant cytotoxic potential. The observed cytotoxic potential against HeLa cell line were 81.50 ± 0.86, 69.00 ± 2.80, 43.66 ± 0.89 and 34.22 ± 0.23 % at concentrations of 500, 250, 125 and 62.5 μg/ml respectively with IC₅₀ value of 151.52 μg/ml. Similarly, the second highest activity has been demonstrated by ethyl acetate fraction i.e., 79.66 ± 0.89, 66.32 ± 1.30, 40.93 ± 0.49 and 29.83 ± 1.36 % cytotoxic activity at concentrations of 500, 250, 125 and 62.5 μg/ml against HeLa cell line with IC₅₀ value of 166.50 μg/ml. The methanolic extract and aqueous fraction demonstrated moderate cytotoxic potentials with IC₅₀ values of 347.33 and 369.68 μg/ml respectively. Among all the samples of R. hastatus, the least activity was shown by that of n-hexane fraction with IC₅₀ of 572.61 μg/ml.

In NIH/3T3 cell line assay, again the chloroform fraction was found dominant exhibiting 82.13 ± 0.88, 70.66 ± 0.49, 64.02 ± 1.11 and 51.43 ± 0.61 % cytotoxic potential at concentrations of 500, 250, 125 and 62.5 μg/ml with IC₅₀ value of 53.37 μg/ml. Similarly, the ethyl acetate fraction revealed the second highest activity against NIH/3T3 cell line i.e., 72.76 ± 0.78, 59.00 ± 0.57, 46.86 ± 0.85 and 31.43 ± 0.81 % at concentrations of 500, 250, 125 and 62.5 μg/ml with IC₅₀ value of 158.73 μg/ml. The IC₅₀ calculated for the rest of the samples were 174.52, 237.62 and 439.26 μg/ml for methanolic extract, aqueous and n-hexane fractions respectively. The cytotoxic potential of all the test samples of R. hastatus against NIH/3T3 cell line has been summarized in Table 1. The standard drug doxorubicin exhibited IC₅₀ value <0.1 μg/ml against both cell lines.

GC-MS analysis
Based on the high potency in both cell lines assays, the chloroform fraction was subjected to GC-MS analysis. A total of 78 phytoconstituents were identified by the GC-MS analysis. The identified compounds contain important bioactive compounds responsible for the cytotoxic potential of the plant. The parameters of
some compounds found in GC-MS analysis have been summarized in the Table 2. It is evident that area wise the highest percentage has been exhibited by linoleic acid ethyl ester with retention time 31.979 (96.29 %) followed by hexadecanoic acid, ethyl ester with retention time 28.475 (94.9 %). A summary of all identified compounds in the chloroform fraction has been shown in Table 3.

The GC-MS chromatogram of the chloroform fraction is shown in Fig. 1 in which some of the important peaks

| Parameters of various components in Chloroform fraction of Rumex hastatus |   |   |   |   |   |   |
|------------------|----|----|----|----|----|----|
| RT (min)         | Height | Height % | Area | Area % | Area Sum % | Base Peak m/z | Width |
| 26.577           | 536469 | 7.54 | 1916591 | 8.14 | 2.26 | 222 | 0.144 |
| 28.475           | 6E + 06 | 87.45 | 22348531 | 94.9 | 26.38 | 88 | 0.204 |
| 31.979           | 7E + 06 | 91.91 | 22675632 | 96.29 | 26.77 | 67.1 | 0.141 |
| 32.106           | 7E + 06 | 100 | 23550533 | 100 | 27.8 | 55.1 | 0.127 |
| 32.173           | 333815 | 4.69 | 496177 | 2.11 | 0.59 | 55.1 | 0.054 |
| 32.525           | 900308 | 12.66 | 2370371 | 10.07 | 2.8 | 88 | 0.107 |
| 34.939           | 467634 | 6.58 | 1286192 | 5.46 | 1.52 | 254 | 0.1 |
| 35.766           | 331299 | 4.66 | 836122 | 3.55 | 0.99 | 88 | 0.097 |
| 37.977           | 340828 | 4.79 | 773168 | 3.28 | 0.91 | 149 | 0.09 |
| 43.667           | 851097 | 11.97 | 2994991 | 12.72 | 3.54 | 43.2 | 0.134 |

Data is represented as mean ± S.E.M; n = 3. ***: P < 0.001
Key: Rh.Cr Crude methanolic extract, Rh.Hex n-hexane fraction, Rh.Chf chloroform fraction, Rh.EtAc ethyl acetate fraction, Rh.Aq aqueous fraction
| S. No | Compound Label | RT   | Common Name               | Formula   | Hits (DB) |
|-------|---------------|------|---------------------------|-----------|-----------|
| 1.    | Diethyl 2,2-Dihydroxy Sulfide | 5.757 | Tedegyl                   | C4H10O2S  | 3         |
| 2.    | Benzenemethanol | 6.438 | Benzyl alcohol            | C7H8O     | 10        |
| 3.    | 2-Pyrrolidinone, 1-methyl | 6.567 | M-Pyro                   | C5H9NO    | 10        |
| 4.    | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | 8.793 | NF                       | C6H8O4    | 10        |
| 5.    | Benzoic acid, ammonium salt | 9.343 | Ammonium benzoate         | C7H6O2    | 10        |
| 6.    | 2-Methoxy-4-vinylphenol | 12.609 | p-Vinylguaicol            | C9H10O2   | 10        |
| 7.    | Trimethylsilyl cyanide | 15.284 | Trimethyl silyl nitrile   | C4H9NSi   | 10        |
| 8.    | Bis(2-hydroxyethyl)lauramide | 17.708 | lauramide                | C16H33NO3 | 10        |
| 9.    | Dodecanic acid, ethyl ester | 18.281 | Ethyl dodecanoate        | C14H28O2  | 10        |
| 10.   | 2-Cyclopenten-1-one, 3-methyl-2-pentyl | 18.547 | Dihydrojasmone           | C11H18O   | 10        |
| 11.   | Ethylalpha-d-glucopyranoside | 19.004 | glucopyranoside         | C8H16O6   | 10        |
| 12.   | Silane, [(1,1-dimethyl-2-propenyl)oxy] dimethyl- | 19.332 | NF                       | C7H16OS   | 10        |
| 13.   | 4-(1S,5-Dimethyl-1,4-Hexadienyl)-1-Methyl-1-Cyclohexene | 19.582 | NF                       | C15H24    | 10        |
| 14.   | Ar-tumerone | 19.755 | Ar-tumerone              | C15H20O   | 10        |
| 15.   | 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol | 21.382 | NF                       | C10H12O3  | 10        |
| 16.   | Tetradecanoic acid | 21.798 | Myristic acid           | C14H28O2  | 10        |
| 17.   | (-)-Loliolide or Loliolide | 22.21 | Calendin                  | C11H16O3  | 10        |
| 18.   | Tetradecanoic acid, ethyl ester | 22.642 | Ethyl myristate         | C16H32O2  | 10        |
| 19.   | 2-Cyclohexen-1-one, 4-hydroxy-3,5,6-trimethyl-4-(3-oxo-1-buteryl) | 22.779 | NF                       | C13H18O3  | 10        |
| 20.   | p-Hydroxycinnamic acid, ethyl ester | 23.832 | p-Hydroxycinnamic acid, ethyl ester | C11H20O3 | 10        |
| 21.   | 7,11,15-Trimethyl-3-Methylene-1-Hexadecene | 24.028 | Neophytadiene          | C20H38    | 10        |
| 22.   | 2-Pentadecanone, 6,10,14-trimethyl | 24.223 | Hexahydrofarnesyl acetone | C18H36O  | 10        |
| 23.   | Pentadecanoic acid, ethyl ester | 25.763 | ethyl pentadecanoate     | C17H34O   | 10        |
| 24.   | Ethyl (2E)-3-(4-hydroxy-3-methoxyphenyl)-2-propenoate | 26.577 | NF                       | C12H14O4  | 6         |
| 25.   | Hexadecanoic acid | 27.756 | Palmitic acid           | C16H32O2  | 10        |
| 26.   | Ethyl 9-Hexadecanoate | 27.899 | NF                       | C18H34O2  | 10        |
| 27.   | 1,9-Tetradecadiene | 28.273 | NF                       | C14H26    | 10        |
| 28.   | Hexadecanoic acid, ethyl ester | 28.475 | Ethyl palmitate        | C18H36O2  | 10        |
| 29.   | (E)-3-(4-Biphenyl)-2-propen-1-ol | 28.518 | NF                       | C15H14O   | 8         |
| 30.   | Peniopholide | 29.798 | Peniopholide            | C15H24O3  | 10        |
| 31.   | Heptadecanoic acid, ethyl ester | 30.025 | Ethyl n-heptadecanoate | C19H38O2  | 10        |
| 32.   | Propyl hexadecanoate | 30.527 | Propyl palmitate        | C19H38O2  | 10        |
| 33.   | Heptadecanoic acid, ethyl ester | 30.607 | Ethyl n-heptadecanoate | C19H38O2  | 10        |
| 34.   | 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-cis-9,cis-12-Octadecadienonic acid | 31.016 | Phytol                 | C20H40O   | 10        |
| 35.   | 3,3'-4,4'-Methylenedithiooctadecane | 31.507 | NF                       | C18H32O2  | 10        |
| 36.   | E-11,13-Tetradecadien-1-ol | 31.616 | NF                       | C14H26O   | 10        |
| 37.   | Linoic acid ethyl ester | 31.979 | Mandenol                | C20H36O2  | 10        |
| 38.   | Ethyl 9-Octadecanoate | 32.104 | Ethyl 9-Octadecanoate  | C20H38O2  | 10        |
| 39.   | exo-4-Methylbicyclo[3.2.1]octan-3-ene | 32.121 | NF                       | C9H14     | 10        |
| 40.   | 16-Methyloxacyclohexadeca-3,5-dien-2-one | 33.111 | NF                       | C16H26O2  | 10        |
| 41.   | 3.beta.-Hydroxydihydroconfertifolin | 33.956 | NF                       | C15H24O3  | 10        |
| 42.   | Ethyl 9-Hexadecanoate | 34.021 | NF                       | C18H34O2  | 10        |
| 43.   | Cis-8-methyl-exo-tricyclo[5.2.1.0(2,6)]decan-1-ol | 34.647 | NF                       | C11H18    | 10        |
| 44.   | 9,10-Anthracenedione, 1,8-dihydroxy-3-methyl | 34.942 | C.I. Natural Yellow 23   | C15H10O4  | 10        |
are clearly visible. Some important bioactive compounds which having a positive role in cytotoxicity are sorted in Fig. 2. Moreover, the integration patterns of some important compounds as elucidated by GC-MS are shown in Fig. 3.

**Table 3** List of compounds in chloroform fraction of *Rumex hastatus* (Continued)

| No. | Compound                                                                 | RI   | Retention time | Concentration |
|-----|--------------------------------------------------------------------------|------|----------------|---------------|
| 45  | 4,8,12-Trimethyltridecan-4-olide                                          | 35.181 | NF            | C16H30O2      | 10  |
| 46  | 5-icosyne                                                                | 35.305 | S-Eicosyne     | C20H38        | 10  |
| 47  | Ethyl 9-Hexadecenoate                                                    | 35.382 | NF            | C18H34O2      | 10  |
| 48  | Heptadecanoic acid, ethyl ester                                          | 35.768 | NF            | C19H38O2      | 10  |
| 49  | 13-Tetradecenal                                                          | 35.985 | NF            | C14H26O       | 10  |
| 50  | 5-Dodecylcyclopropane                                                   | 36.078 | S-Dodecane     | C12H22        | 10  |
| 51  | N-Vanillylnonanoamide                                                    | 37.013 | Nonivamide     | C17H27NO3     | 10  |
| 52  | 1,2-Benzenedecarboxylic acid, bis (2 ethylhexyl) ester                   | 37.978 | DNOP          | C24H38O4      | 10  |
| 53  | N(4-Hydroxy-3-Methoxybenzyl)-8-Methyl-6-Enamide                          | 38.186 | NF            | C18H27NO3     | 10  |
| 54  | delta.13-cis-Docosenoic acid                                            | 38.242 | Erucic acid    | C22H42O2      | 10  |
| 55  | N-(4-Hydroxy-3-Methoxybenzyl)-8-Methyl-Nonanamide                        | 38.489 | NF            | C18H29NO3     | 10  |
| 56  | Docosanoic acid, ethyl ester                                            | 38.566 | Ethyl docosanoate | C24H48O2     | 10  |
| 57  | 9,10-Anthracenedione, 1,8-dihydroxy-3-methoxy-6-methyl                   | 39.322 | Physcion      | C16H12O5      | 10  |
| 58  | Methyl palustrate isomer                                                 | 39.554 | Methyl palustrate | C21H32O      | 1  |
| 59  | 1-Bromo-4,8,12-trimethyl(3(E),7(E)-11-tridecatriene                      | 40.642 | NF            | C16H27Br      | 5   |
| 60  | Oleic acid amide                                                        | 40.909 | Oleamide      | C18H35NO      | 10  |
| 61  | Heptadecanoic acid, ethyl ester                                          | 41.07  | NF            | C19H38O2      | 10  |
| 62  | 1,1-Di(1,1-dimethylethyl)cyclopropane                                    | 41.672 | NF            | C11H22       | 3   |
| 63  | Arachic alcohol                                                         | 41.685 | n-Eicosanol   | C20H42O       | 10  |
| 64  | Aristol-9-en-8-one                                                       | 42.368 | Aristolone    | C15H22O      | 10  |
| 65  | 2-Bromotetradecane                                                      | 42.397 | NF            | C14H29Br      | 10  |
| 66  | Stigmasta-5,22-dien-3-ol, acetate, (3.beta.,22Z)-                        | 42.529 | NF            | C31H50O2     | 10  |
| 67  | Stigmast-5-en-3-ol, (3.beta.,245)- (CAS)                                 | 42.968 | Clionasterol | C29H50O      | 10  |
| 68  | 7-methylpentol                                                          | 43.226 | NF            | C27H46O2      | 2   |
| 69  | Stigmast-5-en-3-ol, acetate, (3.beta.)-                                  | 43.666 | β-Sitosterol acetate | C31H52O2 | 10  |
| 70  | alpha.-Tocopherol                                                       | 44.466 | Vitamin E     | C29H50O       | 7   |
| 71  | Cholesta-4,6-dien-3-ol, benzoate, (3.beta.)                             | 45.533 | NF            | C34H48O2      | 9   |
| 72  | Alpha.-Biisabolol                                                       | 52.989 | Alpha.-biisabolol | C18H32O      | 10  |
| 73  | Methyl Commate E                                                        | 53.773 | NF            | C31H50O5     | 10   |
| 74  | Stigmast-4-en-3-one                                                      | 55.572 | Sitostenone   | C29H48O      | 10  |
| 75  | 2-Ethylthio-2-ethoxy-3-oxo-N phenylbutanamide                           | 57.414 | NF            | C14H19NO3S    | 9   |
| 76  | 2-(Methoxymethoxy)-5-(phenylmethoxpentanal                               | 58.739 | NF            | C14H20O4      | 1   |
| 77  | 13-Epimanool                                                            | 62.472 | Epimanool-    | C20H34O      | 10  |
| 78  | 1,2-Dicyclohexyl-1,1,2,2-tetrafluoroethane                               | 70.638 | NF            | C14H22F4      | 6   |

**Discussion**

HeLa is a type of immortal cell line obtained from cervical cancer cells and for the very first time this cell line has been taken from late Henrietta Lacks in 1951 and abbreviated for her name [30]. Similarly, the NIH/3T3 cell line was originated from swiss mice in 1962 which consists of immortal fibroblast cell and widely used for experimental purposes [31]. To figure out the cytotoxicity in these cells, the MTT assay is considered as a rapid and authentic procedure to appraise the cell viability and death by calorimetric analysis [29]. Previously, the MTT assay has been reported by numerous researchers to evaluate the cytotoxicity [32, 33]. Recently, *Polygonum hydropiper* has been demonstrated with significant cytotoxicity against NIH/3T3 cell line following MTT assay [34]. As this is evidenced from several
reports that a specific pharmacological potential within plant species is basically conferred due to specific group of compounds [35]. Similarly, a specific group of phytoconstituents is responsible for the cytotoxic potential of certain plants [36]. The GC-MS is a quick and easy way of finding out various components in a crude mixture of plant extract [37]. In our current research, the GC-MS analysis of chloroform fraction of *R. hastatus* showed 78 compounds summarized in Table 2. Several compounds identified by GC-MS in the chloroform fraction are reported to have positive role in cell toxicities. For instance, phytol, dihydrojasmine, ethyl α-β-d-glucopyranoside, anthracenedione, silane, nonivamide, eicosanol, aristolone, ar-tumerone and sitostenone are the compounds with cytotoxic/anticancer potential demonstrated along with their spectra in Figs. 2 and 3.

Phytol present in *R. hastatus* has been reported to induce programmed cell death in human lymphoid leukemia Molt 4B cells [38]. Dihydrojasmine, one of the member of jasmonate family, which has been implied as a new family of anticancer agents [39]. Ethyl-α-β-d-glucopyranoside a derivative of glucopyranoside has been reported time and again to possess strong anticancer potential and it is
evident from the GC-MS analysis that \textit{R. hastatus} contain ethyl \(\alpha\)-d-glucopyranoside, which may confer the possible anticancer potential to this plant. Anthracenedione has also been reported to possess anticancer properties [40]. Silane has been proven as an efficient agent in a nanoparticle based drug delivery system for anticancer compounds. The chloroform fraction of \textit{R. hastatus} also possess noni-vamide, which is skin permeation enhancer and used in various ointments etc [41]. Similarly, eicosanol is a \(C_{20}\) alcohol present in \textit{R. hastatus} and \(C_{20}\) aliphatic alcohols has been employed in the treatment of hyperproliferative skin disordersone [42]. Aristolone and Ar-tumerone are sesquiterpenes, and the derivatives of sesquiterpene have been reported to possess the cytotoxic potential [43]. Likewise, vitamin E a phenolic compound with pronounced free radical scavenging and anticancer potential has also been evidenced from Table 2 [44, 45]. Another compound i.e., a natural steroid named sitostenone has also been analyzed in GC-MS spectra and steroids have also been used since long for the treatment of cancer, so this compound may also be involved in cytotoxicity observed in our current studies [46]. The current investigational study demonstrates
that the chloroform fraction of \textit{R. hastatus} was the most active one against two types of cell lines. The regression and correlation analysis shows that this plant has a parallel cytotoxic potential against both the cell lines as depicted in the Fig. 4 with \( r^2 \) value of 0.881. The current study can also be correlated with the previous cytotoxic activity of \textit{R. hastatus} against brine shrimps in which the chloroform fraction was the most active fraction [22]. Based on the marked potential of this fraction, it has been chemically characterized and based on the literature survey; the active compounds have been sorted out.

**Conclusion**

Based on our current results, we can conclude that \textit{Rumex hastatus} is a potential source of cytotoxic compounds. Moreover, the chloroform fraction is the active one among other solvent fractions of \textit{R. hastatus}. Based on the GC-MS analysis of chloroform fraction, we can conclude that the chloroform fraction of \textit{R. hastatus} is a rich source of bioactive compounds responsible for cytotoxicity.

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**Availability of data and materials**
The data presented in this manuscript belong to the PhD work of Mr. Sajjad Ahmad and has not been deposited in any repository yet. However, the materials are available to the researchers upon request.

**Authors’ contributions**
SA and AZ carried out experimental work, data collection and literature search. FU designed the project and helped in supervision. MA and FU drafted the manuscript for publication. AS make the final version of publication. All the authors have read and approved the final manuscript for publication.

**Competing interests**
The authors declare that they have no competing interests.

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