Chemical composition, antioxidant and anticholinesterase potentials of essential oil of *Rumex hastatus* D. Don collected from the North West of Pakistan

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**Abstract**

**Background:** Ethnomedicinally *Rumex hastatus* D. Don has been used since long for various ailments especially in neurological disorders. The reported data and the importance of *Rumex* genus demonstrate the vital medicinal value of *R. hastatus*.

**Methods:** In the current investigational study, isolation of essential oil and its antioxidant and anticholinesterase assays were performed. The essential oil of *R. hastatus* was analyzed by GC-MS for the first time. The essential oil was evaluated for anticholinesterase and antioxidant assays. The anticholinesterase assay was conducted at various concentrations (62.5 to 1000 μg/ml) against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Similarly, the antioxidant potential was determined using DPPH and ABTS free radicals.

**Results:** The GC-MS analysis of essential oil showed 123 components. The result recorded for the anticholinesterase assays demonstrated a marked potential against AChE and BChE with IC₅₀ values of 32.54 and 97.38 μg/ml respectively which were comparable with the positive control i.e., galanthamine (AChE, IC₅₀ = 4.73 μg/ml and BChE, IC₅₀ = 11.09 μg/ml). The antioxidant assays against DPPH and ABTS free radicals also exhibited significant scavenging potential with IC₅₀ values of 3.71 and 6.29 μg/ml respectively, while for ascorbic acid the IC₅₀ value was <0.1 μg/ml against both free radicals.

**Conclusions:** Based on the current investigational studies, it may be concluded that *R. hastatus* is an effective source of essential oil's components having anticholinesterase and antioxidant potentials, which after subjecting to drug development may lead to novel drug candidates against neurodegenerative disorders.

**Keywords:** Essential oil, Acetylcholinesterase, Butyrylcholinesterase, Antioxidant, GC-MS, Free radicals, *Rumex hastatus*

**Background**

A brief history of medicine demonstrates the use of herbal medicine for the effective treatment of various ailments. Herbal medicine has been used since long in various forms including the decoction, powdered sample, oleoresins, crude extracts, fixed oil, essential oil etc [1]. Various plants have been used in multiple types of food items for preservation and therapeutic effects [2]. In this regards, essential oils have been manifested by several reporters to play a major role. Essential oils have the property to attenuate the effects of free radicals, e.g., reactive oxygen species (ROS) which are derived from metabolism of oxygen and exogenous agents [3]. ROS are responsible for wide variety of diseased conditions including oxidative stress and nervous disorders [4]. Essential oils are well-known for their radicals scavenging properties and amelioration of various cognitive disorders. Among the cognitive disorders, Alzheimer’s disease...
(AD) is the most common in elderly people [5]. One of the best therapeutic approaches for AD is to increase the concentration of the neurotransmitter (Acetylcholine) by inhibiting the enzyme (acetylcholinesterase) responsible for its breakdown. Various drugs originated either from natural or synthetic sources are being used for the management of AD and other nervous disorders [6]. Similarly, it has also been reported that oxidative stress are responsible for wide variety of mental diseases due to neuronal degeneration and other factors. Oxidative stress is mainly developed due to increase in concentration of free radicals within the body. The free radicals have been reported by numerous researchers to possess multiple destructive properties, due to which interest has been focused to scavenge the free radicals somehow and avoid their deteriorating effects [7]. In this context, investigators are trying to explore more and more sources of natural and synthetic bioactive principles [8]. The natural drugs are being preferred over the synthetic due to their negligible harmful and deleterious effects [9]. That's why researchers are trying to explore novel sources of natural medicine [10–18]. Among the natural sources, herbal medicines have been shown promising results due to the presence of numerous secondary metabolites and essential oils. Essential oils isolated from various plants have been reported to possess marked acetylcholinesterase inhibitory and radicals scavenging potential [19–21]. Traditional knowledge also demonstrates the use of essential oils for various nervous system disorders [22].

*R. hastatus* D. Don belongs to the family Polygonaceae. Various members of this family have been reported to be used against paralysis, headache and other nervous system disorders [23–26]. Various solvent samples of *R. hastatus* have recently been reported to possess strong anticholinesterase and antioxidant potentials [26]. To date, the chemical composition of essential oil of *R. hastatus* has not been reported or evaluated for any pharmacological activity. Based on the literature survey and medicinal importance of *R. hastatus*, the current investigational study is arranged to isolate the essential oil, analyze the chemical composition and to evaluate for the anticholinesterase and antioxidant potentials, which may be a possible remedy for oxidative stress and nervous system disorder.

**Methods**

**Plant sample collection**

The aerial parts of *R. hastatus* were collected from the proximity of University of Malakand. The plant was identified by plant taxonomist Ali Hazrat and deposited with voucher number (10155S) in the herbarium of Department of Botany, Shaheed Benazir Bhutto University Sheringal, Dir (U), KPK, Pakistan. Extraction of essential oil of *R. hastatus* was performed by hydrodistillation using clevenger type apparatus [27]. The essential oil obtained was stored at -20 °C until required.

**Chemicals and drugs**

DPPH (Sigma Aldrich CHEMIE GmbH USA, code 101341986), K$_2$S$_2$O$_4$ (Riedel-de Haen Germany), ABTS (Sigma Aldrich USA, code 1001551916), Gallic acid (GmbH USA), Folin Ciocalteu reagent (Merck Co. Germany), AChE (Electric eel type-VI-S, Sigma-Aldrich GmbH USA, code 1001596210), BChE (Equine serum Lyophilized Sigma-Aldrich GmbH USA, code 101292670), Acetylthiocholine iodide (Sigma-Aldrich UK, code 101303874), Butyrylthiocholine Iodide (Sigma-Aldrich Switzerland, code 101334643), DTNB (Sigma-Aldrich Germany, code 101261619), Galanthamine hydrobromide Lycoris Sp. (Sigma-Aldrich France, code G1660), K$_3$HPO$_4$, KH$_2$PO$_4$, KOH. All the chemical used were of analytical grade.

**Gas Chromatography (GC) analysis**

The GC analysis of essential oil was carried out via gas chromatograph Agilent USB-393752 (Agilent Technologies, Palo Alto, CA, USA) with HHP-5MS 5% phenylmethyl siloxane capillary column (30 m × 0.25 mm × 0.25 μm film thickness; Restek, Belleville, PA) connected with FID detector. The oven was set at temperature of 70 °C for one minute and then increased to 180 °C at the rate of 6 °C/min for 5 min and lastly to 280 °C at the rate of 5 °C/min for 20 min. The temperature of injector and detector were maintained at 220 °C and 290 °C correspondingly. The flow rate of carrier gas i.e., Helium was 1 ml/min and the diluted samples (1/1000 in n-pentane, v/v) of 1 μl were manually injected in the split-less mode.

**Gas Chromatography–Mass Spectrometry (GC-MS) analysis**

The GC/MS of the essential oil was performed via USB-393752 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with a HHP-5MS 5% phenylmethyl siloxane capillary column (30 m × 0.25 mm × 0.25 μm film thickness; Restek, Belleville, 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**

The recognition of all the major constituents of oil was performed by comparing their retention times with the authentic compounds in the literature. Identification of compounds was further processed through the spectral data obtained from the Wiley and NIST libraries as well...
as fragmentation patterns’ comparisons of the mass spectra with data reported in literature or with those of mass spectra from literature [28, 29]. Each determination was processed in duplicate.

Anticholinesterase assays

Anticholinesterase (AChE and BChE inhibitions) activity was performed for the essential oil of *R. hastatus* by spectrophotometric analysis following the method of Ellman’s assay [30]. The substrates used were acetylthiocholine iodide and butyrylthiocholine iodide. Briefly, 5 \( \mu \text{L} \) of 0.03 U/mL AChE and 0.01 U/mL BChE were taken in a cuvette and 205 \( \mu \text{L} \) of essential oil having concentration of 62.5–1000 \( \mu \text{g/mL} \) were transferred to them using micropipette. Similarly, 5 \( \mu \text{L} \) of DTNB was also added to this afterwards. The mixtures obtained were kept in water bath for 15 min at the temperature of 30 °C. After incubation, 5 \( \mu \text{L} \) of the Substrates were added to the mixture to optimize the reaction. A double beam spectrophotometer was used to measure the reaction time at 412 nm via a double beam spectrophotometer (Thermo electron corporation USA). Absorption values were obtained for 4 min. Meanwhile, the yellow colored mixtures indicated the formation of 5-thio-2-nitrobenzoate anion as a reaction product of thiocholines and DTNB. White assay was also performed without enzymes and plant samples to check the non-enzymatic hydrolysis of substrate. The mixture which contained all the components excluding essential oil was marked as control. Percent enzyme activity and percent inhibition were recorded as follows.

\[
V = \frac{\Delta \text{Abs}}{\Delta t}
\]

% enzyme activity = \( \frac{V}{V_{\text{max}}} \times 100 \)

% enzyme inhibition = 100–% enzyme activity

(Where V symbolizes the rate of reaction in the presence of inhibitor and \( V_{\text{max}} \) stands for rate of reaction without inhibitor)

DPPH radical scavenging assay

The DPPH radical scavenging potential was evaluated for essential oil of *R. hastatus* following previously described procedure [31]. DPPH solution (0.004 %) was prepared in methanol to get a deep violet colored solution. Similarly, stock solution of essential oil was prepared in ethanol having concentration of 1 mg/mL. The stock solution was serially diluted to get the concentrations of 62.5 to 1000 \( \mu \text{g/mL} \). Afterwards,

| Table 1 Anticholinesterase activity of essential oil of *Rumex hastatus* at various concentrations |
|-----------------------------------------------|
| Samples | Enzymes | Conc. \( \mu \text{g/mL} \) |
|--------|---------|-----------------|
|        |         | 62.5 | 125 | 250 | 500 | 1000 |
| EO     | AChE    | 54.32 ± 1.33 | 61.64 ± 1.60 | 67.26 ± 1.24 | 71.70 ± 1.63 | 74.90 ± 0.52 | 32.54 |
| EO     | BChE    | 46.32 ± 3.50 | 52.73 ± 0.78 | 57.00 ± 2.80 | 66.33 ± 0.49 | 71.32 ± 4.8 | 97.38 |
| Gal    | AChE    | 72.08 ± 1.04 | 78.58 ± 1.12 | 83.70 ± 1.60 | 89.00 ± 1.15 | 96.65 ± 1.34 | 04.73 |
| Gal    | BChE    | 66.87 ± 1.27 | 73.67 ± 0.88 | 79.95 ± 2.01 | 86.62 ± 1.67 | 91.61 ± 0.43 | 11.09 |

Data is expressed as Mean ± SEM; EO and Gal are abbreviated for Essential oil and Galanthamine respectively.

Table 2 Parameters of various components of essential oil of *Rumex hastatus*

| RT (min) | Height | Height (%) | Area | Area (%) | Area Sum % | Base Peak m/z | Width |
|----------|--------|------------|------|----------|------------|---------------|-------|
| 6.447    | 254413 | 18.51      | 620057 | 20.82 | 5.87 | 83 | 0.127 |
| 6.818    | 324110 | 23.59      | 626045 | 21.02 | 5.93 | 57.1 | 0.077 |
| 10.958   | 430958 | 31.36      | 822529 | 27.61 | 7.79 | 55.1 | 0.074 |
| 11.363   | 250143 | 18.2       | 592697 | 19.9  | 5.61 | 59.1 | 0.09  |
| 11.761   | 278058 | 20.23      | 665761 | 22.35 | 6.31 | 59.1 | 0.094 |
| 12.97    | 177060 | 12.88      | 399792 | 13.42 | 3.79 | 43.1 | 0.097 |
| 13.171   | 312841 | 22.77      | 664487 | 22.31 | 6.29 | 55.1 | 0.08  |
| 13.308   | 1E + 06 | 100       | 3E + 06 | 100  | 28.21 | 57.1 | 0.1    |
| 15.063   | 159790 | 11.63      | 336861 | 11.31 | 3.19 | 55.1 | 0.08  |
| 19.213   | 450356 | 32.77      | 782083 | 26.26 | 7.41 | 133.1 | 0.064 |
Table 3 List of components of essential oil of *Rumex hastatus*

| S.No | Compound Label | Common name | RT   | Formula          | Hits (DB) |
|------|----------------|-------------|------|------------------|-----------|
| 1.   | Trans-dideuteroxy-cyclopentene | NF | 5.757 | C5H6D2O2 | 10 |
| 2.   | 1-Nonen-4-ol | NF | 5.884 | C9H18O | 10 |
| 3.   | Ethyl 2-hydroxybutyrate | NF | 6.169 | C6H12O3 | 10 |
| 4.   | (SH)-Furanone, 5-ethyl | NF | 6.445 | C6H8O2 | 10 |
| 5.   | Pentanoic acid, 4-oxo | Levulinic acid | 6.68 | C5H8O3 | 10 |
| 6.   | 2,2-Dimethylpropanoic anhydride | Trimethylacetic anhydride | 6.819 | C10H18O3 | 10 |
| 7.   | Heptanoic acid | Enanthetic acid | 7.117 | C7H14O2 | 10 |
| 8.   | Ethanethioic acid, S-(2-methylpropyl) ester | NF | 7.374 | C6H12OS | 10 |
| 9.   | 4-Octanol, 7-methyl | NF | 7.511 | C9H20O | 10 |
| 10.  | 4-(Tetrahydrofuranyl-2-oxy)-4-methyl-2-pentanone | NF | 7.619 | C10H18O3 | 10 |
| 11.  | Cyclopropane, 1,2-dimethyl-1-pentyl | NF | 7.698 | C10H20 | 10 |
| 12.  | n-Nonanal | Nonanal | 7.852 | C9H18O | 10 |
| 13.  | Cyclooctanone | NF | 8.275 | C8H14O | 10 |
| 14.  | 1,4,4-Trimethylcyclohexa-2-en-1-ol | NF | 8.494 | C9H16O | 10 |
| 15.  | 3-Octanol, 2-methyl | NF | 8.716 | C9H20O | 10 |
| 16.  | 2-Oxatricyclo[3.3.1.1(3,7)]decan-1-methyl- | NF | 9.116 | C10H16O | 10 |
| 17.  | Succinimide, N-methoxy | NF | 9.338 | C5H7NO3 | 10 |
| 18.  | 4-Heptanol, 2-methyl | NF | 9.547 | C8H18O | 10 |
| 19.  | Ethanone, 1-(methylphenyl) | Methylacetophenone | 9.712 | C9H10O | 10 |
| 20.  | Decanal | NF | 10.099 | C10H20O | 10 |
| 21.  | 3-Heptanol, 2,4-dimethyl | NF | 10.328 | C9H20O | 10 |
| 22.  | Cyclooctanone | NF | 10.957 | C8H14O | 10 |
| 23.  | 1-Decyne (CAS) $$Octylacetylene | NF | 11.165 | C10H18 | 10 |
| 24.  | 3-Heptanol, 5-methyl | NF | 11.364 | C8H18O | 10 |
| 25.  | Nonanoic acid | Pelargic acid | 11.456 | C9H18O2 | 10 |
| 26.  | ETHYL AMYL CARBINOL | NF | 11.763 | C8H18O | 10 |
| 27.  | CIS-SABINE HYDRATE | NF | 11.96 | C10H18O | 10 |
| 28.  | 1,8-Bisoxiranylnonane | NF | 12.047 | C13H24O2 | 10 |
| 29.  | 3-Heptanone, 4-methyl | NF | 12.817 | C8H16O | 10 |
| 30.  | Methyl 2-vinylbutanoate | NF | 12.972 | C7H12O2 | 10 |
| 31.  | trans-3-Nonen-2-one | NF | 13.171 | C9H16O | 10 |
| 32.  | Octane, 2,4,6-trimethyl | NF | 13.309 | C11H24 | 10 |
| 33.  | 2H-Pyran-2-one, 6-heptyltetrahydro | Delta-laurolactone | 13.471 | C12H22O2 | 10 |
| 34.  | Decanoic acid | Capric acid | 13.601 | C10H20O2 | 10 |
| 35.  | 3-Octanol | NF | 14.002 | C10H22O | 10 |
| 36.  | Ethyl 3,3-dimethylbutyrate | NF | 14.246 | C8H16O2 | 1 |
| 37.  | 5-Hexenal | NF | 14.547 | C6H10O | 10 |
| 38.  | 2-Pentenoic acid, 4-hydroxy | NF | 14.878 | C5H8O3 | 10 |
| 39.  | Nonanoic acid, 9-oxo-, methyl ester | Azelaadehydic acid | 15.065 | C10H18O3 | 10 |
| 40.  | Thiophene, 2-methoxy | NF | 15.345 | C5H6O5 | 3 |
| 41.  | Octanoic acid, 8-hydroxy | NF | 15.49 | C8H16O3 | 10 |
| 42.  | Oxirane, octyl | NF | 15.604 | C10H20O | 10 |
| 43.  | Butane, 1,1'-oxybis[3-methyl | NF | 15.875 | C10H22O | 5 |
| 44.  | 3-Hydroxy-4-methoxystyrene | NF | 16.153 | C9H10O2 | 7 |
| No. | Component                                                                 | Mol. Formula | MW | Purity |
|-----|---------------------------------------------------------------------------|--------------|-----|--------|
| 45  | Octanoic Acid                                                             | n-Caprylic acid | 16.355 | C8H16O2 | 10 |
| 46  | 3-Hexanol, 3,5-dimethyl                                                   | NF           | 16.55  | C8H18O | 10 |
| 47  | 2-Tridecan-1-ol, (E)                                                      | NF           | 16.643 | C13H26O | 10 |
| 48  | 1-Isopropyl-4,7-dimethyl-1,2-dihydronephthalene                           | Alpha-Calcorene | 16.877 | C15H2O | 10 |
| 49  | 4-(5',5'-dimethyl-2'-methylidene-3',8'-dioxacyclo[5.1.0]oct-4-ylidene)-2-b... | NF           | 17.084 | C13H18O3 | 5 |
| 50  | 9-Methyl-5-octahydrophenanathrene                                        | NF           | 17.192 | C15H2O | 10 |
| 51  | Z-10-Tetradecen-1-ol acetate                                             | NF           | 17.373 | C13H30O2 | 10 |
| 52  | Dodecanamide, N,N-bis(2-hydroxyethyl)                                     | NF           | 17.737 | C16H33NO3 | 10 |
| 53  | 5,8-Dimethyl-1,2,3,4-tetrahydro-1-naphthol                                | NF           | 17.847 | C12H16O | 3 |
| 54  | 3-Hexen-1-ol, benzoate, (Z)                                               | NF           | 17.917 | C13H16O2 | 10 |
| 55  | Nonanoic acid                                                             | Pelargic acid | 18.014 | C9H18O2 | 10 |
| 56  | Nonanedioic acid, monomethyl ester                                        | NF           | 18.153 | C10H18O4 | 10 |
| 57  | (-)-Caryophyllene oxide                                                   | Caryophyllene oxide | 18.311 | C15H24O | 10 |
| 58  | (+-)-Andirolactone                                                        | Andirolactone | 18.513 | C11H14O2 | 10 |
| 59  | Ledol                                                                    | NF           | 18.64  | C15H26O | 10 |
| 60  | (4-5'-dimethyl-6-p-tolylheptanol (diasteroisomer II)                      | NF           | 18.693 | C15H24O | 10 |
| 61  | Propanal, 2,2-dimethyl                                                    | NF           | 18.777 | C5H10O | 1 |
| 62  | 2,6,10-Trimethylundecan-(SE)-2,5,9-trien-4-one                             | NF           | 18.869 | C14H22O | 10 |
| 63  | 7-oxacyclo[4.1.0]heptane, 1-(1,3-dimethyl-1,3-butadienyl)-2,2,6-trimethyl... | NF           | 19.004 | C15H24O | 10 |
| 64  | Octanoic acid, 6,6-dimethoxy-, methyl ester                              | NF           | 19.087 | C11H22O4 | 10 |
| 65  | 2-(p-methylphenyl)-2-nitropropane                                        | NF           | 19.212 | C10H13NO2 | 10 |
| 66  | Azelaic Acid                                                             | Anchoic acid | 19.589 | C10H16O4 | 4 |
| 67  | cis-9-oxacyclo[6.1.0]non-2-ene                                            | NF           | 19.736 | C9H12O | 10 |
| 68  | 1-Buten-3-one, 1-(2-carboxy-4,4-dimethylcyclobutenyl)                      | NF           | 19.864 | C11H14O2 | 10 |
| 69  | Campherenone                                                             | Campherenone | 20.056 | C15H24O | 10 |
| 70  | 11-Hexadecyn-1-ol                                                        | NF           | 20.231 | C16H30O | 10 |
| 71  | Cycloexene, 1-ethyl-2-methyl-                                             | NF           | 20.385 | C13H24 | 10 |
| 72  | 1,3-Doxolane-4,5-dicarboxylic acid, 2,2-dimethyl-, dimethyl ester         | NF           | 20.627 | C9H14O6 | 5 |
| 73  | 10-(1-Methylethyl)tricyclo[6.3.1.0(2,7)]dodeca-2(7),3,5-trien-10-ol       | NF           | 20.768 | C16H20O | 4 |
| 74  | 2-Acetoxy-1,1,10-trimethyl-6,9-epidioxidecaralin                          | NF           | 20.894 | C15H24O4 | 10 |
| 75  | Farnesyl Acetone                                                         | Farnesyl Acetone | 21.18 | C18H30O | 10 |
| 76  | 17-Octadecynoic acid                                                     | NF           | 21.401 | C18H32O2 | 10 |
| 77  | Tetradecanoic acid                                                       | Myristic acid | 21.82 | C14H28O2 | 10 |
| 78  | Drimol                                                                  | Drimenol     | 22.167 | C15H26O | 10 |
| 79  | 2,2,6-Trimethyl-1-(3-methylbuta-1,3-dienyl)-7-oxacyclo[4.1.0]heptan-3-ol | NF           | 22.272 | C14H22O | 10 |
| 80  | 1,3,5-trimethyl-6-methylen-tricyclo[3.2.1.0(2,7)]oct-3-en-8-endo-ol        | NF           | 22.677 | C12H16O | 9 |
| 81  | 1-Methyl-2-acetyl-6-methoxy-3,4-dihynadrophthalene                        | NF           | 22.933 | C14H16O2 | 10 |
| 82  | N-(1-Cyanoethyl)(7,7-dimethyl-2-oxacyclo[2.2.1]hept-1-ylmethanesulfoxamide| NF           | 23.386 | C13H20N2O3S | 10 |
| 83  | 5-(ethylamino)-1,6-dimethyl-2(1H)-quinolinone                            | NF           | 23.511 | C13H16N2O | 10 |
| 84  | (-)-Isolongifolol                                                        | Isolongifolol | 23.926 | C15H26O | 10 |
| 85  | Neophytadiene                                                            | Neophytadiene | 24.02 | C20H38 | 10 |
| 86  | Naphthalene, 1-(1,1-dimethylethyl)-7-methoxy-                             | NF           | 24.123 | C15H18O | 2 |
| 87  | 2-Pentadecanone, 6,10,14-trimethyl                                       | NF           | 24.218 | C18H36O | 10 |
| 88  | 2,5,8-Trimethyltricyclo[5.3.1.1(3,9)]dodecane-2-anti,8-tnti-diol           | NF           | 24.561 | C15H26O2 | 3 |
| 89  | Pentadecanoic acid                                                       | Pentadecyclic acid | 24.74 | C15H30O2 | 10 |
0.1 mL of each concentration was added to the 3 mL of DPPH solution. The mixture obtained was incubated at 23 °C for 30 min in dark. After incubation the absorbance of each sample were recorded at the wavelength of 517 nm using double beam spectrophotometer. Ascorbic acid was used as positive control. All the samples were processed in triplicates and the percent activity was recorded as mean ± SEM. The percent radical scavenging potential was figured out using the following formula:

\[
\% \text{ scavenging} = \left(\frac{\text{absorption of control} - \text{absorption of test sample}}{\text{absorption of control}}\right) \times 100
\]

**Table 3** List of components of essential oil of *Rumex hastatus* (Continued)

| No. | Component | Identification | Formula | Molecular Weight | Retention Time (min) |
|-----|-----------|----------------|---------|------------------|---------------------|
| 90  | 9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate | NF | C32H52O2 | 450.376 | 25.047 |
| 91  | 8-Keto-10-dehydrobrominated-beta-snyderol | NF | C15H22O | 262.174 | 25.298 |
| 92  | Widdrol | Widdrol | C15H26O | 278.360 | 25.848 |
| 93  | 2,4,7,9-Tetramethyl-5-decyn-4,7-diol | NF | C15H22O | 250.246 | 26.036 |
| 94  | Phenol, 2-methyl-4-(1,1,3,3-tetramethylbutyl) | NF | C15H24O | 258.252 | 26.54 |
| 95  | Benzene, 1,1'-(1,2-diethyl-1,2-ethanediyl)bis[4-methoxy-| NF | C20H26O2 | 266.360 | 26.548 |
| 96  | (1R,3S)-2,2,3-Trimethyl-6-methylidenecyclohexane-1-carbaldehyde | NF | C11H18O | 26.624 | 26.624 |
| 97  | Hexadecanoic acid, methyl ester | Methyl palmitate | C17H34O2 | 267.334 | 27.731 |
| 98  | 1-Hexadecen-3-ol, 3,5,11,15-tetramethyl- | NF | C20H40O | 27.585 | 27.585 |
| 99  | Benzo[e]isobenzofuran-1,4-dione,1,3,4,5,5a,6,7,8,9,9a-decahydro-6,6,9a-trime... | Palmitic acid | C16H32O2 | 27.984 | 27.984 |
| 100 | Hexadecanoic acid | NF | C13H20O | 28.431 | 28.431 |
| 101 | Butane-1,1-dicarbonitrile, 1-cyclohexyl-3-methyl- | NF | C8H14O | 29.064 | 29.064 |
| 102 | 2-Methyl-2-propyl-2,5-dihydrofuran | NF | C8H14O | 29.247 | 29.247 |
| 103 | 5A-Methyl-3,8-dimethylene-2-oxooctodehydroxireno[2',3';6,7]naphtho[1,2-b]fur... | NF | C20H24O2 | 29.621 | 29.621 |
| 104 | 4-(3,7,7-Trimethyl-2-oxacyclo[3.2.0]hept-3-en-1-yl)but-3-ene-2-one | NF | C13H18O2 | 29.89 | 29.89 |
| 105 | Cyclobutanecarboxylic acid, 2-methyloct-5-yn-4-yl ester | NF | C14H26O2 | 30.064 | 30.064 |
| 106 | Cyclooctenone, dimer | NF | C14H28O2 | 30.439 | 30.439 |
| 107 | Undecane, 6-cyclohexyl- | NF | C17H34 | 30.639 | 30.639 |
| 108 | 2,4,5,7-Tetramethyl-2,6-octadiene | NF | C18H36 | 31.291 | 31.291 |
| 109 | Cyclohexane, 1,2,3,4,5,6-hexaethyl | NF | C19H38 | 31.577 | 31.577 |
| 110 | Cyclopentanone, 3-methyl-2-(2-pentenyl)- | NF | C10H18O | 31.791 | 31.791 |
| 111 | 2-Propanon | Acetone | C3H6O | 31.44 | 31.44 |
| 112 | beta-Ionol $$ 3$-Buten-2-ol, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)- | NF | C14H22O | 31.703 | 31.703 |
| 113 | Velleral | Velleral | C15H20O | 32.121 | 32.121 |
| 114 | 2-Hydrazino-2-imidazoline | NF | C14H20O | 32.733 | 32.733 |
| 115 | 2H-cycloprop[2]benzofuran, 4,5,5A,6,6A,6B-hexahydro-4,4,6b-trimethyl-2-(1-m... | NF | C15H22O | 33.658 | 33.658 |
| 116 | Hexadecane | Cetane | C16H34 | 37.132 | 37.132 |
| 117 | Docosane | Docosane | C22H44 | 38.808 | 38.808 |
| 118 | 4,4-6-Trimethyl-7-oxabicyclo[4.1.0]heptan-2-one | NF | C9H14O | 39.247 | 39.247 |
| 119 | 1,2-Benzenedicarboxylic acid, bis(2-ethyl/hexyl ester | NF | C24H38O4 | 39.623 | 39.623 |
| 120 | 4-Allyl-1-ethoxy-3-phenylbenzo[c]-1,2-oxaphosphinine - 1-Oxide | NF | C19H19O3P | 40.4 | 40.4 |
| 121 | Hexadecane | Cetane | C16H34 | 41.915 | 41.915 |
| 122 | Undecane, 3,8-dimethyl- | NF | C13H28 | 44.76 | 44.76 |
| 123 | 4-Methyl-7-ethyldizidine $$ 8$-Methyl-5-ethylindolizidine | NF | C18H36 | 58.237 | 58.237 |

**ABTS radical scavenging assay**

The 2, 2-azinobis [3-ethylbenzthiazoline]-6-sulfonic acid (ABTS) free radicals scavenging assay of the essential oil was evaluated followed standard procedure [11]. ABTS solution 7 mM and potassium persulfate solution 2.45 mM were prepared and mixed thoroughly. The
solution prepared was put in dark overnight for the production of free radicals. After incubation time the absorbance of solution was adjusted at 745 nm to 0.7 by the addition of 50 % methanol. Test samples having volume of 300 μl was taken in a test tube and 3 mL ABTS solution was added to it. The solution was transferred to the cuvette and absorbance values were taken for six minutes using double beam spectrophotometer. Ascorbic acid was used as positive control. All the samples were run in triplicate and percent ABTS radical scavenging potential was figured out using the following formula;

\[
\% \text{ scavenging activity} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100
\]

**Estimation of IC\textsubscript{50} values**

The median inhibitory concentration i.e., IC\textsubscript{50} values of AChE, BChE, DPPH and ABTS were determined by a linear regression analysis of the percent inhibition versus the concentrations of test samples through MS Excel program.

**Statistical data analysis**

All the tests were conducted in triplicate and the values were tabulated as mean ± S.E.M. Significant difference of the percent inhibition of various test samples was analyzed via two way ANOVA following Bonferroni's post test using GraphPad Prism software in which the \( P < 0.05 \) were considered significant.
Results and discussion

In the current investigational study the radical scavenging potential of volatile oil was studied based on spectrophotometric analysis. The sources of free radicals employed were DPPH and ABTS, which have maximum absorbance values at 517 nm and 745 nm respectively. After getting scavenged by antioxidant compounds the colors of DPPH (violet) and ABTS (blue) solution change into yellow. Change in the color results in decrease of absorbance values which is directly proportional to the amount of radical scavenging compounds in the solution [32, 33].

Similarly, the anticholinesterase activity is based on the hydrolysis of acetylthiocholine iodide and butyrylcholine iodide by the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholines, catalyzed by enzymes at a wavelength of 412 nm using spectrophotometer or microplate reader. Acetylthiocholine iodide and butyrylthiocholine iodide work as substrate of the reaction, while the DTNB is utilized for the measurement of cholinesterase activity. The percent inhibition of enzymatic activity is calculated from the rate of change in absorption of the reaction mixture [34].

The available literature on etiology of diseases demonstrate multiple causative agents responsible for specific disease [35]. In the context of Alzheimer’s disease, numerous investigators have reported the role of various causative agents along with various successful approaches [36]. Like all neurodegenerative disorders, the free radicals have a prominent role in the induction and progression of AD [37]. By avoiding or attenuating the causative agents one can hinder the progression of a specific disease. In case of neurodegenerative disorders, the scavenging of free radicals can be a vital target. Various researchers have demonstrated the effective role of natural antioxidants especially the essential oils to combat the free radicals [38]. Similarly, one of the most widely employed treatment strategies for AD i.e., the inhibition of AChE to increase the concentration of neurotransmitter is highly recommended [39]. In this regard, essential oils are being investigated by advanced researchers with better results. Essential oils obtained from various plants possess marked anti-Alzheimer’s potential due to the presence of wide variety of valuable compounds in it [40, 41]. The anticholinesterase potential of essential oil of Rumex hastatus has been summarized in the Table 1, while the Table 2 shows various parameters of the compounds present in the essential oil of this plant. The GC-MS analysis of essential oil of R. hastatus demonstrates a total of 123 components as shown in Table 3. The anticholinesterase activity of essential oil of
R. hastatus might be due to its hydrophobic nature because of the good affinity of hydrophobic active site of AChE [42, 43]. Some of the most common components of essential oils i.e., palmitic acid, myristic acid, palmitoleic acid, caprylic acid, docosane, cetane, velleral, acetone, methyl palmitate, widdrol, isolongifolol, ophytadiene, drijenol and levulinic acid have been found in the essential oil of R. hastatus. Some of these components have been reported previously by other investigators to possess antioxidant and anticholinesterase potentials [44–49]. The percent antioxidant potential of essential oil is illustrated in the Fig. 1. The peaks given in the Table 2 shows various volatile compounds like 5-ethyl-2(5H)-furanone, trimethylacetic anhydride, cyclooctanone, 5-methyl-3-heptanol, methyl 2-vinylbutanoate, 2-(p-methylphenyl)-2-nitropropane, azelaaldehydic acid, 2,4,6-trimethyloctane and trans-3-nonen-2-one with retention times of 6.447, 6.818, 10.958, 11.363, 11.761, 12.97, 13.171, 13.308, 15.063 and 19.213 min respectively. Going to the detail of various components of essential oil of R. hastatus, it is clear that the marked anticholinesterase potential shown by essential oil is observed due to the presence of wide variety of compounds in it. Essential oil demonstrated 74.90, 71.70, 67.26, 61.64, 54.32 % AChE inhibition at 1000, 500, 250, 125, 62.5 μg/ml respectively. Similarly, the BChE inhibition exhibited by essential oil was recorded as 71.32, 66.33, 46.32, 52.73, 57.00 % at 1000, 500, 250, 125, 62.5 μg/ml respectively. The essential oil attain IC50 values of 32.54 and 97.38 μg/ml for AChE and BChE inhibitions respectively. The anticholinesterase potential shown by essential oil goes parallel with the positive control which is also obvious from the Fig. 2 (a & b) with the correlation coefficient of 0.961 and 0.988 for essential oil versus AChE and BChE respectively. Apart from the anticholinesterase potential of essential oil, the antioxidant potential of essential oil of various plants has been reported with discrimination by various investigators [50, 51]. In our current investigational study, the free radicals scavenging assay of essential oil of R. hastatus against DPPH and ABTS was significant and almost comparable with the positive control. From Fig. 1, it is clear that essential oil exhibited marked potential with IC50 of 3.71 and 6.29 μg/ml against DPPH and ABTS respectively, which is also comparable with the previously reported literature. The previously reported data of R. hastatus verifies its anticholinesterase and antioxidant potentials which may be linked to the current investigational studies [26]. Some important components of essential oil and the chromatogram have been given in Figs. 3 and 4 respectively.

**Conclusion**

Essential oil isolated for the first time from the R. hastatus and its chemical composition demonstrates that R. hastatus is a source of valuable volatile components. Based on the anticholinesterase and antioxidant results of essential oil, it can be concluded that R. hastatus plant may be an effective source of compounds which may lead to possible palliative therapy and cure of oxidative stresses and neurodegenerative diseases.

**Competing interests**

All authors declare that they have no competing interests.

**Authors’ contributions**

FU, AS and MRS conceived the idea and did literature survey. SA, MA, IA and AZ conducted practical work. MI and FU analyzed the data. All the authors contributed in drafting of the manuscript.
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