Study on the Anti-Inflammatory Mechanism of Volatile Components of Hebei Aster tataricus Before and After Honey-Fried Based on Gas Chromatography-Mass Spectrometry and Network Pharmacology

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ABSTRACT

Aster tataricus (AT) and honey-fried Aster tataricus (HAT) have a significant effect on relieving cough and reducing sputum, both of which contain many volatile components. Studies have shown that the volatile components of AT and HAT may have an anti-inflammatory effect, but the mechanism is unclear. This study aimed to analyze the daodi herb of Hebei AT and HAT qualitatively and quantitatively using gas chromatography-mass spectrometry and systematically explored the similarities and differences of anti-inflammatory molecular mechanisms of volatile components Hebei AT and HAT by using network pharmacology. These results indicate that there are significant differences in volatile compositions and percentage contents between AT and HAT. Moreover, the anti-inflammatory mechanism of volatile components of Hebei AT and HAT have more prominent similarities and fewer differences. AT and HAT’s similar potential active components such as humulene, γ-muurolene, α-phellandrene, and acetic acid were nine. The similar key gene targets were forty-seven, such as CAT, GAPDH, HMOX1, and CTH. The potential active ingredients peculiar to HAT were furfural, β-elemene, methyleugenol, and unique targets of EIF6 and PKIA. It suggests that HAT had its characteristics in clinical anti-inflammatory. Their active anti-inflammatory components and percentage contents were different, and HAT was higher than that of AT. The anti-inflammatory effect of volatile components of HAT may be better than that of AT. These results provide a theoretical basis for the study of the anti-inflammatory molecular mechanism of AT and HAT.

Keywords: Anti-inflammatory; Aster tataricus; honey-fried; volatile components
INTRODUCTION

*Aster tataricus* (AT) is derived from the dry roots and rhizomes of Compositae plant *Aster tataricus* L.f., which is first recorded in ‘Shen Nong Ben Cao Jing’, has a long history of clinical application in China (Su & Liu 2011). It has the effect of moistening the lung to lower qi, relieving cough and reducing sputum (National Pharmacopoeia Commission 2020). AT is mainly produced in Hebei, Anhui, Henan, Gansu, North and Northeast China. Among them, the annual output of Hebei AT was more than 60% of the whole country, with high yield and good quality. AT plays an important role in medicine for relieving cough and reducing sputum; it is often used in raw thick slices and honey-fried processing (Fang et al. 2012). The processing method of honey-fried *Aster tataricus* (HAT) is to take cooked honey, add an appropriate amount of boiling water to dilute it, add it into AT slices, mix well, moisten it until it is transparent, put it in a frying container, heat it with soft fire, fry it until it is brown, and take it out to cool when it is not sticky (Gong 2016). The method of processing traditional Chinese medicine (TCM) is the characteristics and advantages of TCM, and it is the first batch of intangible cultural heritage in China (Li et al. 2020).

It has been reported that HAT has a better effect than AT in eliminating phlegm, which indicated that the processing method affected AT’s eliminating phlegm effect (Wu et al. 2006). Pharmacodynamic experiments showed that relieving cough and reducing sputum is closely related to inflammation (Li et al. 2021), volatile components of TCM have an anti-inflammatory effect (Zhang & Li 2017), and AT has an anti-inflammatory effect after compatibility of TCM (Li et al. 2009). It is suggested that the volatile components of AT and HAT may have an anti-inflammatory effect. Inflammation is a common clinical symptom associated with the occurrence and development of various diseases (Dutta et al. 2019). Therefore, how to reduce the occurrence of inflammation effectively has become a hot topic in clinical research.

Network pharmacology is a new technology that integrates many subjects. It is consistent with the holistic and systematic research concept of TCM and the characteristics of multi-component and multi-level coordination. It can comprehensively explore the correlation between active components and active targets and diseases (Guo et al. 2021). The combination of network pharmacology and gas chromatography-mass spectrometry (GC-MS) are helpful to clarify the potential complex relationship between multi-component and multi-target when the volatile components of the dao-di herb Hebei AT before and after honey-fried play an anti-inflammatory role. Finally, the screening results of network pharmacology were verified by molecular docking. The research results will provide a basis for clinical rational drug use of AT.

MATERIALS AND METHODS

EXPERIMENTAL MATERIALS AND INSTRUMENTS

Samples of Hebei AT and HAT were purchased from the medicinal materials market and identified as AT and HAT derived from the dry roots and rhizomes of Compositae plant *Aster tataricus* L.f. by professor Xiangpei Wang of Guizhou Minzu University. GC-MS analysis was carried out using an HP6890/5975C GC-MS spectrometer (Agilent USA) equipped with an HP-5MS (60 m × 0.25 mm × 0.25 µm) stone elastic capillary column.

THE SOLID-PHASE MICROEXTRACTION PROCEDURE

The accurately weighed AT and HAT (1.000g) were placed into 25 mL of solid-phase microextraction sampling bottles, respectively, and then, inserted into a manual injector with a 2 cm-50/30 um DVB/CAR/PDMS Stableflex fibre head. The temperature of the headspace vial was kept at 60 °C, exposed to the sample headspace for 60 min. The extraction head was removed from sample vials and immediately inserted onto the gas chromatography injection port (temperature of 250 °C), the sample thermal desorption and then directly injected into gas chromatography.

GC-MS ANALYSIS

The analysis of gas chromatography used an HP-5MS (60 m × 0.25 mm × 0.25 µm) stone elastic capillary column. High purity helium (purity 99.99 %) and the pre-column pressure was 15.85 psi, which were used as carrier gas with a flow rate of 1.0 mLmin⁻¹, samples were injected in splitless mode. The gas chromatography initial temperature was programmed to hold at 40 °C for 2 min, temperature increased to 180 °C at 3.5 °Cmin⁻¹, then to increase to 260 °C at 10 °Cmin⁻¹, running 50 min. The injector temperature was set at 250 °C. The solvent delay was 3 min. The mass spectrometer was operated in the electron impact (EI) mode by using ionization energy at 70 eV with an ionization source temperature.
of 230 °C and a quadrupole temperature set of 150 °C. The emission current was 34.6 uA, the multiplier voltage was 1847 V, the interface temperature was 280 °C, and the mass range was 29-500 amu.

DATA ANALYSIS
The mass spectrometer computer data system retrieved each peak in the total ion flow map. The volatile chemical components were determined by the standard mass spectra of NIST17 and Wiley 275. Then the relative concentration of each chemical composition was determined by the peak area normalization method.

MOLECULAR STRUCTURE AND TARGET PROTEIN PREDICTION
The molecular structures of the volatile compounds were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), and the 3D structures were downloaded in .sdf format. The action chemical constituents were screened by the TCMSP database with ADME parameters (oral bioavailability≥30% and drug-likeness≥0.18), and the related anti-inflammatory active ingredients reported in the literature were used as the standard (Feng et al. 2015; Fink et al. 2015; Labib et al. 2017; Li et al. 2015; Lin 2011; Lin et al. 2020; Ninomiya et al. 2013; Queiroz et al. 2014; Saeed et al. 2012; Sakhaee et al. 2020; Sousa et al. 2020; Yang et al. 2019). Inflammation related target proteins were retrieved from a comprehensive database of human genes and gene phenotypes (OMIM, http://www.omim.org/) and GeneCards database (https://www.genecards.org/). Finally, the screened targets were converted into UniProt ID format with the UniProt database (https://www.uniprot.org/).

NETWORK CONSTRUCTION AND TOPOLOGIC PROFILE ANALYSIS
AT and HAT’s active components, targets, and inflammatory targets were connected to form a ‘component-target-disease’ network. The above network was visually analyzed using Cytoscape 3.7.2 software, the degree, betweenness centrality and closeness centrality of each node were obtained. Proteins showing values greater than the median value for the above three topological parameters at all nodes were regarded as the anti-inflammatory response’s key target (Huang et al. 2020).

GO AND KEGG ENRICHMENT ANALYSIS
Protein interaction analysis of the selected targets was carried out by using the STRING database (http://string-db.org). Then, the database DAVID (https://david.ncifcrf.gov/) was carried out to analyze the KEGG pathway and the biological process of GO (Gene Ontology).

MOLECULAR DOCKING
Molecular docking was carried out using the DockThor (Santos et al. 2020), which applied a rigid protein and a flexible ligand to docking and illustrates how a ligand acts on a complex molecular network. Finally, it was visualized in Ligplot.

RESULTS AND DISCUSSION
IDENTIFICATION AND ANALYSIS OF VOLATILE COMPONENTS
A total of sixty-three volatile components were identified in AT sample by GC-MS analysis (Figure 1), the main components were 1-pentadecene (7.226%), β-pipene (3.091%), longicyclene (1.618%), acetic acid (1.591%), and α-terpinolene (1.418%). A total of fifty-nine volatile components were identified in the HAT sample by GC-MS analysis (Figure 2), the main components were 1-pentadecene (2.310%), acetic acid (2.060%), β-pipene (1.805%), furfural (1.661%), and bicyclogermacrene (1.631%). The main volatile components were alkenes, aldehydes, alcohols, and esters. There were forty-seven similar volatile components in AT and HAT, and the main components were 1-pentadecene, β-pipene, and acetic acid. AT had sixteen unique volatile components, the main components were ledene; p-(1-propenyl)-toluene; 1, 8, 11, 14-heptadecatetraene; cedrol. HAT had 12 unique volatile components, and the main component was furfural, bicyclogermacrene, 5-methyl-2-furancarboxaldehyde. The results showed that there were significant differences in volatile compositions and percentage contents between AT and HAT. The chemical constituents of AT changed during honey-fried. After processing, HAT reduced sixteen volatile components and increased twelve volatile components. The corresponding volatile components are listed in Figure 3 and Tables 1 and 2.
TABLE 1. Percentages of volatile components extracted from AT

| No. | Retention time (min) | Compound name               | Formula | Molecular weight | The percentage (%) |
|-----|----------------------|-----------------------------|---------|------------------|--------------------|
| 1   | 4.437                | Ethanol                     | C\textsubscript{2}H\textsubscript{5}O     | 46                | 0.033              |
| 2   | 5.726                | Acetic acid                 | C\textsubscript{2}H\textsubscript{4}O\textsubscript{2} | 60                | 1.591              |
| 3   | 6.484                | 3-Methylbutanal             | C\textsubscript{8}H\textsubscript{12}O   | 86                | 0.006              |
| 4   | 6.673                | 2-Methylbutanal             | C\textsubscript{8}H\textsubscript{12}O   | 86                | 0.005              |
| 5   | 15.917               | α-Thujene                   | C\textsubscript{10}H\textsubscript{16} | 136               | 0.020              |
| 6   | 16.23                | α-Pinene                    | C\textsubscript{10}H\textsubscript{16} | 136               | 1.016              |
| 7   | 16.843               | α-Fenchene                  | C\textsubscript{10}H\textsubscript{16} | 136               | 0.010              |
| 8   | 16.923               | Camphene                    | C\textsubscript{10}H\textsubscript{16} | 136               | 0.074              |
| 9   | 17.713               | Benzaldehyde                | C\textsubscript{8}H\textsubscript{8}O   | 106               | 0.128              |
| 10  | 18.033               | Sabinene                    | C\textsubscript{10}H\textsubscript{16} | 136               | 0.018              |
| 11  | 18.196               | β-pipene                    | C\textsubscript{10}H\textsubscript{16} | 136               | 3.091              |
| 12  | 18.747               | β-Myrcene                   | C\textsubscript{10}H\textsubscript{16} | 136               | 0.394              |
| 13  | 19.422               | α-Phellandrene              | C\textsubscript{10}H\textsubscript{16} | 136               | 0.169              |
| 14  | 19.977               | α-terpinene                 | C\textsubscript{10}H\textsubscript{16} | 136               | 0.099              |
| 15  | 20.333               | p-Cymene                    | C\textsubscript{10}H\textsubscript{14} | 134               | 0.276              |
| 16  | 20.533               | Limonene                    | C\textsubscript{10}H\textsubscript{16} | 136               | 0.655              |
| 17  | 20.554               | β-Phellandrene              | C\textsubscript{10}H\textsubscript{16} | 136               | 0.935              |
| 18  | 21.868               | γ-Terpine                   | C\textsubscript{10}H\textsubscript{16} | 136               | 0.128              |
| 19  | 22.425               | 1-(1H-pyrrol-2-yl)-Ethanone | C\textsubscript{13}H\textsubscript{14}NO | 109               | 0.053              |
| 20  | 22.506               | Acetophenone                | C\textsubscript{8}H\textsubscript{8}O   | 120               | 0.085              |
| 21  | 23.189               | α-terpinolene               | C\textsubscript{10}H\textsubscript{16} | 136               | 1.418              |
| 22  | 23.230               | p-(1-Propenyl)-toluene      | C\textsubscript{10}H\textsubscript{12} | 132               | 0.839              |
| No. | Retention Time | Compound                        | Molecular Formula | Molecular Weight | RI     |
|-----|----------------|---------------------------------|-------------------|------------------|-------|
| 23  | 23.649         | Linalool                        | C10H14O            | 154              | 0.065 |
| 24  | 24.264         | 1,3,8-p-Menthatriene            | C10H14              | 134              | 0.030 |
| 25  | 24.363         | Fenchol                         | C10H14O            | 154              | 0.057 |
| 26  | 26.541         | Pinocarvone                     | C10H14O            | 150              | 0.015 |
| 27  | 26.657         | endo-Borneol                    | C10H14O            | 154              | 0.131 |
| 28  | 27.117         | Terpinen-4-ol                   | C10H14O            | 154              | 0.987 |
| 29  | 27.419         | p-Cymen-8-ol                    | C10H14O            | 150              | 0.140 |
| 30  | 27.665         | α-Terpineol                     | C10H14O            | 154              | 0.610 |
| 31  | 27.818         | Dodecane                        | C12H26              | 170              | 0.081 |
| 32  | 29.825         | Benzaldehyde, 4-[(1-methylethyl)- | C10H14O            | 148              | 0.015 |
| 33  | 30.164         | Bicyclo[2.2.1]heptane-2-carboxylic acid, 3,3-dimethyl-, methyl ester | C11H18O2 | 182 | 0.554 |
| 34  | 31.541         | Bornyl acetate                  | C12H20O2           | 196              | 0.767 |
| 35  | 33.072         | Myrtenyl acetate                | C12H18O2           | 194              | 0.082 |
| 36  | 34.076         | α-Cubebene                      | C12H20             | 204              | 0.027 |
| 37  | 34.257         | α-Longipinene                   | C12H20             | 204              | 1.335 |
| 38  | 34.377         | Neryl acetate                   | C12H18O2           | 196              | 0.363 |
| 39  | 34.989         | Ylangene                        | C12H20             | 204              | 0.183 |
| 40  | 35.098         | longicyclene                    | C12H20             | 204              | 1.618 |
| 41  | 35.22          | (-)-trans-Myrtanyl acatate      | C12H20O2           | 196              | 0.690 |
| 42  | 35.687         | Tetradecane                     | C14H30             | 198              | 0.181 |
| 43  | 36.164         | β-Longipinene                   | C12H20             | 204              | 0.060 |
| 44  | 36.428         | Longifolene                     | C12H20             | 204              | 0.519 |
| 45  | 37.212         | γ-Elemene                       | C12H20             | 204              | 0.863 |
| 46  | 37.584         | Aromandendrene                  | C13H24             | 204              | 0.407 |
| 47  | 37.837         | cis-β-Farnesene                 | C13H24             | 204              | 0.334 |
| 48  | 37.993         | α-Himachalene                   | C13H24             | 204              | 0.232 |
| 49  | 38.109         | Humulene                        | C13H24             | 204              | 0.691 |
| 50  | 38.823         | γ-Muurolene                     | C13H24             | 204              | 0.139 |
| 51  | 39.045         | 1-Pentadecene                   | C15H30             | 210              | 7.226 |
| 52  | 39.58          | Ledene                          | C15H28             | 204              | 1.170 |
| 53  | 39.628         | α-Muurolene                     | C15H28             | 204              | 0.337 |
| 54  | 39.763         | (3S,3aS,8aR)-6,8a-Dimethyl-3- (prop-1-en-2-yl)-1,2,3,3a,4,5,8,8a-octahydroazulene | C15H28 | 204 | 1.272 |
| 55  | 40.447         | δ-Cadinene                      | C15H28             | 204              | 0.252 |
| 56  | 41.218         | α-Calacorene                    | C15H28             | 200              | 0.188 |
| 57  | 41.327         | Germacrene B                    | C15H28             | 204              | 0.078 |
| 58  | 42.458         | Spathulenol                     | C15H20O2           | 220              | 0.425 |
| 59  | 42.657         | (-)-Spathulenol                 | C15H20O2           | 220              | 0.118 |
| 60  | 43.29          | Cedrol                          | C15H20             | 222              | 0.593 |
| 61  | 44.148         | Isopathulenol                   | C15H20O2           | 220              | 0.277 |
| 62  | 44.564         | 1,8,11,14-Heptadecatetraene, (Z,Z,Z)- | C15H20 | 232 | 0.812 |
| 63  | 49.171         | Hexadecanoic acid, methyl ester | C18H36O2           | 270              | 0.039 |
### TABLE 2. Percentages of volatile components extracted from HAT

| No. | Retention time (min) | Compound name                              | Formula | Molecular weight | The percentage (%) |
|-----|----------------------|---------------------------------------------|---------|------------------|--------------------|
| 1   | 4.423                | Ethanol                                     | C₂H₅O   | 46               | 0.093              |
| 2   | 5.715                | Acetic acid                                 | C₂H₄O₂  | 60               | 2.060              |
| 3   | 6.467                | 3-Methylbutanal                             | C₄H₁₀O  | 86               | 0.010              |
| 4   | 6.66                 | 2-Methylbutanal                             | C₄H₁₀O  | 86               | 0.013              |
| 5   | 11.764               | Pyrazine, methyl-                           | C₅H₈N₂  | 94               | 0.025              |
| 6   | 11.967               | Furfural                                    | C₅H₈O₂  | 96               | 1.661              |
| 7   | 13.145               | 2-Furanmethanol                             | C₅H₈O₂  | 98               | 0.107              |
| 8   | 15.424               | Ethanone, 1-(2-furanyl)-                    | C₆H₁₀O₂ | 110              | 0.273              |
| 9   | 15.909               | α-Thujene                                   | C₁₀H₁₆  | 136              | 0.066              |
| 10  | 16.229               | α-Pinene                                    | C₁₀H₁₆  | 136              | 0.280              |
| 11  | 16.921               | Camphene                                    | C₁₀H₁₆  | 136              | 0.025              |
| 12  | 17.609               | Benzaldehyde                                | C₇H₈O   | 106              | 0.085              |
| 13  | 17.685               | 5-methyl-2-Furancarboxaldehyde              | C₆H₁₀O₂ | 110              | 0.534              |
| 14  | 18.02                | Sabinene                                    | C₁₀H₁₆  | 136              | 0.207              |
| 15  | 18.187               | β-Pipene                                    | C₁₀H₁₆  | 136              | 1.805              |
| 16  | 18.749               | β-Myrcene                                   | C₁₀H₁₆  | 136              | 0.246              |
| 17  | 19.421               | α-Phellandrene                              | C₁₀H₁₆  | 136              | 0.131              |
| 18  | 19.98                | α-terpinene                                 | C₁₀H₁₆  | 136              | 0.028              |
| 19  | 20.336               | p-Cymene                                    | C₆H₁₄   | 134              | 0.140              |
| 20  | 20.553               | Limonene                                    | C₁₀H₁₆  | 136              | 0.612              |
| 21  | 21.867               | γ-Terpine                                   | C₁₀H₁₆  | 136              | 0.037              |
| 22  | 22.145               | Ethanone, 1-(1H-pyrrol-2-yl)-               | C₁₀H₁₉NO| 109              | 0.330              |

FIGURE 2. TIC of volatile components extracted from HAT
| No. | Retention Time | Name | Molecular Formula | Molecular Weight | Density |
|-----|----------------|------|-------------------|------------------|---------|
| 23  | 22.4           | Acetophenone | C₆H₅NO       | 120              | 0.185   |
| 24  | 23.189         | α-terpinolene | C₁₀H₁₆O        | 154              | 1.283   |
| 25  | 24.261         | 1,3,8-p-Menthatriene | C₁₅H₂₈O₄     | 244              | 0.091   |
| 26  | 25.745         | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | C₉H₆O₄        | 144              | 0.018   |
| 27  | 27.128         | Terpinen-4-ol | C₁₀H₁₈O        | 154              | 0.098   |
| 28  | 27.426         | p-Cymen-8-ol | C₁₀H₁₄O        | 150              | 0.159   |
| 29  | 27.671         | α-Terpineol | C₁₀H₁₈O        | 154              | 0.058   |
| 30  | 28.811         | Dodecane | C₁₂H₂₆        | 170              | 0.046   |
| 31  | 29.742         | Pulegone | C₁₀H₁₆O        | 152              | 0.036   |
| 32  | 30.164         | Bicyclo[2.2.1]heptane-2-carboxylic acid, 3,3-dimethyl-, methyl ester | C₁₁H₁₈O₂      | 182              | 0.714   |
| 33  | 31.541         | Bornyl acetate | C₁₃H₂₀O₂      | 196              | 0.667   |
| 34  | 33.073         | Myrtenyl acetate | C₁₃H₂₀O₂      | 194              | 0.095   |
| 35  | 34.075         | α-Cubebe | C₁₅H₂₄        | 204              | 0.106   |
| 36  | 34.255         | α-Longipinene | C₁₅H₂₈O₂      | 204              | 1.288   |
| 37  | 34.375         | Neryl acetate | C₁₅H₂₈O₂      | 196              | 0.353   |
| 38  | 34.994         | Ylangene | C₁₅H₂₄        | 204              | 0.132   |
| 39  | 35.099         | longicyclene | C₁₅H₂₄       | 204              | 1.485   |
| 40  | 35.22          | (-)-trans-Myrtanyl acetate | C₁₃H₂₀O₂      | 196              | 1.167   |
| 41  | 35.701         | β-elemene | C₁₅H₂₄        | 204              | 0.402   |
| 42  | 35.984         | Methyleugenol | C₁₅H₂₆O₂      | 178              | 0.142   |
| 43  | 36.164         | β-Longipinene | C₁₅H₂₈O₂      | 204              | 0.064   |
| 44  | 36.429         | Longifolene | C₁₅H₂₄        | 204              | 0.513   |
| 45  | 37.213         | γ-Elemene | C₁₅H₂₄        | 204              | 1.502   |
| 46  | 37.584         | Aromandendrene | C₁₅H₂₄       | 204              | 0.544   |
| 47  | 37.835         | cis-β-Farnesene | C₁₅H₂₄       | 204              | 0.410   |
| 48  | 37.995         | α-Himachalene | C₁₅H₂₄       | 204              | 0.257   |
| 49  | 38.111         | Humulene | C₁₅H₂₄        | 204              | 0.350   |
| 50  | 38.826         | γ-Muurolene | C₁₅H₂₄       | 204              | 0.208   |
| 51  | 39.005         | 1-Pentadecene | C₁₅H₃₀       | 210              | 2.310   |
| 52  | 39.627         | Bicyclogermaene | C₁₅H₂₄       | 204              | 1.631   |
| 53  | 39.758         | (3S,3aS,8aR)-6,8a-Dimethyl-3-(prop-1-en-2-yl)-1,2,3,3a,4,5,8,8a-octahydroazulene | C₁₅H₂₄     | 204              | 0.421   |
| 54  | 40.44          | δ-Cadinene | C₁₅H₂₄       | 204              | 0.506   |
| 55  | 41.215         | α-Calacorene | C₁₅H₂₂O       | 220              | 0.169   |
| 56  | 41.628         | Nerolidol | C₁₅H₂₈O       | 222              | 0.156   |
| 57  | 41.781         | Germacrene B | C₁₅H₂₄      | 204              | 0.159   |
| 58  | 42.456         | Spathulene | C₁₅H₂₈O       | 220              | 1.270   |
| 59  | 49.171         | Hexadecanoic acid, methyl ester | C₁₆H₃₂O₂     | 270              | 0.048   |
MOLECULAR STRUCTURE AND TARGET PROTEIN PREDICTION

This study screened thirty-two volatile anti-inflammatory components of AT and twenty-six volatile anti-inflammatory components of HAT. A total of ten thousand four hundred and thirty genes related to inflammation and protein targets were screened by OMIM and GeneCards database. The targets of AT, HAT and inflammation were drawn in the Venn diagram (Figure 4). There were nine active anti-inflammatory components in AT and twelve active anti-inflammatory components in HAT, of which there were forty-seven intersection targets of inflammation and AT, forty-nine intersection targets of inflammation and HAT. They represent the potential anti-inflammatory targets of AT and HAT, respectively. The anti-inflammatory targets peculiar to HAT were twenty-six. There were forty-seven similar intersection targets of inflammation in AT and HAT. Which showed similarities and differences in the anti-inflammatory effect of volatile components of AT and HAT.

FIGURE 3. Comparison of volatile components in AT before and after honey-fried

FIGURE 4. Target Venny diagram of inflammation, AT and HAT
NETWORK CONSTRUCTION AND SCREENING OF KEY TARGET PROTEINS

The interaction network of the anti-inflammatory effect of AT and HAT were constructed through Cytoscape 3.7.2 software. The network was visualized with different colours and shapes. The results can be directly shown the network relationship between active ingredients and disease targets. AT and HAT’s similar potential active components were nine, including ethanol, acetic acid, benzaldehyde, α-phellandrene, p-cymene, acetophenone, humulene, γ-muurolene and methyl palmitate. The similar potential key targets were forty-seven, including CTSB, CTSD, GPI, HDC, PNP, CBS, F7, KYNU, TNF, AHCY, CAT, CES2, PFKFB4, LY, GYS1, GAPDH, ABAT, HMOX1, FDXR, SHMT2, SHMT1, F13A1, UROD, APRT, GSTM1, ANXA3, CPA1, LCMT2, CTH, GNMT, HAGH, GPHN, GSTM4, HDAC8, GAMT, ALOX5, MMUT, PPOX, MPST, ASL, PYCR2, SPR, GGT5, MPO, REN, TREH, and NCOA2. The potential active ingredients peculiar to HAT were furfural, β-elemene, methyleugenol, and unique targets of EIF6, PKIA. It illustrating the anti-inflammatory mechanism of volatile components of Hebei AT before and after honey-fried had more prominent similarities and smaller differences. The results are shown in Figure 5.

FIGURE 5. ‘Component-target-disease’ interactive network of the anti-inflammatory of AT and HAT (The yellow triangle represents the active components of anti-inflammatory drugs. The green circle represents the direct targets of anti-inflammatory drugs. The blue diamond represents medicine, and the red hexagon represents inflammation).

AT’s three topological parameters (degree, betweenness centrality and closeness centrality) were calculated as 3, 0.00004509 and 0.46825397. The parameters of HAT were calculated as 3, 0.00005237 and 0.46616541. The similar essential gene targets of AT and HAT were 47, including CTSB, CTSD, GPI, HDC, PNP, CBS, F7, KYNU, TNF, AHCY, CAT, CES2, PFKFB4, LY, GYS1, GAPDH, ABAT, HMOX1, FDXR, SHMT2, SHMT1, F13A1, UROD, APRT, GSTM1, ANXA3, CPA1, LCMT2, CTH, GNMT, HAGH, GPHN, GSTM4, HDAC8, GAMT, ALOX5, MMUT, PPOX, MPST, ASL, PYCR2, SPR, GGT5, MPO, REN, TREH, and NCOA2, which were all more significant than the median mean. These targets were considered as key targets of the anti-inflammatory effects of the volatile component. Our results predicted that the key anti-inflammatory targets of AT and HAT were the same. The top ten key targets of AT and HAT are shown in Table 3.
Protein protein interaction (PPI) network was constructed in the String database and visualized in Cytoscape 3.7.2. The results are shown in Figure 6. The analysis results showed that CAT, GAPDH, HMOX1, CTH, SHMT2, SHMT1, TNF, MPO, AHCY and GPI, played an essential role in the anti-inflammatory process of AT and HAT.

GO AND KEGG ENRICHMENT ANALYSIS
DAVID database was used to analyze the functional enrichment analysis of the key targets to study the functions of these targets holistically. Forty-nine biological processes (BPs), fifteen molecular functions (MFs) and eleven cellular components (CCs) were enriched from AT and HAT. The top twenty biological processes are shown in Figure 7. These targets were involved in various biological processes, including protein homotetramerization, hydrogen sulfide biosynthetic process, transsulfuration, L-serine metabolic process and protein tetramerization. The results illustrated that the volatile components of AT and HAT play an anti-inflammatory role by regulating these similar biological processes.

**TABLE 3. Topological parameter analysis of direct-acting targets in the network**

| Names  | Gene names | Name of the target protein          | Betweennesscentrality | Closeness centrality | Degree |
|--------|------------|-------------------------------------|------------------------|----------------------|--------|
| AT     | LYZ        | Lysozyme                            | 0.08914553            | 0.5                  | 10     |
| AT     | NCOA2      | Nuclear receptor coactivator 2       | 0.07629411            | 0.5                  | 10     |
| AT     | TNF        | Tumor necrosis factor               | 0.02738993            | 0.48360656           | 7      |
| AT     | ABAT       | 4-aminobutyrate aminotransferase    | 0.00596034            | 0.47580645           | 7      |
| AT     | GPI        | Glucose-6-phosphate isomerase       | 0.00596034            | 0.47580645           | 5      |
| AT     | CTSD       | Cathepsin D                         | 0.00596034            | 0.47580645           | 5      |
| AT     | CTSB       | Cathepsin B                         | 0.00596034            | 0.47580645           | 5      |
| AT     | UROD       | Uroporphyrinogen decarboxylase      | 4.51E-05              | 0.46825397           | 3      |
| AT     | Trehalase  | Trehalase                           | 4.51E-05              | 0.46825397           | 3      |
| HAT    | NCOA2      | Nuclear receptor coactivator 2       | 0.04944516            | 0.496                | 10     |
| HAT    | LYZ        | Lysozyme                            | 0.06879739            | 0.496                | 10     |
| HAT    | TNF        | Tumor necrosis factor               | 0.02504945            | 0.48062016           | 7      |
| HAT    | ABAT       | 4-aminobutyrate aminotransferase    | 0.00544716            | 0.47328244           | 7      |
| HAT    | GPI        | Glucose-6-phosphate isomerase       | 0.00544716            | 0.47328244           | 5      |
| HAT    | CTSD       | Cathepsin D                         | 0.00544716            | 0.47328244           | 5      |
| HAT    | CTSB       | Cathepsin B                         | 0.00544716            | 0.47328244           | 5      |
| HAT    | UROD       | Uroporphyrinogen decarboxylase      | 5.24E-05              | 0.46616541           | 3      |
| HAT    | Trehalase  | Trehalase                           | 5.24E-05              | 0.46616541           | 3      |

**Protein protein interaction (PPI) network was constructed in the String database and visualized in Cytoscape 3.7.2. The results are shown in Figure 6. The analysis results showed that CAT, GAPDH, HMOX1, CTH, SHMT2, SHMT1, TNF, MPO, AHCY and GPI, played an essential role in the anti-inflammatory process of AT and HAT.**

**GO AND KEGG ENRICHMENT ANALYSIS**
**DAVID database was used to analyze the functional enrichment analysis of the key targets to study the functions of these targets holistically. Forty-nine biological processes (BPs), fifteen molecular functions (MFs) and eleven cellular components (CCs) were enriched from AT and HAT. The top twenty biological processes are shown in Figure 7. These targets were involved in various biological processes, including protein homotetramerization, hydrogen sulfide biosynthetic process, transsulfuration, L-serine metabolic process and protein tetramerization. The results illustrated that the volatile components of AT and HAT play an anti-inflammatory role by regulating these similar biological processes.**
KEGG pathways were mainly associated with glycine, serine and threonine metabolism; biosynthesis of amino acids; biosynthesis of antibiotics; and cyanoamino acid metabolism (Figure 8). Using the KEGG mapper function in the KEGG signalling pathway database, the key target genes and the target proteins associated with inflammation were marked on the most closely related signal pathway, and similar anti-inflammatory targets of AT and HAT were marked in red. The results are shown in Figure 9.

DOCKING RESULTS
The nodes with a high degree value in PPI were considered as the essential proteins. In this study, AT and HAT’s top ten key proteins include SHMT1, CAT, GPI, AHCY, HMOX1, CTH, MPO, TNF, SHMT2, and GAPDH, were used for molecular docking with the active ingredients with the highest content in AT and HAT. The results showed the ten proteins with a strong affinity for humulene, γ-muurolene, α-phellandrene, and acetic acid. Therefore, the volatile components of AT and HAT play an essential role in anti-inflammatory action. The docking scores are shown in Figures 10 and 11.
FIGURE 8. KEGG pathways analysis of AT and HAT volatile oil anti-inflammation

FIGURE 9. Glycine, serine and threonine metabolism annotated signalling pathway diagram
FIGURE 10. Molecular docking results (A: humulene; B: γ-muurolene; C: α-phellandrene; D: acetic acid)

FIGURE 11. Molecular docking scores of AT and HAT (A: humulene; B: γ-muurolene; C: α-phellandrene; D: acetic acid)
THE SIMILARITIES AND DIFFERENCES OF AN ANTI-INFLAMMATORY MECHANISM

The volatile components of AT and HAT predicted by GC-MS and network pharmacology have anti-inflammatory effects. These findings are consistent with those reported in related literatures. The results are shown in Tables 4 and 5.

**TABLE 4.** similarities of anti-inflammation between AT and HAT

| Type                      | Similarities                                                                                       | Reference                                                                                      |
|---------------------------|---------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|
| Potential active components | ethanol, acetic acid, benzaldehyde, α-phellandrene, p-cymene, acetophenone, humulene, γ-muurolene, methyl palmitate | The pharmacodynamic experiment showed that p-cymene has an anti-inflammatory effect (Leonardo et al. 2012) |
|                           |                                                                                                  | Methyl palmitate is a universal macrophage inhibitor, and it has an anti-inflammatory effect (Ebtehal 2011) |
| Key targets               | CTSB, CTSD, GPI, HDC, PNP, CBS, F7, KYNU, TNF, AHCY, CAT, CES2, PFKFB4, LYZ, GYS1, GAPDH, ABAT, HMOX1, FDXR, SHMT2, SHMT1, F13A1, UROD, APRT, GSTM1, ANXA3, CPA1, CTH, GNMT, HAGH, GPHN, GSTM4, HDAC8, GAMT, ALOX5, MMUT, PPOX, MPST, ASL, LCMT2, PYCR2, SPR, GGT5, MPO, REN, Treh, NCOA2 | Catalase (CAT) is the most important intracellular enzyme, and it could suppress periodontal inflammation in beagles (Petelin et al. 2000) One of the bacterial surface Plg receptors is the multifunctional glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Recent research suggested that when upon inflammation, macrophages recruit GAPDH onto their surface to carry out a similar task of capturing Plg to digest extracellular matrix to aid rapid phagocyte migration and combat the invading pathogens (Chauhan et al. 2017) |
| Pathways                  | Metabolic pathways; Glycine, serine and threonine metabolism; Biosynthesis of amino acids; Biosynthesis of antibiotics; Cyanoamino acid metabolism; Cysteine and methionine metabolism; Carbon metabolism; Glyoxylate and dicarboxylate metabolism; Starch and sucrose metabolism; Porphyрин and chlorophyll metabolism; Glutathione metabolism | According to the reports, the increased proliferation and rapid activation of immune cells during inflammation require a switch in cell metabolism from a resting regulatory state to a highly metabolically active state to maintain energy homeostasis. This metabolic shift occurs in many inflammatory conditions such as colitis, diabetes, psoriasis, obesity and rheumatoid arthritis (Ping et al. 2015; Trudy et al. 2017) Glycine, serine and threonine metabolism were the main biomarkers of the antiarthritic mechanism of moxibustion (Pang et al. 2021) |
**TABLE 5. Differences of anti-inflammation between AT and HAT**

| Type                          | Differences                  | Reference                                                                 |
|-------------------------------|------------------------------|---------------------------------------------------------------------------|
| potential active components   | methyleugenol, β-elemene,    | Methyleugenol (ME) is a natural compound with antiallergic, and anti-inflammatory effects. ME markedly reduced the production of proinflammatory lipid mediators prostaglandin E2, prostaglandin D2, leukotriene B4, and leukotriene C4. Furthermore, it could inhibit allergic response by suppressing the activation of Syk, ERK1/2, p38, JNK, cPLA2, and 5-LO (Feng et al. 2015) β-elemene treatment modulated immune balance in the periphery and the inflamed optic nerve by promoting less downregulation in Treg cells, inhibiting Th17 and Th1 polarization (Zhang et al. 2010). Therefore, β-elemene induces substantial protection in experimental autoimmune encephalomyelitis optic nerve. Meanwhile, β-elemene is a natural antitumor plant drug (Bai et al. 2021) |
| of HAT                        | furfural                     |                                                                           |
| potential active targets of   | EIF6, PKIA                   | Eukaryotic translation initiation factor 6 (EIF6) could inhibit the expression of inflammatory mediators of M2 macrophages and then inhibit the production of vascular endothelial growth factors from preventing the excessive proliferation of blood vessels and granulation tissue (Wen et al. 2015). It suggests that HAT has the potential to prevent the excessive proliferation of blood vessels and granulation tissue Hepatocellular carcinoma, a slow multistep process, eventually starts from long-term inflammation to fibrosis and leads to malignancy. Protein kinase A is one of them, significantly contributes to liver tumorigenesis by stimulating cyclic AMP. Its inhibitor, cAMP-dependent protein kinase inhibitor alpha (PKIA), plays a significant role in inhibiting hepatic tumorigenesis (Riggle et al. 2016), so PKIA can improve the tumour by preventing inflammation. It suggested that HAT had a potential therapeutic effect on tumours The results showed that different anti-inflammatory active components were produced in different processing processes, and then different anti-inflammatory targets were produced |
| HAT                           |                              |                                                                           |

**Conclusions**

In summary, the obtained results by GC/MS indicated significant differences in volatile compositions and percentages between AT and HAT. After honey processing, HAT reduced sixteen volatile components
and increased twelve volatile components. Moreover, the anti-inflammatory mechanism of volatile components of Hebei AT before and after honey-fried had prominent similarities and smaller differences. HAT can play an anti-inflammatory role through its unique active ingredients and key targets, indicating that HAT has its characteristics in clinical anti-inflammatory. Nevertheless, their active anti-inflammatory components and percentage contents were different, and HAT was higher than AT. Therefore, the anti-inflammatory effect of volatile components of HAT may be better than that of AT. HAT has a better effect than AT in eliminating phlegm (Wu et al. 2006). It is possible that the volatile oil components of AT changed in the process of honey-fried, which enhanced the anti-inflammatory effect and then enhanced the expectorant effect of HAT. This study laid a theoretical foundation for the anti-inflammatory mechanism of AT and HAT and provided a theoretical basis for whether AT and HAT should be treated differently in future research.

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REFERENCES

Bai, Z.Q., Yao, C.S., Zhu, J.L. & Xie, Y.Y. 2021. Anti-tumor drug discovery based on natural product β-elemene: Anti-tumor mechanisms and structural modification. *Molecules* 26(6): 1499.

Bonjardim, L.R., Cunha, E.S., Guimarães, A.G., Santana, M.F., Oliveira, M.G., Serafini, M.R., Araújo, A.A., Antoniolli, A.R., Cavalcanti, S.C., Santos, M.R. & Quintans-Júnior, L.J. 2012. Evaluation of the anti-inflammatory and antinociceptive properties of p-cymene in mice. *Zeitschrift für Naturforschung C* 67(1-2): 15-21.

Chauhan, A.S., Kumar, M., Chaudhary, S., Patidar, A., Dhiman, A., Sheokand, N., Malhotra, H., Raje, C.I. & Raje, M. 2017. Moonlighting glycolytic protein glyceraldehyde-3-phosphate dehydrogenase (GAPDH): An evolutionarily conserved plasminogen receptor on mammalian cells. *The Federation of American Societies for Experimental Biology Journal* 31(6): 2638-2648.

Dutta, P., Sahu, R.K., Dey, T., Lahkar, M.D., Manna, P. & Kalita, J. 2019. Beneficial role of insect-derived bioactive components against inflammation and its associated complications (colitis and arthritis) and cancer. *Chemico-Biological Interactions* 313(2019): 108824.

Etnehal, E. 2011. Anti-inflammatory and antifibrotic effects of methyl palmitate. *Toxicology and Applied Pharmacology* 254(3): 238-244.

Fang, H.Y., Shan, G.W., Qin, G.F., Zhen, L., Li, M.H. & Hao, L.J. 2012. Advances on chemical components and pharmacological actions of *Aster tataricus*. *Medical Research and Education* 29(5): 73-77.

Feng, T., Feilong, C., Xiao, L., Huang, Y., Zheng, X., Tang, Q. & Tan, X. 2015. Inhibitory effect of methyleugenol on IgE-mediated allergic inflammation in RBL-2H3 cells. *Mediators of Inflammation* 2015: 463530.

Fink, T., Wolf, A., Maurer, F., Albrecht, F.W., Nathalie, H., Beate, W., Hauschild, A.C., Bertram, B., Baumbach, J.I. & Thomas, V. 2015. Volatile organic compounds during inflammation and sepsis in rats: A potential breath test using ion-mobility spectrometry. *Anesthesiol* 122(1): 117-126.

Gong, Q.F. 2016. *Traditional Chinese Medicine Processing: Chapter XII*. Beijing: China Press of Traditional Chinese Medicine.

Guo, C., Kang, X.D., Cao, F., Yang, J. & Fu, X. 2021. Network pharmacology and molecular docking on the molecular mechanism of Luo-Hua-Zi-Zhu (LHZZZ) granule in the prevention and treatment of bowel precancerous lesions. *Frontiers in Pharmacology* 12: 1-14.

Huang, X., Gao, Y., Xu, F., Fan, D. & Wu, H. 2020. Molecular mechanism underlying the anti-inflammatory effects of volatile components of *Ligularia fischeri* (Ledeb) Turcz based on network pharmacology. *BMC Complementary Medicine and Therapies* 20(1): 1-13.

Labib, R.M., Youssef, F.S., Ashour, M.L., Abdel-Daim, M.M. & Ross, S.A. 2017. Chemical composition of *Pinus roxburghii* bark volatile oil and validation of its anti-inflammatory activity using molecular modelling and bleomycin-induced inflammation in albino mice. *Molecules* 22(9): 1384.

Li, C., Huang, F., Dou, C.G., Zhang, M. & Ma, S.P. 2009. Effect of compatibility of *Aster tataricus* and Flos Farfarae on anti-inflammatory. *Chinese Journal of Clinical Pharmacology and Therapeutics* 14(2): 155-159.

Li, M.Q., Luo, L., Shang, N.N., Meng, B.H. & Huang, H.Z. 2020. Contradictions and countermeasures from cultural inheritance to industrial modernization. *Chinese Traditional Patent Medicine* 42(11): 2999-3003.

Li, P., Wang, J., Wang, C., Cheng, L. & Zhao, B. 2021. Therapeutic effects and mechanisms study of Hanchuan Zupa Granule in a guinea pig model of cough variant asthma. *Journal of Ethnopharmacology* 269(6): 113719.

Li, S.M., Zeng, B.Y., Ye, Q., Ao, H. & Li, H.X. 2015. Correlation analysis between GC-MS fingerprint of essential oil of *amomi fructus* and antiinflammatory activity. *Chinese Journal of Experimental Traditional Medical Formulae* 21(9): 133-136.

Lin, Z.X. 2011. Benzylamine and methylamine, substrates of semicarbazide-sensitive amine oxidase, attenuate inflammatory response induced by lipopolysaccharide. Thesis. Shantou University (Unpublished).

Lin, Y.M., Badrealam, K.F., Kuo, W.W., Lai, P.F., Chen, W.S., Day, C.H., Ho, T.J., Viswanadha, V.P., Shibu, M.A. & Huang, C.Y. 2020. Nerolidol improves cardiac function in spontaneously hypertensive rats by inhibiting cardiac inflammation and remodelling associated TLR4/ NF-κB signalling cascade. *Food and Chemical Toxicology* 147(2021): 111837.

McGarry, T., Binecka, M., Gao, W., Cluxton, D., Canavan, M., Wade, S., Wade, S., Gallagher, L., Orr, C., Veale, D.J. & Fearon, U. 2017. Resolution of TLR2-induced inflammation through manipulation of metabolic pathways in Rheumatoid Arthritis. *Scientific Reports* 7: 43165.
National Pharmacopoeia Commission. 2020. The Pharmacopoeia of the People’s Republic of China: Part I. Beijing: China Medical Science and Technology Press.

Ninomiya, K., Hayama, K., Ishijima, S.A., Maruyama, N., Irie, H., Kurihara, J. & Abe, S. 2013. Suppression of inflammatory reactions by terpinen-4-ol, a main constituent of tea tree oil, in a murine model of oral candidiasis and its suppressive activity to cytokine production of macrophages in vitro. Biological and Pharmaceutical Bulletin 36(5): 838-844.

Ping, X.T., Zhang, Y.Y., Leng, Y.F., Yao, Y., Zhang, R., Wang, D.W., Xu, X. & Sun, Z.L. 2021. Metabolomics study of biochemical changes in the serum and articular synovium tissue of moxibustion in rats with collagen-induced arthritis. World Journal of Acupuncture-Moxibustion 31(1): 30-43.

Petelin, M., Pavlica, Z., Ivanuša, T., Šentjurc, M. & Skalerič, U. 2000. Local delivery of liposome-encapsulated superoxide dismutase and catalase suppress periodontal inflammation in beagles. Journal of Clinical Periodontology 27(12): 918-925.

Ping, J., Hao, L. & Xiao, L. 2015. Diabetes mellitus risk factors in rheumatoid arthritis: A systematic review and meta-analysis. Clinical and Experimental Rheumatology 33(1): 115-121.

Queiroz, J.C.C., Antoniolli, Â.R., Quintans-Júnior, L.J., Brito, R.G., Barreto, R.S., Costa, E.V., da Silva, T.B., Prata, A.P.N., de Lucca, W., Almeida, J.R. & Lima, J.T. 2014. Evaluation of the anti-inflammatory and antinociceptive effects of the essential oil from leaves of Xylopia laevigata in experimental models. The Scientific World Journal 2014: 816450.

Riggle, K.M., Richle, K.J., Kenerson, H.L., Turnham, R., Homma, M.K., Kazami, M., Samelson, B., Bauer, R., McKnight, G.S. & Scott, J.D. 2016. Enhanced cAMP-stimulated protein kinase A activity in human fibrolamelar hepatocellular carcinoma. Pediatric Research 80: 110-118.

Saeed, N.M., Ebeihal, E.D., Hanan, M.A., Algandaby, M.M., Fahad, A.A. & Ashraf, B.A. 2012. Anti-inflammatory activity of methyl palmitate and ethyl palmitate in different experimental rat models. Toxicology and Applied Pharmacology 264(1): 84-93.

Santos, K.B., Guedes, I.A., Karl, A.L. & Dardenne, L.E. 2020. Highly flexible ligand docking: Benchmarking of the DockThor program on the LEADS-PEP protein–peptide data set. Journal of Chemical Information and Modeling 60(2): 667-683.

Su, G.Y. & Liu, Y. 2011. Production process of Aster tataricus and honey-fried Aster tataricus. Capital Medicine 18(3): 49.

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