VARIATION IN THE COMPOSITION OF THE ESSENTIAL OIL OF COMMERCIAL ARTEMISIA ABSINTHIUM L. HERB SAMPLES FROM DIFFERENT COUNTRIES

Ain Raal, Tetiana Ilina, Alla Kovalyova, Anne Orav, Margit Karileet, Mariana Džaniašvili, Taras Koliadzhyn, Andriy Grytsyk, and Oleh Koshovyi

Wormwood (Artemisia absinthium L., Asteraceae) is an aromatic bitter herb that contains bitter-tasting metabolites and essential oil. The composition and biological effects of A. absinthium essential oil have been widely studied. However, the data on the content of the individual components vary significantly.

The aim. The aim of the study was to research the compositions of essential oils from A. absinthium herb, which are on the market in various European countries and determine difference in their compositions, possible chemotypes, and compliance of the essential oil samples with the European Pharmacopoeia requirements.

Materials and methods. The composition of 16 essential oil samples of A. absinthium herb from different countries was investigated using the gas chromatography method. Samples were obtained from retail pharmacies in 14 different countries.

Research results. A total of 41 compounds were identified in the studied A. absinthium essential oils. In all the samples, monoterpenes and monoterpenoids dominate (28.0–92.2 %), much less sesquiterpenes and sesquiterpenoids (0–18.9 %). The dominant components among the identified ones were sabinine (traces(tr.)–21.2 %), myrcene (0.1–25.6 %), p-cymene (0.2–6.5 %), 1,8-cineole (0.1–18.0 %), artemisia ketone (tr.–14.9 %), borneol (tr.–10.8 %), β-thujone (0.1–38.7 %), (E)-epoxyocymene (tr.–59.7 %), (E)-verbenol (tr.–7.9 %), sabinene (tr.–11.7 %), (E)-sabinal acetate (tr.–70.5 %), neryl butyrate (0–13.9 %), spathulenol (tr.–9.2 %), carophyllene oxide (tr.–7.3 %). Both “pure”-chemotypes and “mixed”-chemotypes of A. absinthium have been defined.

Conclusions. Two “pure”-chemotype consist of 70.5 % (E)-sabinal acetate and 59.2 % (E)-epoxyocymene, respectively. Also, eleven “mixed”-chemotypes of A. absinthium essential oils have been defined. Some correlations were established between the content of terpenes in the A. absinthium essential oils

Keywords: Artemisia absinthium L., essential oil, terpenes, chemotypes, (E)-sabinal acetate, (E)-epoxyocymene, β-thujone


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1. Introduction

The wormwood genus Artemisia belongs to the Asteraceae L. family and includes more than 500 species in the world flora [1]. Artemisia absinthium L. ranges from Europe to Siberia and W. Himalaya, N. Africa; it has been introduced and naturalized as a ruderal species in many countries [2, 3]. A large number of representatives of this genus grow in the countries of Western Europe [4, 5], as well as in Estonia [6]. They are also found in China, Japan, Russia, the countries of Central Asia [7], North Africa and the Middle East, India, the USA, and Canada [8, 9].

The wormwood herb (Absinthii herba) is an official plant raw material in European Pharmacopoeia (PhEur) [10]. The herb is collected at the beginning of flowering (July–August). In the case of gathering in a later period, the herb acquires a dark grey colour and flowers turn brown and fall apart. Wormwood is an aromatic bitter herb, which is known for bitter-tasting metabolites and essential oils [3, 11].

The essential oil of A. absinthium contains acyclic, monocyclic and bicyclic monoterpenoids (oils) and their esters [12, 13]. These are: myrcene, nerol, lavandulol, α-terpinene, γ-terpinene, linalool, β-ocimene, cis- and trans-epoxyocimene, limonene, β-phellandrene, chrysanthenol, α-pinene, α-tuyol, α-thujone, β-thujone, camphene, camphor, 1,8 cineole [14], terpinolene, sabinal acetate, thujyl acetate, neryl acetate [15], geraniacetate, geranylisovalerianate, isoborneol, thujene, thujyl alcohol, sabinene, sabinal, β-caryophyllene [16, 17], carophyllene oxide, α-terpineol, geraniol, β-pinene [18, 19].

The sesquiterpenes of A. absinthium belong to different series of terpenoids, these are the acyclic (linear) and the guaianes, benzocycloheptene, germacrane, cadinane and eudesmane (selinane) groups [20, 21]. Acyclic sesquiterpenes are represented by β-farnesene and farnesol [22, 23]. Among the monocyclic sesquiterpene-oils of wormwood, the main ones are elemol, germacrone D, α-humulene, β-curcumene, bisabolene and α-bisabolol [24, 25].
Bicyclic sesquiterpenes of the azulene series are represented by 5,6-dihydrochamazulene, 3,6-dihydrochamazulene, chamazulene, methylchamazulene, ethylchamazulene [21, 22]. Bicyclic sesquiterpene(-oids) of the cadinene series [23, 24], naphthalene derivatives [25, 26]: α-cadinene, 7-epi-α-cadinene; eudesman series – eudesman and γ-selinene [27]. Bicyclic sesquiterpene(-oid)s of benzocycloheptene series: α-himachalene, himachalenol [28, 29]. Tricyclic sesquiterpenes of the azulene series are represented by aromadendren, copaene [30]. The aromatic monoterpane p-cymene (p-isopropyltoluene, 4-isopropyltoluene) is also typical of wormwood [31, 32].

Sesquiterpene lactones from A. absinthium have been well studied [33, 34]. Among the guayan lactones (guayanolides) are known artabsin, matricen, nor-sesquitenoid abilsactone, artalin, artabsinolides A, B, C, D; dimers – absintin, absintolide, anabsintin. Among the germacrane lactones (germacranolides) are artabine, artabolide (artabolide), dihydrocostunolide, costunolide, 3α-hydroxyepenolid, ketopelenolid A, ketopelenolid B. Among the eudesmane lactones: hansonolactone, arabsin, α-santonin [33, 35]. Among the lactones, absin, 3α-hydroxypelenolide, ketopelenolide A, ketopelenolide B.

The composition and biological effects of the essential oil (EO) of A. absinthium have been widely studied. However, the data on the content of the individual components varies significantly. The composition of the EO exhibits a large intraspecific variability. It may vary depending on the growing conditions, the period of harvesting raw materials, and the conditions of its drying and storage [11, 15]. There are studies on changes in the composition of wormwood EO in the process of ontogeny, which confirm that the content of thujones changes during the growing season [2, 5]. Several chemotypes of volatile oils are known from different parts of the world according to geographic regions and determine differences in content. About their chemotypes is very important. Identification of various chemotypes of wormwood EO allows us to predict, modulate and establish the mechanism of pharmacological activity at the molecular and cellular levels and predict their toxicity.

2. Planning (methodology) of research

The study aims to research the compositions of samples of A. absinthium herb essential oils from various geographic regions and determine differences in content composition, possible chemotypes, and compliance of the essential oil samples with the PhEur requirements [10]. This study continues our previous article [9] and brings new samples and research aspects. Identification of various chemotypes of wormwood EOs allows us to predict, modulate and establish the mechanism of pharmacological activity at the molecular and cellular levels and predict their toxicity.

3. Material and methods

Materials. The A. absinthium herbs commercial samples were obtained from retail pharmacies or health shops in different countries: Estonia 1–3, France, Belgium, Greece, Ukraine, Latvia, Lithuania, Italy, Spain, Germany, The Netherlands 1–2, England, Austria, and Georgia (in 2000–2011). They were partially studied previously [7, 9], and these results were used in our present research for settling correlations and chemotypes. Several packages of raw materials from each country were used, and average samples were made. Essential oils from the raw material samples were obtained by hydrodistillation according to the European Pharmacopoeia method and monograph “Absinthii herba” [10]. These essential oils were immediately analyzed by gas chromatography. All samples of A. absinthium herbs met the requirements of European Pharmacopoeia [10].

Capillary gas chromatography: GC analysis was carried out using a Chrom-5 chromatograph (Laboratori
Pristroe Prague, Czech Republic) with FID on two fused silica capillary columns with a bonded stationary phase: poly(5 % diphenyl-95 % dimethyl) siloxane) SPB-5 (30 m × 0.25 mm, Supelco) and polyethylene glycol SW-10 (30 m × 0.25 mm, Supelco). The film thickness of both stationary phases was 0.25 µm. Carrier gas was He with a split ratio of 1:150, and the flow rate 35–40 (SPB-5) and 30–35 (SW-10) cm/s was applied. The temperature program was from 50–250 °C at 2 °C/min; the injector temperature was 200 °C. A Hewlett-Packard Model 3390A integrator was used for data processing [21].

The identification of the EO components was accomplished by comparing their retention indices (RI) using n-alkanes C6–C24 as standards on two columns with the RI values of reference standards, our RI data bank and with literature data [22, 23]. GC/MS confirmed the results obtained [45, 46]. The percentage composition of the EOs was calculated in peak areas using normalization method without correction factors. The relative standard deviation of percentages of oil components of three repeated GC analyses of a single EO sample didn’t exceed 5 % [47, 48].

### 4. Results and Discussion

A total of seventeen wormwood EOs were used in our analyses. A total of 41 compounds were identified in A. absinthium EO samples studied. All identified components have been reported previously in wormwood herb EO [7, 9]. All samples of A. absinthium herbs met the requirements of European Pharmacopoeia monograph “Absinthii herba” [10]. The content of essential oils in the raw materials was between 2.4 and 3.7 ml/kg. The identified compounds in EO samples from different countries, their range and mean %, and variation coefficients are presented in Table 1. The main components, whose content in EOs could be over 6 %, were sabine (tr.–21.2 %), myrcene (0.1–25.6 %), p-cymene (0.2–6.5 %), α-pinene (0.2–18.0 %), terpinolene, α-terpineol, neryl acetate, α-copaene, neryl propionate, (E)-β-caryophyllene, γ-terpinene (tr.–7.9 %), α-Fellandrene (tr.–2.7 %), and carvomenthyl oxide (tr.–7.3 %) (Table 1, Fig. 1).

High variation coefficients of the majority of compounds (>1) showed that the content of these compounds strongly differed from sample to sample. Low variation coefficients (0.56–0.76) could be seen for α-pinene, terpinolene, 1,8-cineole, 1,8-cineole, 1,8-cineole, α-copaene, neryl propionate, (E)-β-carvomenthyl, (E)-nerolidol and carvomenthyl oxide.

In all the studied samples, monoterpenes and monoterpenoids dominate (28.0–92.2 %), much less sesquiterpenes and sesquiterpenoids (0–18.9 %). Aromatic monoterpenes are represented by p-cymene (0.2–6.5 %) (Fig. 3). A high content of oxygenated sesquiterpenes (6.8–16.6 %) characterized the samples from France, Austria, Ukraine, Germany, Spain, Greece, Latvia and Italy. The principal compounds in these groups were (E)-β-caryophyllene (tr.–2.1 %), carvomenthyl oxide (tr.–7.3 %) and spathulenol (tr.–9.2 %). The EO from Germany, Italy and France contained more than 1 % chamazulene (2.1 %, 1.5 % and 1.4 %, respectively). Only Austrian wormwood EO was rich in viridiflorol (2.3 %).

In the samples from the Netherlands, England, Austria, and Georgia, the main (>10 %) compounds were also (E)-epoxyocymene (tr.–32.7 %), myrcene (0.8–29.9 %), sabine (8.1–25.3 %), and (E)-sabinyl acetate (tr.–18.6 %) (Table 2).

**Table 1**

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<th>α-Pinene</th>
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<th>Myrcene</th>
<th>α-Copaene</th>
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**Note:** tr. – traces (<0.05 %); Bold – >1 %. The deviations of the results are within the deviations of the device.
The EO composition of three wormwood samples from Estonia was quite different. Sample 1 was dominated by monoterpenes, and samples 2 and 3 were dominated by oxygen-containing monoterpenes [7, 9]. Three samples that differ from the others in their dominant monoterpene content are those from Estonia 1, England and Hungary [7, 9]. All three have the highest myrcene content (25.6 %, 29.9 % and 17.1 %, respectively) and sabinene (21.2 %, 25.3 % and 18.1 %, respectively). Comparatively large contents of myrcene were also found in oils from Ukraine (5.9 %) and The Netherlands (5.4 %), sabinene – in the EOs from The Netherlands, Austria (9.3 % each) and Georgia (8.1 %). According to the literature, sabinene has antimicrobial, antifungal [49], anticonvulsant and antispasmodic effects [50, 51].

In samples from Estonia 2, Georgia and Austria (E)-epoxyocymene predominated (59.7 %, 32.7 % and 22.1 %, respectively). According to the literature, EO *A. absinthium* from France, containing (Z)-epoxyocimene and chrysanthenylacetate as the main components, inhibited the growth of both the yeast *Candida albicans* and *Saccharomyces cerevisiae* var. *chevalieri* [34].

The five EOs studied, such as samples from Estonia 3, The Netherlands, Canada, Latvia and Lithuania, contain the maximum content of (E)-sabinyl acetate (70.5 %, 18.6 %, 26.4 %, 23.6 % and 13.7 % respectively). Comparatively large contents of (E)-sabinyl acetate were also found in the EOs from Estonia 2 (23.6 %), Italy (11.4 %) and France (9.3 %). It should be noted that the composition of the four dominant components, namely (E)-sabinyl acetate>borneol>β-thujone>-caryophyllene oxide, EOs from Latvia and Lithuania, coincide entirely [9].

β-Thujone predominated in EOs from Greece and Italy (38.7 % and 12.3 %). β-Thujone-rich EO was characteristic of samples from Ukraine (6.3 %), Latvia and Spain (6.2 % each) [9].

Wormwood EO is neurotoxic, embryo-fetotoxic and abortifacient; its toxicity is associated with the combined effects of thujone and sabinyl acetate. It is best to completely avoid sabinyl acetate-rich EOs and any medications containing them during pregnancy, especially in the first trimester [52].

α-Thujone is more neurotoxic than β-thujone. When taken orally, thujone can affect the central nervous system and cause seizures, suggesting that it may cross the blood-brain barrier [53]. Thujone diastereomers have been shown to inhibit human gamma-aminobutyric acid type A (GABA) receptor current, a mechanism that causes muscle spasms and convulsions [53, 54]. α-Thujone has been found to inhibit CYP2A6 (IC50=2.34 mg/L) and CYP2B6 (IC50=2.66 mg/L), which may contribute to the long-term and enhanced toxicity of α-Thujone [55–57].

Thujone-rich EOs have been shown to have acaricidal, insecticidal and fungicidal effects and myrtenol-rich EOs repelled fleas, flies, mosquitoes and ticks [10]. Thujone-rich *A. absinthium* EO from Uruguay demonstrated antifungal activity against *Alternaria* sp. and *Botrytis cinerea* [58].
Spathulenol predominated in EOs from Ukraine (7.4 %) and Germany (5.5 %). Comparatively large amounts of spathulenol were also found in the EOs in France (9.2 %) and Austria (7.6 %). Spathulenol is one of the immunomodulatory compounds capable of inhibiting lymphocyte proliferation and inducing apoptosis in these cells; treatment of activated lymphocytes with a concentrated fraction containing 62 % spathulenol shows a decrease in lymphocyte proliferation with an IC50 of 85.4±11.08 µg/ml [59]. Spathulenol has moderate to low cytotoxicity with an IC50 above 6 μM and is a good candidate for use in combination chemotherapy for multidrug-resistant cancers and, therefore, merits further in vivo studies [44, 60].

In samples from France neryl butyrate predominated (13.9 %). Neryl butyrate rich EO was also characteristic of samples from Austria (4.9 %) and Ukraine (3.8 %) [9].

In samples from Spain 1,8-cineole and Artemisia ketone predominated (18.0 % and 14.9 %, respectively). Wormwood EO from a Turkish population, whose main components are camphor, 1,8-cineole, and chamazulene, has been described as fungicidal against 34 species of fungi, including Fusarium solani and Fusarium oxysporum [21].

p-Cymene rich EO was characteristic of samples from England, The Netherlands and Hungary (6.5 %, 6.1 %, 4.1 %, respectively) [61]. p-Cymene exhibits antioxidant, anti-inflammatory, antiparasitic, antidiabetic, antiviral, antitumor, antibacterial and antifungal activity [62, 63].

Accordingly, in “Assessment report on A. absinthium L., herba” [44], the composition of EO depends on the different chemotypes. "Pure"-chemotype: α-Thujone is typical for plants grown in areas below 1,000 m a.s.l. (Z)-epoxy-ocimene is the main component in plants grown in Europe at altitudes higher 1,000 m a.s.l. In France, there are different chemotypes with trans-sabinyl-acetate and chrysanthenyl-acetate as main components, while Eastern European plants are mostly mixed types. In Spain, species were established the two most characteristic chemotypes of A. absinthium: chemotype A, with (Z)-epoxyocimene as the main component, and B, with (Z)-epoxyocimene and (Z)-chrysanthemyl acetate as major ones [30].

In our studies, both “pure”-chemotypes and “mixed”-chemotypes have been defined. "Pure"-chemotype are EOs from Estonia 3, which consists of 70.5 % (E)-sabinyl acetate and from Estonia 2, which consists of 59.2 % (E)-epoxyocimene. The obtained data are different from those previously known. This applies to the sabinyl acetate and epoxyocimene isomers. It was established that not only (Z)-epoxy-ocimene could be the main component of the essential oil of wormwood, but also (E)-epoxyocimene. At the same time, this is in no way related to the height of the plant growing. This also applies to isomers of sabinyl acetate: not only trans-sabinyl-acetate, but also cis-, as well (E)-sabinyl acetate can be the dominant component of the essential oil.

Accordingly, “Assessment report on A. absinthium L., herba” [44], “mixed”-chemotype:

- (Z)-epoxy-ocimene+chrysanthenyl-acetate+thujone-chemotype;
- (Z)-epoxy-ocimene+β-thujone-chemotype;
- (Z)-epoxy-ocimene+chrysanthenyl-acetate-chemotype;
- β-thujone-sabinyl-acetate-chemotype;

In the previously published work, three additional chemotypes of *A. absinthium* were revealed: sabine-n+myrcene, neryl butyrate, and 1,8-cineole [9]. Some of the results we obtained confirm the existence of similar chemotypes:

- CT-(E)-epoxycymene+β-thujone+sabinene: sample from Georgia (32.7 %+8.5 %+8.1 %, respectively);
- CT-β-thujone+(E)-sabinyl acetate+spathulenol+borneol and carophyllene oxide: sample from Italy (12.3 %+11.4 %+3.3 %+2.6 %+2.6 %);
- CT-myrcene+sabinene: samples from Estonia 1 (25.6%+21.2 % respectively) [9], England (29.9%+25.3 % respectively) and Hungary (17.7%+18.1 % respectively) [9];
- CT-neryl butyrate+linalool+(E)-sabinyl acetate+spathulenol: sample from France (13.9 %+10.3 %+9.3 %+9.2 %) [9];
- CT-1,8-cineole+Artemisia ketone+linalool+β-thujone: sample from Spain (18.0 %+14.9 %+10.8 %+6.2 %) [9, 33].

The results of this present study shows that there are numerous additional "mixed" chemotypes (CT) of *A. absinthium*:

- CT-(E)-epoxycymene+sabinene+spathulenol: sample from Austria (22.1 %+9.3 %+7.6 %);
- CT-(E)-sabinyl acetate+sabinene+(E)-verbenol+1+α-cymene: sample from The Netherlands (70.5 %+9.3 %+7.9 %+6.1 %);
- CT-(E)-sabinyl acetate+borneol-β-thujone-caryophyllene oxide: samples from Latvia (23.6 %+9.2 %+6.2 %+4.7 %) and Lithuania (13.7 %+11.7 %+4.6 %+4.1 %);
- CT-β-thujone+linalool+caryophyllene ox-ride+spathulenol and sabine: sample from Greece: (38.7 %+4.5 %+3.7 %+3 %+3 %);
- CT-spethulenol+caryophyllene oxide+β-thujone+myrcene: sample from Ukraine (7.4 %+7.3 %+6.3 %+5.9 %);
- CT-spethulenol+geranyl isovaleriate+caryophyllene oxide+1,8-cineole: sample from Germany (5.5 %+4.9 %+3.8 %+3.4 %).

Literature data indicate a good correlation between some monoterpenes (e.g. nerol or limonene) and the antioxidant capacity of the natural extract [64]. However, the study of the correlation between the content of individual groups of compounds or between the content of individual substances in the essential oil of wormwood has not been conducted before.

Our results revealed relationships between the quantitative content of components in EOs that are characteristic of all objects (Table 3).

<table>
<thead>
<tr>
<th>Couples</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sabinene – Myrcene</td>
<td>0.91</td>
</tr>
<tr>
<td>Spathulenol – Caryophyllene oxide</td>
<td>0.81</td>
</tr>
<tr>
<td>Neryl butyrate – Spathulenol</td>
<td>0.81</td>
</tr>
<tr>
<td>Spathulenol – Geranyl isovaleriate</td>
<td>0.68</td>
</tr>
<tr>
<td>Sabinene – p-Cymene</td>
<td>0.66</td>
</tr>
<tr>
<td>Linalool – Caryophyllene oxide</td>
<td>0.62</td>
</tr>
<tr>
<td>Myrcene – p-Cymene</td>
<td>0.61</td>
</tr>
<tr>
<td>1,8-Cineole – Linalool</td>
<td>0.58</td>
</tr>
<tr>
<td>Neryl butyrate – Geranyl isovaleriate</td>
<td>0.58</td>
</tr>
<tr>
<td>Neryl butyrate – Caryophyllene oxide</td>
<td>0.57</td>
</tr>
<tr>
<td>Monoterpenes – aromatic monoterpenes</td>
<td>0.64</td>
</tr>
<tr>
<td>Sesquiterpenes – Oxygenated sesquiterpenes</td>
<td>0.55</td>
</tr>
<tr>
<td>Monoterpenes – Oxygenated monoterpenes</td>
<td>–0.69</td>
</tr>
<tr>
<td>Monoterpenes – Sesquiterpenes</td>
<td>–0.60</td>
</tr>
</tbody>
</table>

There is a strong inverse correlation between the content of monoterpenes and oxygenated monoterpenes (r=–0.69) and a moderate inverse correlation between the content of monoterpenes and sesquiterpenes (r=–0.60). The identified correlations can serve as a generalized characteristic of EOs of wormwood of different origins.

**Practical Relevance.** The established chemotypes make it possible to more accurately classify and sort *Artemisia absinthium* L. raw material and essential oils, predict the pharmacological activity of its medicinal products, and provide the possibility of a targeted search for the necessary raw materials according to the specified parameters. The correlation of substances’ content allows us to predict the content of target molecules in *Artemisia absinthium* L. essential oils.

**Study limitations.** The used samples of *Artemisia absinthium* L. herb were only from European countries. It would be appropriate to expand the geography of the origin of raw materials.

**The prospects for further research.** The prospects for further research consist of expanding the geography of the origin of commercial raw materials and establishing new chemotypes and correlations with growth regions. The results of the research create conditions for a targeted search for biologically active substances and seed materials.

**6. Conclusions**

Based on research, 16 brands of EOs and bitters are sold in 15 countries: Estonia, France, Hungary, The Netherlands, England, Austria, Greece, Ukraine, Georgia, Canada, Latvia, Lithuania, Italy, Spain, Germany, 2 "pure"-chemotypes and 11 "mixed"-chemotypes have been defined. "Pure"-chemotype are EOs from Estonia 3, which consists of 70.5 % (E)-sabinyl acetate and from
Estonia 2, which consists of 59.2 % (E)-epoxyocymene. For the first time, correlative deposits have been installed between instead of adjacent shelves in the warehouse of EO of Pauline bitter. The strongest correlations are the pairs sabinene – myrcene ($r=0.91$), spathulenol – caryophyllene oxide and neryl butyrate – spathulenol ($r=0.81$). The revealed patterns can be used to predict, modulate and establish the mechanism of possible pharmacological activity and toxicity of polyhydric EO.

**Conflicts of interest**
The authors declare that they have no conflict of interest concerning this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

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**Data availability**
The datasets used and/or analyzed during the current study are available from the author and/or corresponding author upon reasonable request.

**Use of artificial intelligence**
The authors confirm that they did not use artificial intelligence technologies when creating the current work.

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**References**


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