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GC-MS analysis and cytotoxic activity of essential oils from the leaves of *Abrus precatorius* L. Gaertn

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ABSTRACT

Objective: To determine the chemical constituents, cytotoxic activity and possible applications of the essential oils from the leaves of *Abrus precatorius* L. Gaertn.
Methods: Hydro-distillation using Clevenger-type apparatus was employed to obtain the essential oils. Oil analysis was performed using an HP 6890 gas chromatograph coupled with an HP 5973 mass selective detector. The cytotoxicity bioassay was carried out using the brine shrimp lethality test.
Results: Forty-five compounds were identified representing 100% of the oil. The principal components were γ-cadinene (19.1%), α-selinene (15.3%), α-cubenene (12.8%), β-caryophyllene (8.2%), germacrene B (7.9%), α-copaene (7.7%) and linalool (6.3%). Others are caryophyllene oxide (5.5%), β-elemene (5.4%) and α-caryophyllene (4.0%). The oil was potent with the LC_{50} value of 0.45 μg/mL.
Conclusions: The essential oil from the leaves of *Abrus precatorius* L. Gaertn. could hold promise for future applications in the treatment of cancer-related diseases, in addition to flavor and fragrance industry.

1. Introduction

Abrus precatorius L. Gaertn (*A. precatorius*), which is widely known as rosary pea and crab's eyes, belongs to Fabaceae family. It is a slender perennial climbing plant that twines around trees, shrubs and hedges. It has attractive red-black coloured seeds that are used as beads in many countries[1,2]. It grows in tropical and sub-tropical regions and is commonly found in Nigeria. In traditional medicine, the decoction of its leaves is used in the treatment of diabetes mellitus, cough, piles and malaria[2]. It is called "idon zakara" or "jigaree-hi" in Hausas at the northern part of Nigeria, where the bark is used to treat jaundice, yellow fever and arthritis[3]. Tea made from the leaves is used to treat fevers, cough and cold. In Southwestern Nigeria, it is referred to "oju-ologbo" or "omisinmisin", and the leaves and seeds are boiled in water and the decoction is ingested for treating eye inflammation. However, the plant is classified as poisonous. Toxin abrin was found in the seeds and ingestion of the chewed seeds can affect the gastrointestinal tract, liver, spleen, kidney and lymphatic system[1].

Essential oils are volatile mixtures of complex compounds from natural products used as raw materials in many fields, including perfumes, cosmetics, aromatheraphy, phytotheraphy, spices and nutrition[4,5]. The various constituents of these oils contribute to the overall beneficial or adverse effects. Therefore, adequate knowledge of the compositions of essential oils allows for a better and specially directed applications[4]. Out of the immense number of flora species, essential oils have been well-characterized and identified from only a few thousand plants[6]. A thorough search of the available literatures showed that there is no report on the chemical compositions of the essential oil from *A. precatorius*. However, abrusoside A–E, abrusogenin and subprogenin D were reported to have been isolated from the non-volatile extracts of the plant. Others include abrusgenic acid, triptotriterpenic acid and abruslactone A[7,8].

This paper is reporting the cytotoxic activity, chemical compositions and possible applications of the essential oil from *A.*

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precatorius which hitherto is unavailable.

2. Materials and methods

2.1. Plant material

The whole plant of mature and well established *A. precatorius* was collected in September, 2013 from Ilorin, north-central zone of Nigeria. The plant was identified in Plant Biology Department, University of Ilorin, Nigeria, where the voucher specimen (UIL 00280) was deposited in the herbarium.

2.2. Preparation of the leaf oil

Air-dried leaves (500 g each) were hydrodistilled using distilled water (1 000 mL) for 3 h in an all-glass apparatus constructed according to the specifications of the British Pharmacopoeia using hexane as the collecting solvent. The essential oils collected were separated from water by drying with anhydrous sodium sulphate and stored in well-capped bottles at 4 ℃ prior to analysis.

2.3. Cytotoxic bioassay

The cytotoxicity bioassay was carried out using the brine shrimp lethality test (BST) protocol of McLaughlin and Rogers (1998)[9]. The brine shrimp (*Artemia salina* Leach) eggs were hatched in sea water for 48 h at room temperature. Solutions of the essential oils were made in dimethylsulfoxide at various concentrations (10 000, 1 000, 100, 10 and 1 μg/mL) and incubated in test tubes in triplicates with the brine shrimp larvae. Control brine shrimp larvae were placed in a mixture of sea water and dimethylsulfoxide only. The set up was left for 24 h. After that, the average number of larvae surviving in each test tube was determined. The LC_{50} values of 95% confidence intervals for the statistically significant comparison of potencies were calculated using Finney programme.

2.4. Gas chromatography-mass spectrometry (GC-MS)

For quantification, oil analysis was performed using an HP 6890 gas chromatograph coupled with an HP 5973 mass selective detector. Separation was carried out with an HP 5MS column (30 m × 0.25 mm × 0.25 μm). GC operating conditions were as follows: injector: split ratio 20:1; carrier gas: hydrogen at a flow rate of 1.0 mL/min; temperature: 250 ℃. The GC oven temperature was 40 ℃ for 5 min, rose to 200 ℃ at a rate of 5 ℃ per minute, then kept constant at 220 ℃ for 2 min. The inlet temperature was set at 150 ℃. Mass spectra were acquired at 70 eV with a mass range of 50–300 *m/z*. Data were analysed using HP ChemStation software.

2.5. Identification of constituents

The components were identified by comparing the retention times and mass spectra of the chromatographic peaks with those of standards analysed under the same conditions. The assignment of the peaks of other volatile constituents was based on computer matching of the mass spectra obtained with the Wiley 275, National Institute of Standards and Technology and Adams libraries, taking into account the coherence of the retention indices of the analysed compounds with those reported by Adams and National Institute of Standards and Technology 08 libraries.

3. Results

The air-dried leaves of *A. precatorius* yielded 0.37% (v/w) greenish essential oil by hydrodistillation. Forty-five chemical compounds were identified from the leaf oil representing 99.990% of the total oil profile (Table 1). The oil was dominated by γ-cadinene (19.145%), α-selinene (15.291%), α-cubebene (12.760%), β-caryophyllene (8.235%) and germacrene B (7.904%). Other constituents found in high quantities included α-copaene (7.740%), linalool (6.300%), caryophyllene oxide (5.496%), β-elemene (5.355%) and α-caryophyllene (4.032%). This oil also contained a low percentage (⩽ 2.0%) significant number of monoterpene hydrocarbons such as α-pinene, β-pinene, limonene and γ-terpinene, along with monoterpenoids such as borneol and terpinen-4-ol. The oil analysed gave a LC_{50} value of 0.45 μg/mL using BST.

Table 1

Percentage composition of essential oil of air-dried leaves of *A. precatorius*.

Constituents[a]	RI[b]	RI[c]	Percentage (%)
Cymene	1029	1028	0.022
Cinnamic aldehyde	1267	1267	0.025
Tricyclene	921	921	0.025
Camphene	954	956	0.025
Sabinene	969	969	0.032
Limonene	1029	1029	0.032
α-Pinene	934	932	1.620
β-Pinene	979	974	0.025
Benzyl alcohol	1026	1026	0.060
Cis ocimene	1032	1032	0.007
Myrcene	991	992	0.083
Allo-ocimene	1133	1132	0.054
Pinene-2-ol	1123	1123	0.036
α-Thujene	926	930	0.030
Thymol	1287	1289	0.022
Carvacrol	1298	1298	0.022
3-Methoxyacetophenone	1020	1020	0.022
γ-Terpinene	1020	1020	0.065
Citronellal	1148	1148	0.022
Neral	1240	1240	0.052
Geranial	1272	1272	0.203
Borneol	1167	1169	0.050
Citronellol	1223	1223	0.022
1,8-Cineole	1031	1031	0.010
Linalool	1097	1097	6.300
α-Terpineol	1189	1189	0.027
Terpinen-4-ol	1183	1183	0.028
Germacrene B	1554	1561	7.904
Thymyl methyl ether	1199	1199	0.033
Linalyl acetate	1237	1237	0.045
Borneol acetate	1289	1289	0.074
Geranyl acetate	1381	1381	0.494
α-Cubebene	1351	1351	12.760
β-Caryophyllene	1421	1419	8.235
γ-Cadinene	1514	1514	19.145
β-Elemene	1391	1391	5.355
Germacrene D	1482	1485	1.595
α-Caryophyllene	1408	1408	4.032
α-Copaene	1377	1374	7.740
Acetyleugenol	1521	1521	0.021
α-Selinene	1498	1498	15.291
γ-Muurolene	1477	1477	0.022
Elemicin	1555	1555	0.012
β-Selinene	1585	1585	2.783
Caryophyllene oxide	1583	1587	5.496
Total			99.990

[a]: Compounds are listed in order of elution from a HP-5MS column; RI[b]: Retention indices relative to *n*-alkanes on HP-5MS column; RI[c]: Retention indices from literature.

4. Discussion

The leaf oil of *A. precatorius* was characterized by a high proportion of sesquiterpenes and monoterpenes. The oil was analysed by using BST to determine its relative potency. There is a well established correlation between brine shrimp toxicity and 9KB (human epidermoid carcinoma of nasopharynx) cytotoxicity (P = 0.036 and κ = 0.560). Many novel antitumor agents from natural products have been discovered using BST[9]. The oil was highly active with a LC_{50} value as low as 0.45 μg/mL, because plants having LC_{50} values less than 200 μg/mL in case of extracts and 5 μg/mL in case of pure compounds are considered as highly active[10,11]. The potency could be as a result of higher percentage compositions of linalool, β-caryophyllene, α-copaene, β-elemene, germacrene B, α-cubenene and a significant number of monoterpenes present in the oil.

Previous reports had shown that monoterpenes have multiple pharmacological effects on mevalonate metabolism, which could account for the terpene-tumor suppressive activity. Monoterpenes have been demonstrated to exert chemopreventive as well as chemotherapeutic activities in mammary tumor models and hence may present a novel category of therapeutic agents. Chemoprevention usually occurs during the initiation phase of carcinogenesis to stall the interaction of chemical carcinogens with DNA, by induction of phase I and phase II enzymes to detoxify the carcinogen; while chemotherapy works during the promotion phase, in which inhibition of tumor cell proliferation, acceleration of the rate of tumor cell death and/or induction of tumor cell differentiation may occur[12-14]. Various findings had shown linalool, a monoterpenoid and one of the major constituents, to be a potential anti-cancer and anti-inflammatory agent[15-17]. Linalool is also used for scent in 60%–80% perfumed hygiene products and washing agents such as detergents, shampoos and so on[18]. The research also shows that inhaling linalool can reduce stress and anxiety[19,20]. Linalool-rich essential oil from the leaves of *Croton cajucara* was demonstrated to be potent against *Leishmania amazonensis*[21].

The presence of high levels of sesquiterpenes in the oil could be a bioresource for germacrene D, an antibacterial and antifungal compound[22]. (E)-β-caryophyllene, a sesquiterpenoid, is reported to have significant insecticidal and repellant activities against leaf-cutting ants and termites, which suggests that the leaf oil can be a good source of an environmentally friendly natural pesticides[23-25]. Another study proved β-caryophyllene to exhibit anaesthetic and anti-inflamatory activities[26,27]. Turkez together with his team investigated cytotoxic and cytogenic effects of α-copaene on rat neuron and N2a neuroblastoma cell lines. They concluded that α-copaene exhibited mild cytotoxic effects on N2a neuroblastoma cell lines thus showing potential anti-cancer activity[28].

Anti-cancer properties have indeed been reported for β-elemene[29-31]. A study showed that β-elemene had an anti-proliferative effect on androgen-insensitive prostate carcinoma cells. The effect of β-elemene on cancer cells was dose-dependent with IC_{50} values ranging from 47–95 μg/mL. Treatment with β-elemene also inhibited the growth of brain, breast, prostate, cervical, colon, gastric and lung carcinoma cells[30,32,33]. Germacrene B, a sesquiterpenoid identified in the oil, is reported to exhibit good cytotoxicity against human ovarian cell line A2780, with a IC_{50} value of 6.4 μg/mL[29].

It is clear that only a detailed knowledge of the constituents of an essential oil will lead to an appropriate use. Considering its potency and all the aforementioned therapeutic effects of the principal constituents of the oil, thus, the essential oil from the leaves of *A. precatorius* could hold promise for future applications in the treatment of cancer-related diseases, in addition to flavor and fragrance industry. Further scientific evaluation of the oil against cancer and others should be considered. However, this is the first available study on chemical constituents and cytotoxic activity of the essential oils from the leaves of *A. precatorius*.

Conflict of interest statement

We declare that we have no conflict of interest.

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