Anti-trypanosomal activity and DNA fingerprinting of fifteen *Euphorbia* species using ISSR and SCoT markers

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Abstract

Background: *Euphorbia* is the largest genus in family Euphorbiaceae with a great biological and genetic diversity. The anti-trypanosomal activity of the crude extract of 15 *Euphorbia* species against *Trypanosoma brucei brucei* was carried out. Furthermore, DNA fingerprinting of the tested species using ISSR and SCoT markers was also investigated.

Results: The anti-trypanosomal activity of the 15 *Euphorbia* species revealed the highest activity of *E. officinarum* L. and *E. milli* Des Moul. against *Trypanosoma brucei brucei* with IC_{50} values < 10 µg/mL after 48- and 72-h incubation. Moreover, the assessment of the genetic diversity among the 15 tested species showed similar correlation coefficients of 0.76–0.98 which classified *Euphorbia* species into two main groups, one contained two species and the other contained 13 species.

Conclusions: Some *Euphorbia* species exhibited significant growth inhibitory activity toward *Trypanosoma brucei* strain TC221. Results also indicated the suitability of both markers for genetic fingerprinting of the tested *Euphorbia* species. To our knowledge, this is the first detailed comparison of the performance of two targeted DNA molecular markers (SCoT and ISSR) on the tested 15 *Euphorbia* species. The results guide future efficient use of these molecular markers in the genetic analysis of *Euphorbia*.

Keywords: *Euphorbia*, Anti-trypanosomal activity, *Trypanosoma brucei brucei*, Genetic diversity, ISSR, SCoT, Molecular markers

1 Background

Human African trypanosomiasis, sleeping sickness, is a mortal vector-borne parasitic disease caused by *Trypanosoma brucei* transmitted by the tsetse fly (*Glossina* spp.), especially in sub-Saharan Africa [26]. Up till now, only a few drugs have been approved to treat human African trypanosomiasis as suramin, pentamidine, melarsoprol, eflornithine, and the combination of nifurtimox/eflornithine. Most of the drugs discovered in the 1940s and 1950s had adverse effects such as nausea, vomiting, diarrhea, abdominal cramping, fever, hypoglycemia, hypertension, heart damage, seizures, peripheral neuropathy, and neutropenia [18].

Natural sources as plants, microorganisms, animals, and marines provide a high number of natural products with various chemical structures and novel pharmacological mechanism of action [16]. Therefore, discovering anti-trypanosomal drugs from natural sources is an urgent requirement. *Euphorbia* was reported for its anti-arthritis, anticancer, anticonvulsant, anti-diabetic, anti-eczema, anti-inflammatory, antimicrobial, antioxidant, antispasmodic, anti-tumor, and myelopoiesis properties [5, 12].

Euphorbia genus contained a wide variety of terpenoids, ranging from mono-, sesqui-, and diterpenes to triterpenoids and steroids [24]. Metabolic profiling of

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15 *Euphorbia* species, using LC-HRMS, annotated of 44 natural compounds [11].

Despite the taxonomic effort discussing the variations and relationships among *Euphorbia* species, morphologically based relationship between different *Euphorbia* species is confusing in many cases [9]. They can be morphologically differentiated by their flower color, but it is difficult to distinguish them at the vegetative stage [7].

[6] identified 600 species of the genus *Euphorbia* and then branded into 26 sections. [27] included only the shrubby, fleshy, and cactus-like species in the genus *Euphorbia*. The final analysis as presented by [29] was to treat Chamaesyce as a separate genus, whereas all other taxa were downgraded to sub-generic status within *Euphorbia*. Thus *Euphorbia* is still a large polymorphic genus with seven subgenera, viz. *Esula, Poinsettia, Euphorbia, Agaloma, Eremophyton, Lyciopsis,* and *Rhizanthium* [25].

Plant identification is the first step to assure the efficacy, quality, and safety of a drug or an extract [23]. Molecular genetic markers have been widely used in the last decades in the search for valuable plant phenotypes [13]. They are mostly used for fingerprinting, diversity analyses, gene mapping, variability, tracking individuals or lines carrying particular genes, and linkage maps [17]. Using more than one marker is continuously recommended for a better analysis of the genetic homogeneity of plants [20]. DNA-based markers illustrate the germplasm and count the degree of genetic diversity more specifically than other markers [19].

Herein, the anti-trypanosomal activity of the methanolic extract of the 15 tested species is evaluated. In addition, two genetic markers, SCoT and ISSR, are also used for identification, authentication, and determining the degree of diversity and similarity between 15 *Euphorbia* species.

2 Methods

2.1 Plant materials

The aerial non-flowering parts of 15 *Euphorbia* species were collected during October 2018 from Helal cactus farm, Abdel Samad village, El-Mansouria, Giza, Egypt. All the collected species consisted mainly of stems and leaves. The species were identified and authenticated by Prof. Dr. Abdel-Halim Mohammed, Professor of Agriculture, Flora Department, Agricultural Museum, Dokki, Giza, Egypt. Fresh plants were stored at -5 °C for the DNA study and coded as follows: *E. abyssinica* J.F. Gmel. (1), *E. caput-medusae* L. (2), *E. trigona* Mill. (3), *E. stenoclada* Baill. (4), *E. tithymaloides* L. (5), *E. tirucalli* L. (6), *E. royleana* Boiss. (7), *E. officinarum* L. (8), *E. horrida* Boiss. (9), *E. canariensis* L. (10), *E. grandialata* R.A. Dyer

(11), *E. obesa* Hook. (12), *E. lactea* Haw. (13), *E. ingens* E. Mey. (14), and *E. millii* Des Moul. (15).

2.2 Methanolic extracts for anti-trypanosomal activity

Methanolic extracts were prepared by maceration of 50 g fresh samples in $(3 \times 200$ mL$)$ 80% methanol (Sigma–Aldrich, Germany). The extracts were separately filtered and evaporated to dryness using a rotary evaporator (Buchi, G., Switzerland). Dried extracts were stored at 4 °C.

2.3 Anti-trypanosomal activity

The anti-trypanosomal activity had been investigated following the method reported by [1, 14]. Complete Baltz medium was used for cultivation of *Trypanosoma brucei* strain TC 221 (Missionsärztliche Klinik, University of Wurzburg), Germany. Trypanosomes were seeded in 96-well plate chambers with a pore size of 0.4 μm at an initial cell density of 3×10^4/mL in 140 μL of the medium. They were tested versus various concentrations of the tested 15 *Euphorbia* methanolic extracts at 0.25–40 μg/mL in 1% DMSO to a final volume of 200 μL. 1% DMSO was used as the negative control, and suramin (Calbiochem-Novabiochem Co., purity $>$ 98% by HPLC) was selected as the positive control (concentration of 1 to 0.008 μg/mL). Suramin gave an anti-trypanosomal activity against *T. brucei* at an MIC of 0.23 μg/mL. The plates were incubated for 48 h at 37 °C, 5% CO_2 with a humidified atmosphere, after which 20 μL of Alamar blue was added. Then, plates were incubated again for another 24 h under the same conditions. The activity was measured by light absorption at *ʎ* 550 nm with a reference wavelength of 650 nm using an MR 700 microplate reader (Dynatech Engineering Ltd., Willenhall, UK) after 48 and 72 h. The quantification of the IC_{50} values of the investigated extracts (the dose of the extract which reduces survival to 50%) was processed by linear interpolation.

2.4 DNA analysis

2.4.1 DNA isolation procedure

Sample from each of the 15 *Euphorbia* species (fresh stem) was ground by liquid nitrogen to a fine powder and transferred to an appropriately sized tube. The bulked DNA extraction was performed via DNeasy Plant Mini Kit (QIAGEN) on Agriculture Research Center in Giza, according to [10, 30] methods.

2.4.2 PCR (Polymerase Chain Reaction) condition for SCoT and ISSR

DNA amplifications were achieved in an automated thermal cycle (model Techno 512) which was programmed for one cycle at 94 °C for 4 min, followed by 45 cycles of 1

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min at 94 °C, 1 min at 57 °C, and 2 min at 72 °C. The reaction was lastly kept at 72 °C for 10 min [28].

2.4.3 Gel preparation procedure

Agarose (1.5 g) was mixed with (100 mL) $l \times$ TBE buffer and boiled in a microwave. Ethidium bromide (5 μL) was added to the melted gel after the temperature became 55 °C. Then , the melted gel was poured into the tray of the mini-gel apparatus and a comb was inserted immediately after that comb was removed when the gel becomes hardened. The gel was covered by the electrophoretic buffer ($1 \times$ TBE) , and DNA-amplified product (15 μL) was loaded in each well. DNA ladder (100 bp) mix was used as standard DNA using molecular weights ranging from 100 to 3000 bp, and the run was done for about 30 min at 80 V in mini-submarine gel BioRad.

2.4.4 Data analysis for genetic study

The similarity matrices were done using Gel works ID advanced software UVP-England Program. The relationships among genotypes as revealed by dendrograms were done using the SPSS windows (version 10) program. DICE computer package was used to calculate the pairwise difference matrix and plot the phenogram among cultivars [8, 31].

3 Results

3.1 Anti-trypanosomal activity

The anti-trypanosomal assay was used here as one way to discriminate between the active and inactive extracts (Table 1). Among 15 *Euphorbia* species, four *Euphorbia* species displayed activity against *T. brucei* where *E. officinarum* L. and *E. milli* Des Moul. were the most active IC_{50} values < 10 μg/mL after 48- and 72-h incubation.

3.2 DNA analysis

3.2.1 Genetic diversity revealed by start codon targeted (SCoT) markers

Ten SCoT polymorphic markers were screened to access the level of genetic diversity and similarity between 15 *Euphorbia* species, only five produced scorable bands and selected for the analysis (Additional file 1: Table 2 and Fig. 1). They produced a total of 25 reproducible and scorable bands (Table 2). Each primer produced an amplification product in the range of 135–2465 bp, and the number of the scorable bands varied from 2 to 8 for each primer. The total polymorphism was estimated as 48%. The distribution of monomorphic (common) and polymorphic bands generated by the five SCoT markers showed a unique band at 1735 bp for SCoT 10 with *E. trigone* Mill. (3), while for

Table 1 IC_{50} μg/mL values of methanolic *Euphorbia* extracts after 48-h and 72-h incubation

Sample	T. brucei TC 221, IC$_{50}$	
	(μg/mL, 48 h)	(μg/mL, 72 h)
E. abyssinica J.F. Gmel	17.3	19.4
E. caput-medusae L	>100	>100
E. trigona Mill	>100	>100
E. stenoclada Baill	>100	>100
E. tithymaloides L	>100	>100
E. tirucalli L	>100	>100
E. royleana Boiss	11.1	13.6
E. officinarum L	6.2	9.5
E. horrida Boiss	>100	>100
E. canariensis L	>100	>100
E. grandialata R.A. Dyer	>100	>100
E. obesa Hook	>100	>100
E. lactea Haw	>100	>100
E. ingens E. Mey	>100	>100
E. milli Des Moul	7.8	9.3

SCoT 12 a unique band at 1500 bp with *E. lactea* Haw. was detected (13) (Table 3). The dendrogram of the tested *Euphorbia* species revealed classification of the 15 species, according to the data obtained by the SCoT into two main clusters. The first cluster included species 1 and 2 corresponding to *E. abyssinica* J.F. Gmel. and *E. caput-medusae* L., indicating close similarity between these two species. The other cluster segregated the remaining species into three more sub-clusters (Additional file 1: Figure 1).

3.2.2 Genetic diversity revealed by inter-simple sequence repeat (ISSR) markers

In the case of ISSR markers, five markers yielded reproducible bands out of 15 screened markers (Additional file 1: Table 3 and Fig. 2). They yielded 26 scorable bands (Table 4). The bands ranged from 130 to 1665 bp, while the band numbers ranged from 3 to 8 in each primer. The distribution of monomorphic and polymorphic bands produced by the five ISSR markers revealed that 14A showed unique bands at 1430 and 740 bp with *E. milli* Des Moul. (15), 44B provided unique band with *E. grandialata* R.A. Dyer (11) at 315 bp, and HB-12 had unique band with the same species at 360 bp. The total polymorphism was estimated as 57.7% (Table 5). The dendrogram of 15 *Euphorbia* species was represented in Additional file 1: Figure 2. The 15 examined species
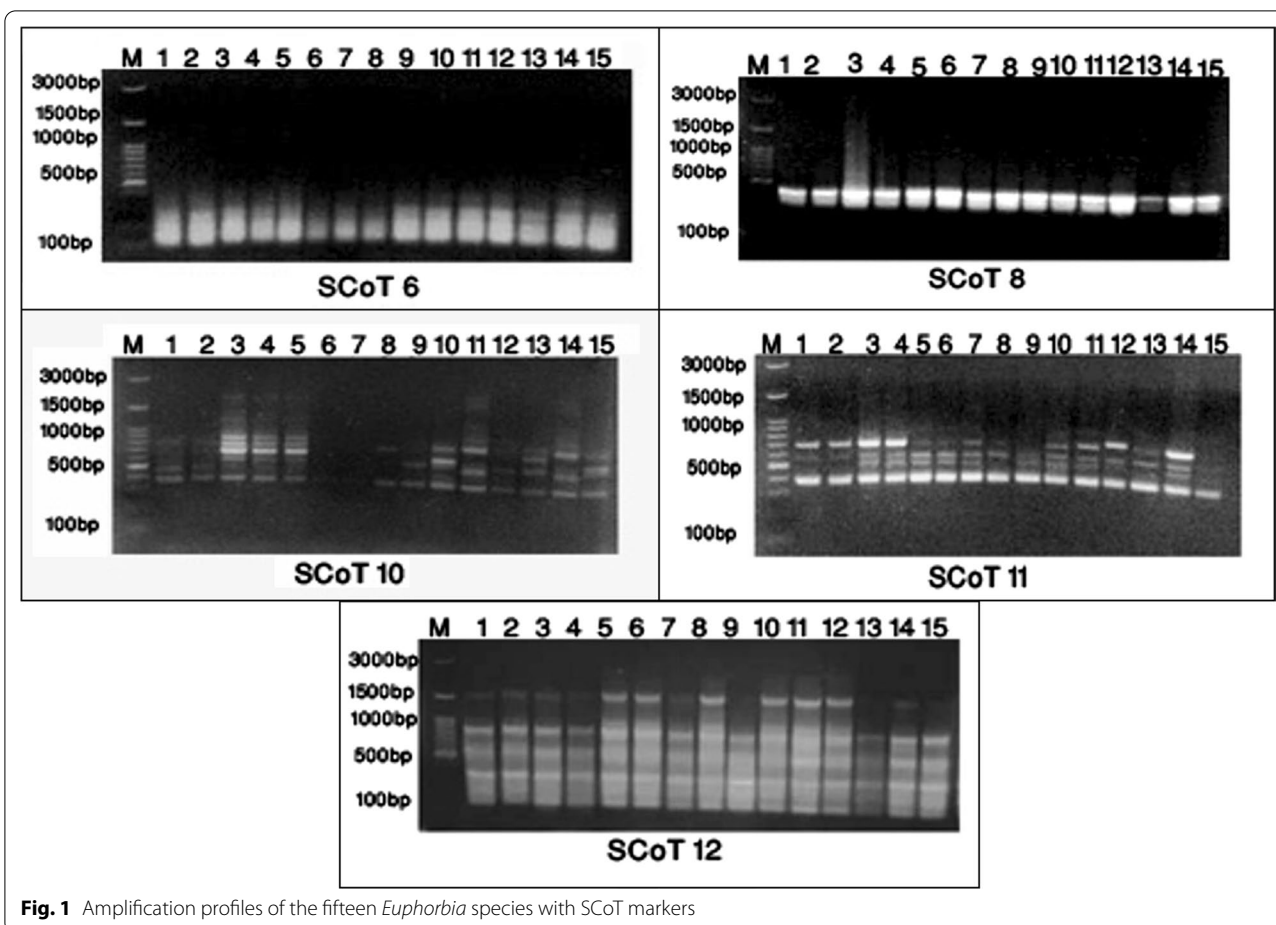
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Fig. 1 Amplification profiles of the fifteen *Euphorbia* species with SCoT markers

were segregated into two main clusters. The first cluster contained seven species, and the second contained the remaining eight.

3.2.3 Combination of ISSR and SCoT data obtained from the 15 Euphorbia species

The markers produced a total of 51 scorable bands (Table 6). The number of bands for each primer varied from 2 to 8 and ranged from 130 to 2465 bp. The maximum number of bands was obtained in SCoT 10 and 44B, and the lowest number of bands was recorded in SCoT 8. A monomorphic banding pattern was observed for all the amplified bands from SCoT and ISSR in all *Euphorbia* species. Dendrograms were generated for the two markers (Additional file 1: Figure 3) and revealed two major groups, one contained *E. abyssinica* J.F. Gmel. (1) and *E. caput-medusae* L. (2) and the other contained the remaining species.

Table 2 Total bands scored by SCoT markers

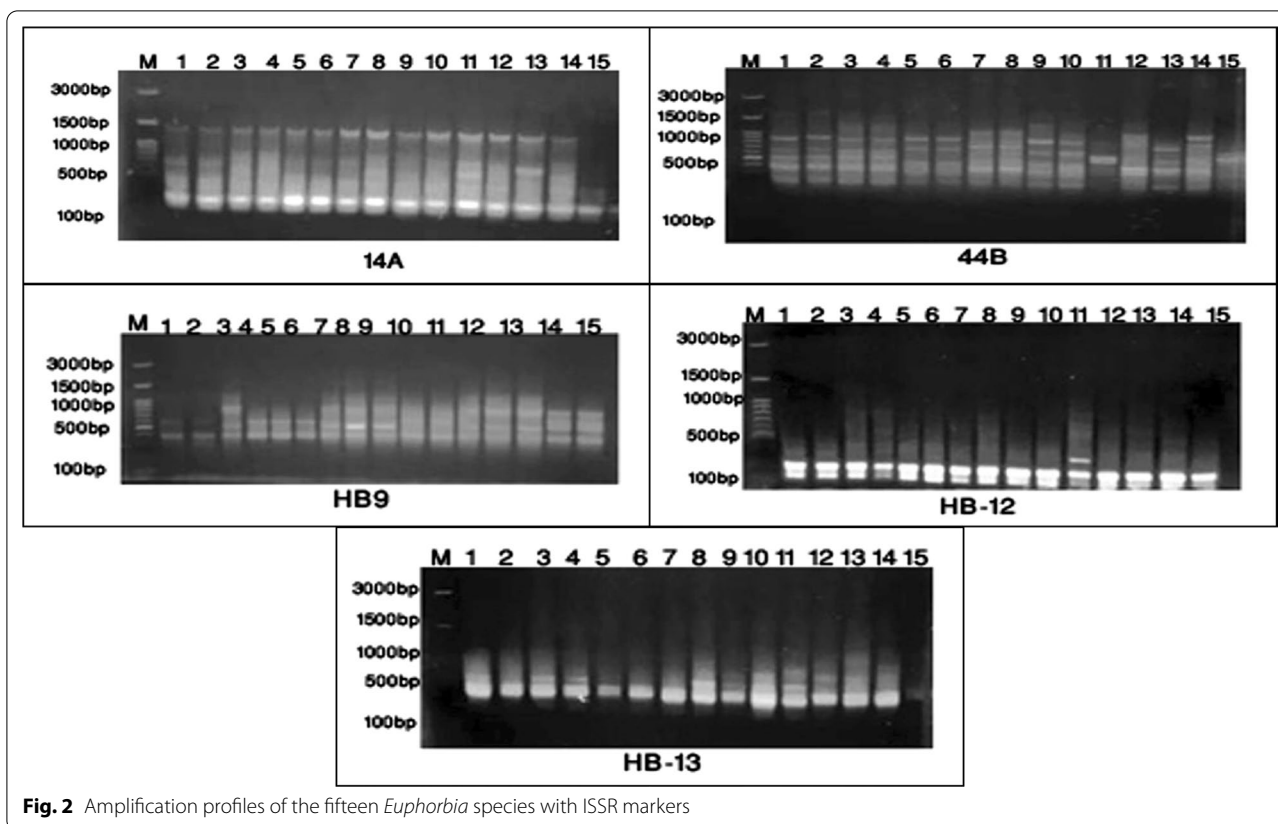| Marker name	Species														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
SCoT 6	3	3	4	4	4	3	3	3	4	4	4	4	4	4	3
SCoT 8	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
SCoT 10	3	3	7	6	6	3	4	4	3	4	7	4	4	6	4
SCoT 11	3	3	4	4	4	4	4	3	3	4	4	3	3	4	3
SCoT 12	6	6	6	6	7	7	6	7	6	7	7	7	5	6	6
Total	17	17	23	22	23	19	19	19	18	21	24	20	18	22	18

Fig. 2 Amplification profiles of the fifteen *Euphorbia* species with ISSR markers

3.2.4 *Similarity index combination of SCoT and ISSR analysis*

Results illustrated that the examined species were segregated according to the data obtained into two main clusters. The first cluster included only two species *E. abyssinica* J.F. Gmel. and *E. caput-medusae* L. with similarity 98.1% to each other and with the lowest similarities with the others. On the other hand, the second cluster was divided into two subclusters: Subcluster 1 contained six species, which showed some similarities with the remained species as it was between the first cluster and subcluster 2 and referred to *E. officinarum* L., *E. obesa* Hook., *E. canariensis* L., *E. lactea* Haw., *E. horrida* Boiss., and *E. milli* Des Moul. and Subcluster 2 contained seven species, which showed the lowest similarities with the first cluster and referred to *E. trigona* Mill., *E. stenoclada* Baill., *E. tithymaloides* L., *E. ingens* E. Mey., *E. grandialata* R.A. Dyer, *E. tirucalli* L., and *E. royleana* Boiss.

4 Discussion

4.1 Anti-trypanosomal activity

The anti-trypanosomal activity of three extracts of *E. hirta* Linn. (hexane, ethyl acetate, and methanol) was

Table 3 The distribution of monomorphic and polymorphic bands generated by the SCoT markers

Marker name	Total band	Total scored	Monomorphic band	Polymorphic band	Unique band	% Polymorphic
SCoT 6	4	54	3	1	–	25%
SCoT 8	2	30	2	–	–	0%
SCoT 10	8	68	1	7	(+)1 1735	87.5%
SCoT 11	4	53	2	2	–	50%
SCoT 12	7	9	5	2	(−)1 1500	28.57%
Total	25	300	13	12	2	48%

(+), positive unique, i.e., give only with this species; (−), negative unique, i.e., give with all species except this one

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Table 4 Total bands scored by ISSR markers

Marker name	Species														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
14A	5	5	6	6	5	5	5	6	6	6	6	6	5	6	3
44B	6	6	7	6	5	5	5	5	5	5	4	4	5	5	3
HB-9	3	2	4	3	3	3	3	4	4	3	4	4	4	4	4
HB-12	2	2	2	2	2	2	2	2	2	2	3	2	2	2	2
HB-13	2	2	3	3	2	2	2	4	3	3	3	4	4	4	3
Total	18	17	22	20	17	17	17	21	20	19	20	20	20	21	15

Table 5 The distribution of monomorphic and polymorphic bands generated by the ISSR markers

Marker name	Total band	Total scored	Monomorphic band	Polymorphic band	Unique band	% Polymorphic
14A	6	81	3	3	(−)2 1430 and 740	50%
44B	8	76	2	6	(−)1 315	75%
HB-9	5	52	2	3	-	60%
HB-12	3	31	2	1	(+)1 360	33.33%
HB-13	4	44	2	2	–	50%
Total	26	284	11	15	4	57.7%

(+), positive unique, i.e., give only with this species; (−), negative unique, i.e., give with all species except this one

evaluated against bloodstream forms of *T. brucei rhodesiense* and displayed activity with IC_{50} 9.91, 8.70, and 54.24 μg/mL, respectively [2]. Furthermore, 6,7-dimethoxynaphthalen-2(1H)-one isolated from *E. poisonii* stem showed activity against *T. brucei brucei* with MIC 1.56 μg/mL [15]. Moreover, 55 semisynthetic terpenoid derivatives obtained through chemical modifications of the major components of *E. resinifera* (α-euphol and α-euphorbol) and *E. officinarum* (obtusifoliol and 31-nor-lanosterol) have been evaluated against *T. cruzi* and 64% of the test compounds demonstrated anti-parasitic effects against *T. cruzi* [21]. The current study investigated the activity of 15 *Euphorbia* species against *T. brucei* strain TC 221, and results indicated high activity of four species with IC_{50} values < 20 μg/mL after 48-h and 72-h incubation. Among the tested species, *E. officinarum* L. was the most active (IC_{50} 6.2, 9.5 μg/mL, 48 and 72 h), followed by *E. milli* Des Moul. (IC_{50} 7.8, 9.3 μg/mL, 48 and 72 h), *E. royleana* Boiss. (IC_{50} 11.1, 13.6 μg/mL, 48 and 72 h),

and *E. abyssinica* J.F. Gmel (IC_{50} 17.3, 19.4 μg/mL, 48 and 72 h).

4.2 DNA analysis

Appropriate identification of plant materials is essential for the conservation of medicinal plant resources and to ensure the proposed biological efficacy. Molecular techniques have recently been used for plant diversity studies as well as for confirmation of the identity of plant species. [9] used multiple regions of DNA sequence data, cpDNA coding region matK including the partial trnK intron, the cpDNA coding region ndhF, and the internal transcribed spacer region of the nuclear ribosomal DNA (ITS), for taxonomic classification of *Euphorbia* species. Furthermore, RAPD and ISSR markers were reported to be used for genetics profiling of three *Euphorbia* species (*E. prostrata* Aiton*, E. peplus* L., and *E. terracina* L.) [22]. The current study uses two markers, SCoT and ISSR. Both markers were previously

Table 6 Combination of SCoT and ISSR scored bands

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Total SCoT	17	17	23	22	23	19	19	19	18	21	24	20	18	22	18
Total ISSR	18	17	22	20	17	17	17	21	20	19	20	20	20	21	15
Total	35	34	45	42	40	36	36	40	38	40	44	40	38	43	33

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used for the diagnostic fingerprinting of *Pistacia vera* cultivars [4]. Moreover, the ISSR marker was previously used to distinguish between three varieties of *E. milli* at the vegetative stage, and it was observed to be a useful molecular marker as it produced 98.7% polymorphism, which proved high genetic diversity [7]. Results of the present study revealed that both markers generated high numbers of polymorphic bands that could be used in the diagnostic fingerprinting of *Euphorbia* species. ISSR was found to be more efficient in clarifying the genetic diversity and homogeneity between the 15 tested *Euphorbia* species. This was indicated by the percentage of polymorphism (57.7%) recorded for ISSR compared to 48% for SCoT. Considering unique bands, that allowed easier and faster genetic identification, ISSR markers produced more unique bands (four bands) than SCoT marker (two bands). Phylogenetic relationships among six *Euphorbia* species were previously, analyzed by RAPD-PCR markers, and the resulted dendrogram indicated similarity between *E. milli* and *E. tirucalli* [3]. However, the current study recorded diversity between these two species according to both SCoT and ISSR markers. Accordingly, these results confirmed that using more than one marker is mostly recommended for a better analysis of genetic homogeneity of plants, as previously stated by [20].

5 Conclusions

The present study highlighted the anti-trypanosomal activity of 15 *Euphorbia* species where four species exhibited high activity against *T. brucei brucei* with *E. officinarum* L. being the most active. Genetic fingerprinting of the 15 tested species, using ISSR and SCoT markers, indicated six unique bands, one for *E. trigone* Mill., one for *E. lactea* Haw., two for *E. milli* Des Moul., and two for *E. grandialata* R.A. Dyer. Moreover, the dendrogram, generated for the two markers, revealed genetic similarity between *E. abyssinica* J.F. Gmel. and *E. caputmedusae* L. picked them in one cluster, while the other remained species were further subdivided under the second cluster. It concluded successful employment of ISSR and SCoT molecular markers for detecting levels of DNA polymorphism and genetic relationship in genus *Euphorbia*. ISSR is recommended for better analysis of homogeneity among *Euphorbia* species.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43088-021-00140-9.

Additional file 1. The nucleotide sequence of each marker, The result of each marker & the dendrograms of SCoT, ISSR and their combination.

Declarations

Ethical approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

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

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References

1. Abdelmohsen UR, Cheng C, Viegelmann C, Zhang T, Grkovic T, Ahmed S, Quinn RJ, Hentschel U, Edrada-Ebel R (2014) Dereplication strategies for targeted isolation of new antitrypanosomal actinosporins A and B from a marine sponge associated-*Actinokineospora* sp. EG49. Mar Drugs 12:1220–1244
2. Abiodun OO, Gbotosho GO, Ajaiyeoba EO, Brun R, Oduola AM (2012) Antitrypanosomal activity of some medicinal plants from Nigerian ethnomedicine. Parasitol Res 110:521–526. https://doi.org/10.1007/s00436-011-2516-z
3. Al-Anbari AK, Al-Hadeethi MA, Sleibi AT, AL-Taie AT, Ahmed LQ (2017) Phylogenetic relationships among *Euphorbia* species according to Random Amplification of Polymorphic DNA (Rapd-Pcr) technique. Int J Sci Nat 8:298–302
4. Baghizadeh A, Dehghan E (2018) Efficacy of SCoT and ISSR markers in assessment of genetic diversity in some Iranian *pistachio* (*Pistacia vera* L.) cultivars. Pist Heal J 1:37–43
5. Bani S, Kaul A, Khan B, Gupta VK, Satti NK, Suri KA, Qazi GN (2007) Anti-arthritic activity of a biopolymeric fraction from *Euphorbia tirucalli*. J Ethnopharmacol 110:92–98
6. Boissier E (1862) Subordo Euphorbieae in A. de Candolle. Prodromus 15:80
7. Chudasama K, Bhatt P, Chudasama K, Thaker V (2018) Molecular marker study in ornamental plant *Euphorbia milii*. J Pharmacogn Phytochem 7:882–888

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8. Dice LR (1945) Measures of the amount of ecologic association between species. Ecology 26:297–302
9. Dorsey BL, Haevermans T, Aubriot X, Morawetz JJ, Riina R, Steinmann VW, Berry PE (2013) Phylogenetics, morphological evolution, and classification of *Euphorbia* subgenus *Euphorbia*. Taxon 62:291–315
10. Douet JP, Castroviejo M, Mabru D, Chevalier G, Dupré C, Bergougnoux F, Ricard JM, Médina B (2004) Rapid molecular typing of *Tuber melanosporum, T. brumale* and *T. indicum* from tree seedlings and canned truffles. Anal Bioanal Chem 379:668–673
11. El-Hawary SS, Mohammed R, Tawfike AF, Lithy NM, AbouZid SF, Amin MN, Abdelmohsen UR, Amin E (2021) Cytotoxic activity and metabolic profiling of fifteen *Euphorbia* Species. Metabolites 11:15
12. Ernst M, Grace OM, Saslis-Lagoudakis CH, Nilsson N, Simonsen HT, Rønsted N (2015) Global medicinal uses of *Euphorbia* L. (Euphorbiaceae). J Ethnopharmacol 176:90–101
13. Hajibarat Z, Saidi A, Hajibarat Z, Talebi R (2015) Characterization of genetic diversity in *chickpea* using SSR markers, Start Codon Targeted Polymorphism (SCoT) and Conserved DNA-Derived Polymorphism (CDDP). Physiol Mol Biol Plants 21:365–373. https://doi.org/10.1007/s12298-015-0306-2
14. Huber W, Koella JC (1993) A comparison of three methods of estimating EC_{50} in studies of drug resistance of malaria parasites. Acta Trop 55:257–261
15. Igoli JO, Gray AI, Clements CJ, Mouad HA (2011) Anti-Trypanosomal activity and cytotoxicity of some compounds and extracts from Nigerian Medicinal Plants. In: Rasooli I (ed) Phytochemicals—bioactivities and impact on health. In Tech, Shanghai, China, pp 375–388
16. Jacobs TR, Nare B, Phillips MA (2011) State of the art in African trypanosome drug discovery. Curr Top Med Chem 11:1255–1274
17. Kalendar R, Flavell AJ, Ellis THN, Sjakste T, Moisy C, Schulman AH (2011) Analysis of plant diversity with retrotransposon-based molecular markers. Heredity (Edinb) 106:520–530
18. Kamal N, Viegelmann CV, Clements CJ, Edrada-Ebel R (2017) Metabolomics-guided isolation of anti-trypanosomal metabolites from the endophytic fungus *Lasiodiplodia theobromae*. Planta Med 83:565–573
19. Kumar H, Priya P, Singh N, Kumar M, Choudhary BK, Kumar L, Singh IS, Kumar N (2016) RAPD and ISSR marker-based comparative evaluation of genetic diversity among Indian germplasms of *Euryale ferox*: an aquatic food plant. Appl Biochem Biotechnol 180:1345–1360. https://doi.org/10.1007/s12010-016-2171-z
20. Lakshmanan V, Reddampalli Venkataramareddy S, Neelwarne B (2007) Molecular analysis of genetic stability in long-term micropropagated shoots of banana using RAPD and ISSR markers. Electron J Biotechnol 10:106–113
21. Mazoir N, Benharref A, Bailén M, Reina M, González-Coloma A, Martínez-Díaz RA (2011) Antileishmanial and antitrypanosomal activity of triterpene derivatives from latex of two *Euphorbia* species. Zeitschrift für Naturforsch C 66:360–366
22. Moustafa M, Mostafa O, Al-Shahrani D, Alrumman S (2016) An application of genetics-chemicals constituents to the relatedness of three *Euphorbia* species. Biologia (Bratisl) 71:1240–1249
23. Rabeh M, El-Hawary SSE-D, El-Tantawy ME-M, Abdelmohsen UR, Badr WK (2020) Survey the genetic diversity of eight *Opuntia* species from Egypt using Random Amplified Polymorphic DNA (RAPD) technique. J Adv Biomed Pharm Sci 3:40–44
24. Samidurai K, Mathew N (2014) Bioassay guided fractionation and GC–MS analysis of *Euphorbia lactea* extract for mosquito larvicidal activity. Int J Pharm Pharm Sci 6:344–347
25. Sehgal L, Paliwal GS (1974) Studies on the leaf anatomy of *Euphorbia* II. Venation patterns. Bot J Linn Soc 68:173–208
26. Simarro PP, Diarra A, Postigo JAR, Franco JR, Jannin JG (2011) The human African trypanosomiasis control and surveillance programme of the World Health Organization 2000–2009: the way forward. PLoS Negl Trop Dis 5:e1007
27. Small JK (1933) Manual of the Southeastern Flora (reprint). Univ. North Carolina Press, Chapel Hill, pp 15–54
28. Thakur J, Dwivedi MD, Sourabh P, Uniyal PL, Pandey AK (2016) Genetic homogeneity revealed using SCoT, ISSR and RAPD markers in micropropagated *Pittosporum eriocarpum* Royle-an endemic and endangered medicinal plant. PLoS ONE 11:e0159050
29. Webster GL (1967) The genera of Euphorbiaceae in the southeastern United States. J Arnold Arbor 48:363–430
30. Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535
31. Yang X, Quiros C (1993) Identification and classification of celery cultivars with RAPD markers. Theor Appl Genet 86:205–212

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