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RESEARCH

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Chemical profiling of antifungal *Dicerocaryum senecioides* and *Diospyros mespiliformis* extracts using TLC-*p-iodonitrotetrazolium* violet assay and GC–MS/MS

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Abstract

Background Despite the availability of conventional medication for fungal skin diseases, dermatophytic infections are now a major crisis in the whole world due to multidrug resistance. In an effort to search for complementary and alternative remedies, this study evaluated the chemical composition of *Dicerocaryum senecioides* and *Diospyros mespiliformis* leaf and fruit extracts, respectively, exhibiting significant in vitro antifungal activity. Ethyl acetate extracts of each plant were screened for antifungal activity against three fungi species: *Candida albicans, Trichophyton rubrum* and *Epidermophyton floccosum* using disc diffusion and poisoned food assays. Gas chromatography–tandem mass spectrometry (GC–MS/MS) was used to evaluate the chemical composition after isolation of active bands by thin-layer chromatography-*p*-iodonitrotetrazolium chloride assay.

Results The composite crude ethyl acetate extracts of *D. mespiliformis* and *D. senecioides* (1:1) exhibited concentration-dependent antifungal potencies. *C. albicans* was susceptible to the crude extracts up to a minimum concentration of 6.25%. *E. floccosum* and *T. rubrum* were more susceptible up to a minimum extract concentrations of 1%. GC–MS/MS analysis of the isolated active TLC bands revealed bioactive compounds including flavonoids (quercetin, luteolin), together with their O-glycosylated counter parts and the C-monoglycosylated flavonoids (vitexin, orientin, isovitexin and isoorientin), terpenoids, esters and other phenolic acids. Some of the compounds were common to both plants.

Conclusion The findings of this study show that *D. senecioides* and *D. mespiliformis* extracts consist of important bioactive compounds to warrant their use in complementary and alternative poly-herbal formulations against fungal infections.

Keywords *D. senecioides*, *D. mespiliformis*, Antifungal, Phytocompounds

Background

Gradual betterment in therapy of human diseases has greatly increased the life expectancy of immune-supressed individuals. Consequently, proliferation of risk factors is inevitable and favours the onrush of other diseases, especially infectious diseases. Among the diseases, fungal infections in humans represent a substantial

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quota [1]. Fungal infections are a major problem affecting humans daily and they are many cases of multidrug resistance [2]. Dermatophytosis is a major cause of morbidity related to superficial mycoses, frequently relapsing and predominantly recalcitrant to therapy [3]. *Trichophyton, Microsporum*, and *Epidermophyton* are the predominant causative agents of superficial mycotic infections [4]. Fungi have remained among the most problematic pathogens as evidenced by the use of amphotericin B (discovered in 1956) as the "Gold Standard" for antifungal therapy [1]. Due to the rise in the number of people with compromised immune systems due to HIV infection, chemotherapy and diabetes among other ailments, there has been a noticeable increase in the cases of fungal infections in the last few decades [5, 6]. Fungal species that are becoming resistant to available therapies are on the rise in the systems of individuals due to exposure to many drugs (multidrug resistance) [7]. Identifying natural compounds that have the capability to circumvent multidrug resistance with negligible adverse side effects offers a comely option to production of complementary and alternative poly-herbal remedies. In addition, there is a need to unravel novel drug(s) from the natural sources whose mode of action would selectively act on new target with fewer or no side effects [8]. Various ethnic groups use plants in medicinal applications [9]. Plants are a fountainhead of multiplex potent drugs for the pharmaceutical industry owing to the diverse bioactive compounds (secondary metabolites) they possess [10, 11]. Phytocompounds are ultra-practical in managing of some health disorders through their individual, additive, or synergistic action [12]. Phenolics, stilbenoids, tannins, coumarins, saponins, alkaloids, terpenes and terpenoids are some of the bioactive metabolites found in plant species [13]. Polyphenols, of which flavonoids and phenolic acids are the commanding group that have potential health benefits depending on their antioxidant and many antimicrobial activities including antifungal activity. Phenolic compounds are also well known to provide resistance to plant pathogens such as fungal species [14]. Similarly, terpenes and terpenoids have been reported to have significant antifungal activity [15]. Plant secondary metabolites are of utmost importance in the development of new leads and in preparation of therapeutic agents against fungal infections, [16]. Herein, the aim was to investigate the phytochemical composition of leaf extracts of *D. senecioides* and raw fruit extracts of *D. mespiliformis* isolated using TLC-directed protocol against *Trichophyton rubrum, Epidermophyton floccosum* and *Candida albicans*. Leaf extracts of *D. senecioides* and raw fruit extracts of *D. mespiliformis* are widely used to treat fungal infections in ethnomedicine however bioassay directed extraction and phytochemical identification of the active compounds has not been conducted. It is important to screen and identify the compounds for the benefit of the medicinal and pharmaceutical chemists. The identified compounds can be potential lead compounds for antifungal agents that can work against fungal resistance.

Methods

Plant samples and extraction process

Fresh plant materials of *D. senecioides* were collected from Mberengwa, Zimbabwe, in February 2021 by following WHO guidelines for collecting plants botany 440/540, and voucher specimens were deposited in the university plant library for future reference. The leaves and stems of *D. senecioides* were cleaned with running tap water and then distilled water, and air-dried under a shade after which the plant material was pulverised. Dried powder, 10 g were macerated in ethyl acetate with intermittent shaking for 72 h. The marc was removed by filtration. The extraction process was done in triplicate using fresh ethyl acetate each time. The menstruum was collected, bulked and concentrated on a rotary evaporator, [17], and dried in a fume-hood. Dried extracts were stored in amber bottles at 4 °C until further analysis. Fresh raw fruits of *D. mespiliformis* were collected from Mudzi district, Zimbabwe, and were used as fresh samples. The same process was done for *D. mespiliformis*.

Antifungal assays

Disc diffusion assay against C. albicans

Sterile paper discs saturated with crude extracts separately at 50, 25, 12.5 and 6.25% concentrations were placed using *sterilised* forceps on the surface of Sabouraud dextrose agar medium inoculated with clinical *C. albicans* isolates adjusted to 0.5 McFarland standard by suspending the colonies in 5 ml of sterile 0.85% saline and turbidity adjusted to 1.0×10^5 to 1.0×10^6 spores/ml. The plates were incubated overnight at 25 ℃ after which zones of inhibition around the discs were measured. Miconazole was used as the positive control and ethyl acetate only as negative control. Sterilisation of equipment was conducted in an autoclave.

Poisoned food assay against T. rubrum and E. floccosum

Antifungal activity of the crude plant extracts on radial growth of *T. rubrum* and *E. floccosum* was evaluated using poisoned agar method. Sabouraud dextrose agar (SDA) was aseptically prepared under sterile conditions. The sterile media were supplemented with varying dilutions of extracts 50%, 25% and 1% mixture (1:1) of both plant extracts. Fifteen millimetres of the poisoned agar was dispensed into sterile triplicate plates and left to congeal. Discs of the test organisms with a diameter of 5 mm

were prepared from the growing cultures and placed on the poisoned media. Plain agar and miconazole were used as the negative and positive controls, respectively. The plates were left under incubation at 25 ℃ for 7 days before diameters of fungal colonies were measured. The antifungal effect (percent inhibition of fungal growth) was estimated using the formula:

$$\%\text{Inhibition} = \left(\frac{\text{Dc} - \text{Ds}}{\text{Dc}}\right) \times 100$$

where Dc is diameter of fungal colony in control plate and Ds is the diameter of fungal colony in test agent plate.

Thin-layer chromatography and thin-layer chromatography-p-iodonitrotetrazolium violet assay

Chemical constituents of the plant extracts were separated on aluminium-backed analytical TLC plates (silica gel F_{254}, 10×10 cm, 0.25 mm thickness, Merck). Development was done using solvent systems developed in the laboratory, ethyl acetate/methanol/water (11:6:1.5) for *D. senecioides* and hexane/acetone (7:3) for *D. mespiliformis*. A 0.1 mg/ml solution of each extract was separately loaded on TLC plates in a narrow band and eluted using the solvent systems developed in the laboratory. The developed plates were dried in a fume hood to remove traces of solvent. The fungal isolate was shake-incubated separately in Sabouraud dextrose broth at 25 °C for 7 days. To remove mycelia, the culture was filtered through a triple layer gauze cloth. The filtrate was centrifuged for 5 min. A spray emulsion of spores was prepared in dehydrated Sabouraud dextrose agar. Spores were added to 15 ml of SDA solution to give a concentration of approximately 1×10^6 spores/ml. The inoculated medium was sprayed on the developed TLC plates rapidly to avoid solidification. The plates were incubated at 25 °C. Active bands were revealed by spraying with p-iodonitrotetrazolium chloride.

Isolation of the antifungal compounds

The extracts were applied to preparative TLC plates (silica gel F_{254}, 20×20 cm, 0.25 mm thickness, Merck) and developed using their respective solvent systems as discussed in previous section. The active bands were marked using R_f values. The bands were scrapped off into separate containers and extracted from the silica gel with their original respective extracting solvents. The containers were centrifuged for 15 min. Resulting supernatants were dried. The isolated active compounds were kept in amber vials in a refrigerator until required for GC–MS/MS analysis.

Gas chromatography–tandem mass spectrometry analysis of active bands

GC–MS/MS analysis of active phytocompounds from *D. senecioides* and *D. mespiliformis* was carried out using a PerkinElmer Carus 680 GC-Clarus SQ 8T MS equipped with Elite-5 MS 30 m×250 µm×25 µm column. Spectroscopic GC–MS/MS detection by GC–MS/MS was done using an electron ionisation system (70 eV) over the range of 50–650 mass/charge ratio in full scan mode. The temperatures of the ion source and transfer line were 250 °C and 270 °C, respectively. Carrier gas was ultrapure helium with a constant flow rate of 1 mL/min. The column oven temperature initially set at 50 °C, then increased by 5 °C per minute to 150 °C and holding time of about 10 min then increased to 300 °C at a rate of 30 °C per minute. Solvent delay was 5 min. Diluted samples of 1 µL were automatically injected using auto-sampler AS3000 coupled with GC using the split mode. The components were identified by comparing retention times and mass spectra to those of the National Institute of Standards and Technology, NIST14 Mass Spectral (MS) Library Search Program. Compounds with a library match ≥ 90% were considered.

Results

Antifungal assays

Figure 1 represents the in vitro susceptibility of *C. albicans* to crude ethyl acetate extracts of *D. mespiliformis* (A) and *D. senecioides* (B), where 1, 2, 3, 4, and 5 represent 50, 25, 12.5, 6.25% extract concentration and negative control, respectively, while + is positive control miconazole (Tables 1, 2).

Figures 2 and 3 represent the preliminary antifungal susceptibility test results for the two plants against *E. floccosum* and *T, rubrum*.

Fig. 1 Disc diffusion assay against *C. albicans*, + = miconazole

Table 1 In vitro activity of the plant extracts against *C. albicans*

Disc concentration	Diameter of zone of inhibition/mm	
	A	*B*
1	17.00	16.00
2	16.00	15.00
3	15.00	14.00
4	15.00	14.00
5	–	–
Positive control	–	–

Table 2 Effect of composite extracts (1:1 ratio) on radial growth of *T. rubrum* and *E. floccosum*

Treatment	% Radial growth inhibition	
	E. floccosum	*T. rubrum*
50%	80.00	73.82
25%	75.00	67.88
1%	20.00	63.48
Positive control	73.30	57.50
Negative control	–	–

presentation of the results is given in Fig. 4a-d.

Figure 5a–d shows GC spectra for 4 selected active bands with R_f values of 0.94; 0.88; 0.64 and 0.78 presented as (a) to (d), respectively.

Tables 4, 5, 6 and 7 represent GC–MS/MS phytochemical characterisation of the bioactive bands under study.

Discussion

Poly-herbal medicine is a major part of treatment systems which is gaining popularity everyday due to clinical failures of conventional medicines due to drug resistance fungal strains [2]. The use of herbal medicine is based on practice and concepts emanating from ancient philosophies. In the current era of evidence-based medicine, it is important to accurately assess composition and efficacy of herbal medicine. In the present effort, chemical profiling of antifungal compounds of *D. senecioides* and *D. mespiliformis* was done using disc diffusion, poisoned food, p-iodonitrotetrazolium chloride and GC–MS/MS analyses. The study followed the poly-herbal formulation philosophy which has an advantage of achieving greater efficacy at low concentration that is likely to be safe to humans.

Preliminary anti-dermatophytic potency experiments were done using concentrations of 50, 25 and 1% made up of crude extracts of the 2 plants under study mixed in a 1:1 ratio. Radial growth inhibition of *E. floccosum* colonies was 80.00, 75.00, 20.00% at the aforementioned extract concentrations, respectively, and 73.30% for the positive control. *T. rubrum* was comparably inhibited at

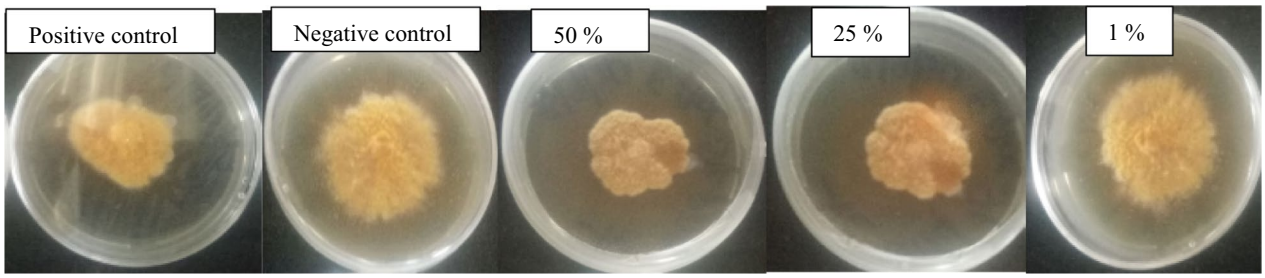

Fig. 2 Inhibition of radial growth in *E. floccosum* by *D. senecioides* and *D. mespiliformis* extracts

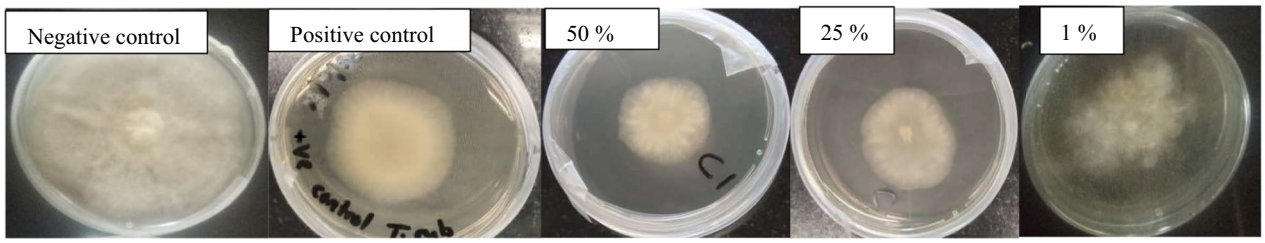

Fig. 3 Inhibition of radial growth in *T. rubrum* by *D. senecioides* and *D. mespiliformis* composite extracts (1:1 ratio), positive control = miconazole, negative control agar and ethyl acetate

GC–MSMS analysis of active bands and TLC and TLC-*p*-iodonitrotetrazolium violet assay

Table 3 summarises results obtained in TLC and TLC-*p*-iodonitrotetrazolium violet assays. A pictorial

Table 3 Summary of TLC and TLC-*p*-iodonitrotetrazolium chloride assay results

Plant	R_f value of active compound	Organism	Figure
D. senecioides	0.94	*T. rubrum*	3.2c
	0.88	*E. floccosum*	3.2d
D. mespiliformis	0.64	*E. floccosum*	3.2a
	0.78	*T. rubrum*	3.2b

R_f = *retention factor*

the same concentrations producing percentage inhibition of 73.82, 67.88 and 63.48, respectively, while the control inhibition was 57.50% (Table 2). At 1% concentration of extract, *E. floccosum* exhibited very low susceptibility compared to *T. rubrum* although the later had visibly disturbed mycelial growth (Figs. 2 and 3). *E. floccosum* was generally more susceptible to the plant extracts at higher concentrations than *T. rubrum*. Generally the extract proved to have potent activity against the test species. The results obtained in this study provide an impetus for further research as very little information is documented on antifungal activity of *D. senecioides*. Research done by [18], on *D. mespiliformis* leaves and bark extracts revealed efficacy in the range between 0.02 and 2.5 mg/ml against *C. albicans* and 0.02–0.16 mg/ml against *T. rubrum.* Little research has been documented on antifungal activity of raw fruit extracts despite their wide use in traditional practices.

In vitro antifungal activity of ethylacetate extracts of *D. senecioides* and *D. mespiliformis* against *C. albicans* is shown in Table 1, while Figs. 2 and 3 show inhibition of radial growth in *E. floccosum* and *T. rubrum*, respectively, by the same extracts. Table 1 gives a numerical summary of the percent inhibition of radial growth in *E. floccosum* and *T. rubrum*. In vitro antifungal analysis of the extracts showed that the extract consist of significant antifungal activity. The 1:1 mixture of *D. senecioides* and *D. mespiliformis* extracts exhibited greater antifungal activity against *C. albicans* (Fig. 1) than the standard drug miconazole. Figure 2 shows that at a concentration of 50 and 25%, the efficacy against *E. floccosum* of the polyherbal mixture was comparable to that of miconazole. No effect was observed at 1%. The polyherbal mixture was very effective against *T. rubrum* even at a low concentration of 1%. The efficacy was significantly greater (Fig. 3 and Table 2) than that of miconazole.

Using the p-iodonitrotetrazolium chloride assay to screen and isolate the active potions and GC–MS/MS analysis, many bioactive phytochemicals were identified (Fig. 5a–d). The list of compounds identified in the active bands of the extracts through GC–MS/MS are presented in Tables 4, 5, 6, and 7 for bands with R_f values of 0.98, 0.88 (*D. senecioides*) 0.64 and 0.78 (*D. mespiliformis*), respectively. Only compounds with match score ≥ 90% were considered. The 0.98 band of *D. senecioides* consisted of flavonoids and flavonoid glycosides amounting to 10 flavonoid compounds. From *D. mespiliformis* extract, a total of 6 flavonoid compounds were obtained from the 0.78 band. In a separate phyto-constituent study by Hawas et al. [19] of *D. mespiliformis* leaf extracts a new acylated flavone isoscutellarein 7-O-(4‴-O-acetyl)-β-allo- pyranosyl(1‴→2″)-*β*-glucopyranoside, together with 8 known flavonoid metabolites, luteolin 3′,4′,6,8-tetramethyl ether, luteolin 4′-O-*β*-neohesperidoside, luteolin 7-O-*β*-glucoside, luteolin, quercetin, quercetin 3-O-*β*-glucoside, quercetin 3-O-α-rhamnoside, and rutin were isolated and identified using spectroscopic data (Ultra Violet, Nuclear Magnetic Resonance, and Mass Spectroscopy). The methylated flavone

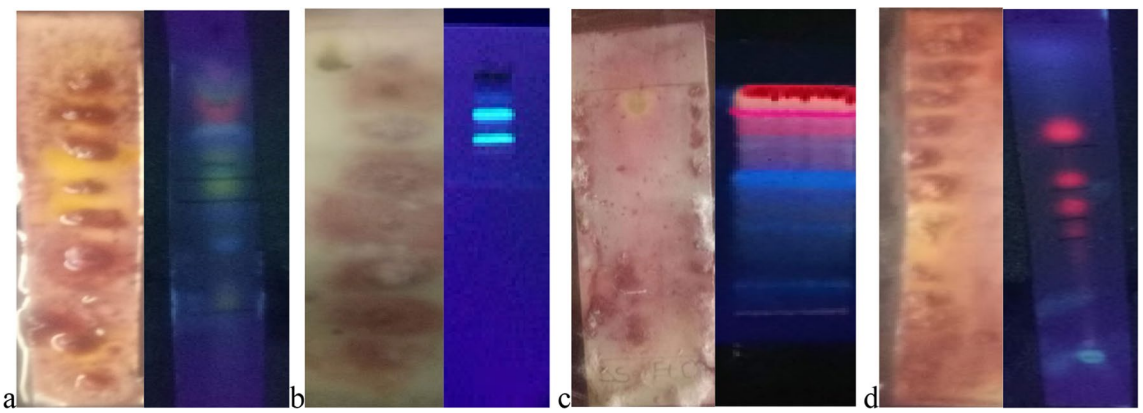

Fig. 4 **a**–**d** Thin-layer chromatograms and TLC-*p*-iodonitrotetrazolium chloride results for *E. floccosum* and *T. rubrum*. **a** TLC-Bioautogram (*E. floccosum*) and chromatogram for *D. mespiliformis* **b** TLC-Bioautogram (*T. rubrum*) and chromatogram for *D. mespiliformis*. **c** TLC-Bioautogram (*E. floccosum*) and chromatogram for *D. senecioides*. **d** TLC-Bioautogram (*T. rubrum*) and chromatogram for *D. senecioides*

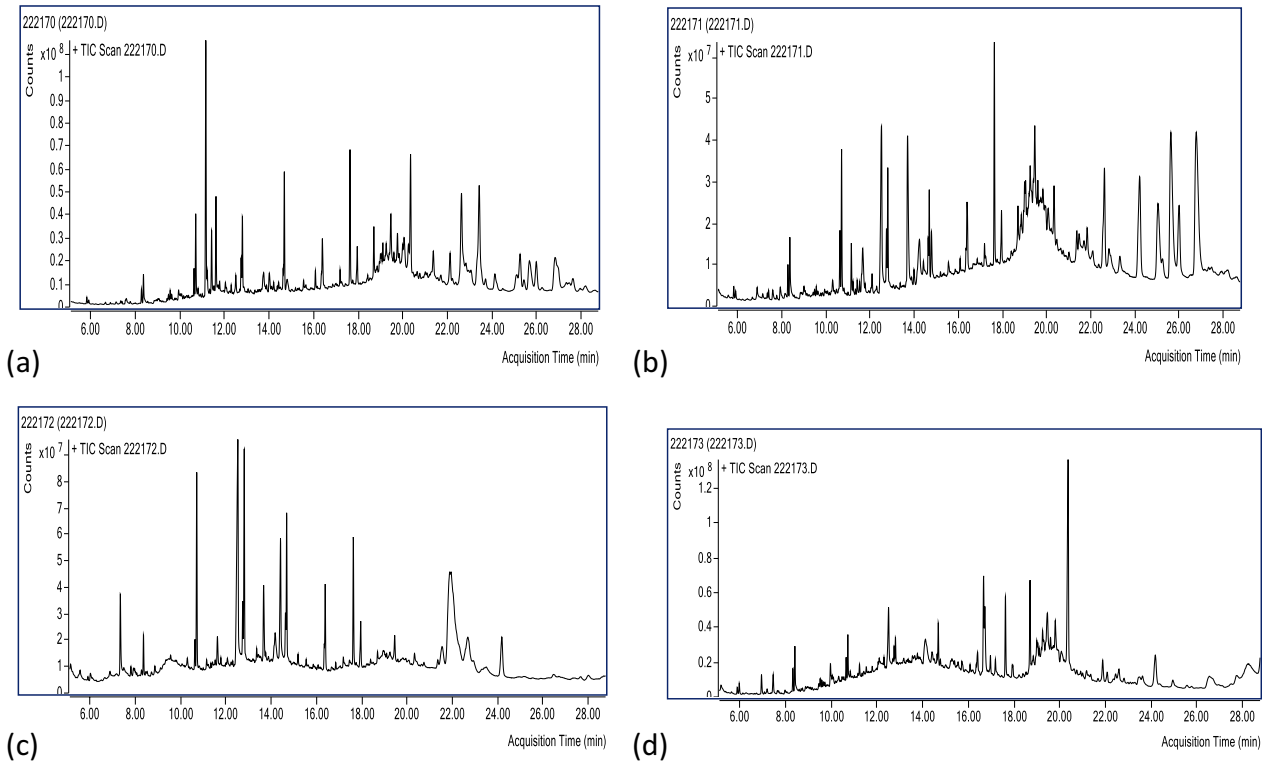

Fig. 5 **a**–**d** Chromatographic profiles of 4 bioactive bands

Table 4 GC–MS/MS characterisation of ethylacetate extract band of *D. senecioides* ($R_f = 0.98$)

Retention time	Compound name	Match score	CAS#	Formula
10.610	Luteolin	95.2	491-70-3	$C_{15}H_{10}O_6$
11.014	Quercetin	96.2	117-38-5	$C_{15}H_{10}O_7$
11.425	Luteolin-3′,4′,6,8-tetramethyl ether	93.9	855-97-0	$C_{19}H_{18}O_6$
11.503	Quercetin-3-O-*β*-glucoside	95.9	482-35-9	$C_{21}H_{12}O_{12}$
12.881	Quercetin-3-O-*α*-rhamnoside	99.2	55696-57-6	$C_{21}H_{20}O_{11}$
14.650	Luteolin-3-O-*β*-glucoside	94.4	154-41-6	$C_{21}H_{20}O_{11}$
17.719	Luteolin-4′-O-*β*-neohesperidoside	95.3	25694-72-8	$C_{27}H_{30}O_{15}$
20.539	Rutin	97.8	153-18-4	$C_{27}H_{30}O_{16}$
22.591	Luteolin-7-O-*β*-glucoside	91.4	5373-11-5	$C_{21}H_{20}O_{11}$
23.533	Quercitrin	90.3	522-12-3	$C_{21}H_{20}O_{11}$

isolated from methanolic leaf extracts of the plant exhibited potent activity against *C. albicans* with an inhibition zone of 25 mm [19], while in preliminary antifungal assay in the current study potency of the crude extracts of the plants under study herein against *C. albicans* was between 15 and 17 mm at 6.25–50% extract concentration. No zones were obtained against *C. albicans* for both the positive and negative controls (Table 1). Stem bark extracts exhibited potency against *C. albicans* at 12.5 mg/ml producing a zone of inhibition of 13.00 ± 0.00 mm [10]. The current results further affirm the potency of *D. mespiliformis* against *C. albicans*. TLC chromatograms and bioautograms of the raw fruit extracts of *D. mespiliformis* and stem/leaves extracts of *D. senecioides* (1:1) mixture (Fig. 4a–d) revealed antifungal compounds with potency against *E. floccosum* and *T. rubrum*. Active compounds against *E. floccosum* had R_f value of 0.88 in *D. senecioides* and 0.64 in *D. mespiliformis* while potency against *T. rubrum* was observed at R_f values of 0.94 and 0.74 in *D. senecioides* and *D. mespiliformis,* respectively.

Table 5 GC–MS/MS characterisation of ethylacetate extract band of *D. senecioides* (R_f = 0.88)

Retention time	Compound name	Match score	CAS#	Formula
10.792	[15-13CD3]-5,6-Dihydroartemisinic acid	92.7	2000312-89-7	$C_{15}H_{24}O_2$
12.590	2-Benzylidene-3-oxo-4-(octylsulfanyl)-2,3-dihydrothiophene-1-dioxide	99.5	0-00-0	$C_{12}H_{22}O_2S_2$
12.766	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	92.1	6422-86-2	$C_{24}H_{38}O_4$
13.866	Terephthalic acid, di(2-ethylhexyl) ester	91.1	2000741-13-7	$C_{24}H_{38}O_4$
14.877	1,6a(3H)-Pentalenedicarboxylic acid, 6-cyano-3a,4,5,6-tetrahydro-2-hydroxy-3,3-dimethyl-, dimethyl ester, (3a.alpha.,6.alpha.,6a.alpha.)-(.+-.)-	90.0	76679-89-5	$C_{15}H_{19}NO_5$
16.398	Phthalic acid, bis(7-methyloctyl) ester	90.1	20548-62-3	$C_{26}H_{42}O_4$
17.667	2-[3′-Methoxy-1′,4′-bis(methoxycarbonyl)-1′,3′-butadien-2′-yl]-1-(methoxyimino)-6,7-bis(methoxy)-1,2,3,4-tetrahydronaphthalene	91.1	2000804-97-5	$C_{22}H_{27}NO_8$
22.541	gamma.-Tocopheryl methyl ether	91.9	0-00-0	$C_{29}H_{50}O_2$
24.228	(.±.)-.alpha.-Tocopherol acetate	94.8	7695-91-2	$C_{31}H_{52}O_3$
25.054	(3methyl,24R)-ergost-5-en-3-ol	93.0	474-62-4	$C_{28}H_{48}O$
25.742	(22E)-Stigmasta-5,22-dien-3-ol	90.5	83-48-7	$C_{29}H_{48}O$
26.057	Benzenamine, 4-(1,1,3,3-tetramethylbutyl)-N-[4-(1,1,3,3-tetramethylbutyl)phenyl]-	91.0	15721-78-5	$C_{28}H_{43}N$
27.100	Hederagenin	96.6	2000843-76-6	$C_{30}H_{48}O_4$

Table 6 GC–MS/MS characterisation of ethylacetate extract band of *D. mespiliformis* (R_f = 0.64)

Retention time	Compound Name	Match Score	CAS#	Formula
7.331	Phenol, 2,4-bis(1,1-dimethylethyl)-	98.1	96-76-4	$C_{14}H_{22}O$
8.518	3-Hydroxy-4-methoxyacetophenone	91.6	2000107-68-7	$C_9H_{10}O_3$
10.670	8-(Acetyloxy)-9-[(acetyloxy)methyl]-5-methylbicyclo[3.3.1]non-3-ene	91.9	2000408-51-4	$C_{15}H_{22}O_4$
11.652	5-Hydroxy-4-methoxy-1,3-benzodioxole	91.5	2000112-00-6	$C_8H_8O_4$
12.596	(.±.)-.alpha.-Tocopherol acetate	96.5	7695-91-2	$C_{31}H_{52}O_3$
12.897	3-(3,5-Dimethoxyphenyl)propanal	94.8	2000181-19-6	$C_{11}H_{14}O_3$
13.810	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	92.9	6422-86-2	$C_{24}H_{38}O_4$
14.580	4-Chloro-4′-methoxy-tetra-deuterobiphenyl	91.0	0-00-0	$C_{13}H_7D_4ClO$
14.767	9H-Purine, 6-chloro-2-fluoro-9-(2,3,5-tri-O-acetyl-.beta.-D-ribofuranosyl)-	95.2	13276-51-2	$C_{16}H_{16}ClFN_4O_7$
16.452	Benzoic acid, 3-[(2,4-dimethoxy-6-propylbenzoyl)oxy]-2-hydroxy-4-methoxy-6-propyl-	90.6	69563-42-4	$C_{23}H_{28}O_8$
17.739	2-Pentene-1,4-dione, 1-(4-methoxyphenyl)-, 4-(dimethylhydrazone)	93.8	80569-12-6	$C_{14}H_{18}N_2O_2$
18.017	(3R,4R)-3,4-Bis(4-Hydroxy-3-methoxybenzyl)tetrahydrofuran	92.0	2000641-41-9	$C_{20}H_{24}O_5$
22.192	N-(2-Chloro-3-(2-cyano-1-hydroxyallyl)-7-phenoxyquinolin-6-yl)methanesulfonamide	90.1	2000799-80-0	$C_{20}H_{16}ClN_3O_4S$
24.220	2-(Phenyl)-4-(4-methylpiperazin-1-yl)-6-iodoquinazoline	94.1	2000800-77-0	$C_{19}H_{19}IN_4$

Table 7 GC–MS/MS characterisation of ethylacetate extract band of *D. mespiliformis* (R_f = 0.78)

Retention time	Compound name	Match score	CAS#	Formula
12.524	Quercetin	97.9	117-39-5	$C_{15}H_{10}O_7$
16.549	Isoquercetin	96.5	482-35-9	$C_{23}H_{32}O_7$
17.563	Vitexin	92.5	3681-93-4	$C_{21}H_{20}O_{10}$
18.649	Isovitexin	94.2	3895-3-85–4	$C_{21}H_{20}O_{10}$
20.262	Orientin	90.2	28608-75-5	$C_{21}H_{20}O_{11}$
24.168	Isoorientin	91.9	4261-42-1	$C_{21}H_{20}O_{11}$

GC spectra of these bands are shown in Fig. 5a–d. The 0.94 and 0.78 bands according to GC-MS/MS analysis consisted of flavonoid compounds (Tables 4 and 5). Flavonoids and flavonoid derivatives have been proved to have potent antifungal activity against many fungal pathogens including *C. albicans* and *T. rubrum* with efficacies ranging from concentrations as low as 1.95 µg/ml, [20]. The R_f 0.64 band was found to be a mixture of phenolic compounds, aromatic compounds and esters (Table 6). Phenolic compounds are known to provide resistance to plant pathogens, fungal species included [21, 22].

The band at R_f (0.88) was mainly made up of terpenoids, terpenes and methyl esters. Terpenes and terpenoids have antifungal potential. Previous studies have proved that crude terpenoids can inhibit mycelial growth in fungi [23], affect DNA (deoxyribonucleic acid), RNA (ribonucleic acid) and protein synthesis, disrupt cell membrane permeability and also disturb metabolic activity in fungi [23]. Other terpenoid compounds interfere with formation and viability of hyphae and induce morphological alterations in the envelopes of fungi [24]. The biological activity of these compounds in this study is indicative of the medicinal potential of the investigated plants. Inarguably, some of these compounds have different pharmacological activities; hence, the possibility of mutually advantageous conjunction in terms of bioactivity of the phytochemicals determined in the two plants cannot be ruled out as possible contributors to the observed activities [25].

Conclusion

The current investigation results show that *D. mespiliformis* and *D. senecioides* ethyl acetate extract consist of bioactive compounds against *C. albicans*, *E. floccosum* and *T. rubrum,* including o-glycosylated and c-glycosylated flavonoids, terpenoids, phenolics and esters. The antifungal activity of the polyherbal mixture as often used in traditional practices was significantly greater or comparable to that of standard antifungal drug. Further studies on isolation of individual active compounds, formulation of poly-herbal alternatives and clinical studies are necessary for the discovery of broad spectrum alternative and complimentary remedies for fungal infections.

Abbreviations

GC	Gas chromatograph
GC-MSMS	Gas chromatography–tandem mass spectrometry
TLC	Thin-layer chromatography
SDA	Sabouraud dextrose agar
NIST	National Institute of Standards and Technology
MS	Mass spectral
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid

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Plant authentication

Plant samples were collected following WHO guidelines for collecting plant botany 440/540. Plant leaves and stem were identified by comparing information given and pictures with published literature and further authenticated by a taxonomist at the Harare national herbarium. Voucher specimen 02/21 spec 9 was deposited in the herbal specimen store of Bindura University of Science Education, Natural Products section.

Author contributions

All authors participated in the formulated of this study. RZ and PD designed the study and carried out experimental work. LG managed literature and wrote the second draft. RZ wrote the first draft. PD and RZ wrote the final draft. All authors proof read and approved the final draft.

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Availability of data and materials

All data and material are available upon request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

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