Bioactivity of Baltic amber – fossil resin

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Abstract: This paper constitutes a review concerning studies on bioactivity of Baltic amber. Baltic amber (succinite) – Eocene fossil resin – is a very complicated mixture consisting of polymeric and low molecular mass components. In folk medicine, succinite is thought to be a remedy for all ailments or diseases. However, there are no scientific results confirming the assumption that succinite can positively influence human body. To confirm it indirectly, many articles examining succinite components were analyzed. Basing on them, we realized that many properties attributed to Baltic amber may originate from its components also released from its polymeric structure. Observed properties are: antioxidative activity, antimicrobial activity, antiphlogistic activity, repellent and insecticidal activity – they coincide with folk medicine applications of succinite.

Keywords: Baltic amber, succinite, fossil resin, biological activity, jewelry wastes, cosmetics.

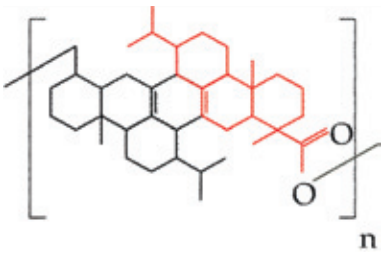
Aktywność biologiczna bursztynu bałtyckiego – żywicy kopalnej

Streszczenie: Artykuł stanowi przegląd literatury dotyczącej badań bioaktywności bursztynu bałtyckiego. Bursztyn bałtycki (sukcynit) – eoceńska żywica kopalna – jest skomplikowaną mieszaniną polimerów i składników małocząsteczkowych. W medycynie ludowej sukcynit używany jest jako cudowny lek na wiele dolegliwości i chorób. Brak jednak naukowych dowodów potwierdzających pozytywny wpływ sukcynitu na organizm człowieka, które uzasadniałyby stosowanie preparatów z bursztynu np. w kosmetykach. Aby pośrednio sprawdzić zasadność doniesień ludowych, przeanalizowano artykuły dotyczące aktywności biologicznej składników bursztynu bałtyckiego zakładając, że właściwości bursztynu muszą wynikać zarówno z właściwości jego składników, jak również z ich umieszczenia w polimerycznej strukturze sukcynitu. Stwierdzono, że właściwości biologiczne składników bursztynu – antyoksydacyjne, przeciwdrobnoustrojowe, przeciwzapalne, repelencyjne oraz insektobójcze mogą być uzasadnieniem doniesień medycyny ludowej o stosowaniu bursztynu.

Słowa kluczowe: bursztyn bałtycki, sukcynit, żywica kopalna, aktywność biologiczna, odpady jubilerskie, kosmetyki.

COMPOSITION OF BALTIC AMBER

Investigation of the chemical composition of Baltic amber (succinite) – Eocene fossil resin – has lasted for more than a century. It is a very complicated mixture of supramolecular structure consisting of macromolecular and low molecular mass components. There are theories that the main component of its polymeric structure is abietic acid and its dimer [1, 2]. The structure of polyabietic acid is shown by formula (I).



(I) **(II)**

Other theories say, that it is a copolymer of communic acid and communol [3–5] shown by formula (II) [4–7].

Low molecular weight compounds might be closed in clathrates. There are mainly monoterpenes and their derivatives in the soluble phase, as they occur in resin, while the insoluble phase consists of resin acids polymers and diterpenes [6]. Due to the presence of different functional groups bonded to resin (*e.g.* hydroxylic ones), the polymeric chains can be cross-linked, for example with succinic acid [4, 6]. A number of amber components

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T a b l e 1. Identification and activity of selected succinite components with indication of the literature sources

Succinite component	Antioxidative	Antibacterial, nematicidal and antiviral	Antifungal	Antiphlogistic	Repellent and insecticidal
Unsaturated monoterpenes					
camphene [5], [10]	[13]	[17, 20, 22]	[20]		[38–40]
limonene [10]	[13]	[17, 19–22]	[16, 20, 21]		[37, 39, 40]
α-pinene [10]	[13]	[19–22]	[20, 21]		[39, 40]
β-pinene [10]	[13]	[15, 19–22]	[20, 21]		[39]
Aromatic monoterpenes					
p-cymene [5], [11]	[13]	[20, 21]	[20, 21]		[35, 39]
Monoterpene alcohols and their esters					
fenchol [5], [7], [11]	[13]	[20]	[20]		
isoborneol [5], [7], [10], [11]		[20]	[20]		
borneol [5], [7], [10], [11]	[13]	[19, 20, 22]	[20]		[39, 40]
bornyl acetate [5]	[13]				[38, 40]
terpinen-4-ol [5]	[13]	[19, 20]	[18, 20]		[39]
Monoterpene ketones					
fenchone [5], [7], [11]	[13]	[20]	[16, 20]		[39]
camphor [5], [7], [11]	[13]	[15, 20, 22]	[15, 20]		[34, 39, 40]
pulegone [3]	[13]	[20]	[20]		
Monoterpene ethers					
eucalyptol [5], [7], [11]	[13], [14]	[20]	[18, 20]		[33, 34, 36, 39, 40]
Polycyclic compounds and their derivatives					
abietic acid [11]		[22, 24]		[29]	
communic acid [11]		[23, 25]			
pimaric acid [11]		[23]			
isopimaric acid [11]		[24]			
abieta-7,13-diene [7]		[26]			
α-amyrin [8]				[30]	
β-amyrin [8]				[30]	
Succinic acid					
succinic acid [9]		[27, 28]			[32]
Fatty acids esters					
methyl palmitate [5], [7]				[31]	

were identified [2, 4, 7–11a]. However, there is no detailed characteristic of raw materials of amber origin, among others used in cosmetics.

APPLICATION OF BALTIC AMBER

Annual processing of amber in Poland is on the level of 200 t. It is our national treasure, mostly used for jewelry and decorative art. Large quantity of wastes left after production of jewelry might be a valuable, natural, inexpensive, potentially bioactive raw material for medicine and cosmetics. Succinite was used in folk medicine for centuries. It was believed being a panacea for any illness: arthritis, rheumatism, but also for severe diseases such as epilepsy, jaundice or plague. Patients were treated with succinite smoke, nuggets hanged on their necks or a tincture used externally and internally [6]. Moreover, due to potential bioactivity (medicinal and skin-care properties) assigned to amber there also exist medical ointments or cosmetics based on succinite. However, there is no scientific evidence specifying and confirming the positive influence of Baltic amber on human body. In that case, every medicament or cosmetic is a "blind shot".

BIOACTIVITY OF BALTIC AMBER

Seeking for the scientific basis of properties assigned to Baltic amber we have found that many components of amber exhibit various kinds of bioactivity which are listed in Table 1 with indication of the sources of such information. Despite the fact that information on bioactivity was not found in the case of all components, one may assume the knowledge of the components' biologi-

T a b l e 2. Antioxidative properties of succinite components

Succinite component	Substance used for determination	Antioxidative index, %
camphene	TBARS	9.8 (1000 ppm); 7.5 (500 ppm); 3.2 (100 ppm) [13]
limonene	TBARS	27.4; 29.4; 24.0 [13]
	linoleic acid	21.0 (10^{-2} M); 15.7 (10^{-3} M) [13]
α-pinene	TBARS	12.6 (1000 ppm); 6.4 (500 ppm) [13]
β-pinene	TBARS	27.6; 18.5; 1.0 [13]
p-cymene	TBARS	42.6; 25.5; 14.9 [13]
fenchol	TBARS	3.0 (1000 ppm) [13]
borneol	TBARS	6.6; 1.8 [13]
bornyl acetate	TBARS	18.7; 17.1; 10.5 [13]
terpinen-4-ol	TBARS	31.0; 21.6; 8.1 [13]
fenchone	TBARS	25.6; 10.6; 7.2 [13]
camphor	TBARS	6.6; 2.3 [13]
pulegone	TBARS	31.1; 17.5; 5.0 [13]
eucalyptol	TBARS	20.3; 3.3 [13]
	aldehyde/carboxylic acid conversion	4 ± 2.8 % (1 μg/mL); 11 ± 0.9 % (50 μg/mL) [14]
α-tocopherol (reference)	TBARS	93.5; 89.3; 82.6 [13]
	linoleic acid	94.8; 91.6 [13]
	aldehyde/carboxylic acid conversion	17 ± 9.9 %; 98 ± 3.0 % [14]

cal properties as indirect indication of amber bioactivity.

We divided the described components of succinite into eight groups:

– unsaturated monoterpenes (camphene, limonene, α-pinene, β-pinene),

– aromatic monoterpenes (*p*-cymene),

– monoterpene alcohols and their esters (fenchol, isoborneol, borneol, bornyl acetate, terpinen-4-ol),

– monoterpene ketones (fenchone, camphor, pulegone),

– monoterpene ethers (eucalyptol),

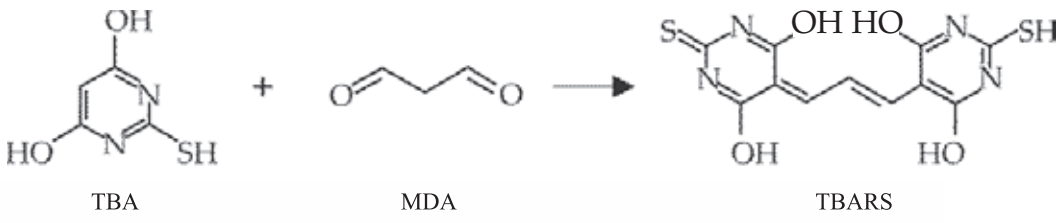
– polycyclic compounds and their derivatives (abietic acid, communic acid, pimaric acid, isopimaric acid, abieta-7,13-diene, α-amyrin, β-amyrin),

– succinic acid and fatty acids esters (methyl palmitate).

These components might be closed in clathrates and/or be released from resin structure, due to *e.g.* depolymeric transesterification during the reaction with an active solvent like alcohol [12]. Several groups of activity were described.

ANTIOXIDATIVE PROPERTIES

An antioxidant inhibits the oxidation of another compound by, for example, eliminating free radicals. The extent of lipid oxidation is measured in the presence of thiobarbituric acid reactive species (TBARS). The analyzed lipid is dissolved in a nonpolar solvent and an aqueous solution of thiobarbituric acid (TBA) is added. Heating of the test-tube for 20 min forms a TBA pink complex the UV absorbance of which is measured using a spectrophotometer at 540 nm. The color intensity is related to the concentration of TBA-reactive substances in the sample. Their amount is increased by such radical initiators as 2,2′-azobis(2-amidinopropane) dihydrochloride (ABAP) and decreased by antioxidants, *e.g.* α-tocopherol or *tert*-butylated *p*-cresol (BHT). Generally, inhibition of lipid oxidation caused by one radical initiator is compared to a few potential antioxidants and a reference compound, *e.g.* α-tocopherol. Usually the pink complex consists of TBA and malondialdehyde (MDA). This complex (TBARS) is a low molecular weight product of lipid peroxidation reaction shown in Scheme A.



TBA MDA TBARS

Scheme A

T a b l e 3. Antibacterial properties of succinite components (pathogenic, or pathogenic and existing on human skin, microorganisms were indicated by the appropriate underlining) determined using agar diffusion test with 15 μL of the tested compound (A) and dilution test (D)

Succinite component	Method	Tested microorganisms	Result
α-pinene	A	*A. calcoacetica, A. hydrophila, A. faecalis, B. subtilis, B. natriegens, B. linens, B. thermosphacta, C. freundii, C. sporogenes, E. faecalis, E. aerogenes, E. carotovora, E. coli, F. suaveolens, K. pneumoniae, L. plantarum, L. cremoris, M. luteus, Moraxella sp., P. vulgaris, P. aeruginosa, S. pullorum, S. marcescens, S. aureus, Y. enterocolitica*	5.7 ± 0.1–9.2 ± 0.1 mm (inhibition zone diameter) [19]
	D	*P. aeruginosa, E. coli, S. aureus*	*MIC* = 16 800 ppm [20]
	A, D	*S. marcescens, E. cloace, K. pneumoniae, A. baumani, S. aureus*	9–24 mm (inhibition zone diameter), *MIC* = 0.8–2 μL/mL [21]
	D	*Propionobacterium acnes*	*MIC* = 25 μL/mL [22]
β-pinene	D	*S. aureus, S. epidermidis, P. aeruginosa, E. cloacae, K. pneumoniae, E. coli* (reference: netylmycin *MIC* = $4 \cdot 10^{-3}$–$10 \cdot 10^{-3}$ mg/mL, amfoterycin B MIC = $0.4 \cdot 10^{-3}$–$1 \cdot 10^{-3}$ mg/mL)	*MIC* = 9.75–16 μL/mL [15]
	A	*A. calcoacetica, A. hydrophila, A. faecalis, B. subtilis, B. natriegens, B. linens, B. thermosphacta, C. freundii, C. sporogenes, E. faecalis, E. aerogenes, E. carotovora, E. coli, F. suaveolens, K. pneumoniae, L. plantarum, L. cremoris, M. luteus, Moraxella sp., P. vulgaris, P. aeruginosa, S. pullorum, S. marcescens, S. aureus, Y. enterocolitica*	4.8 ± 0.8–11.2 ± 0.5 mm (inhibition zone diameter) [19]
	D	*P. aeruginosa, E. coli, S. aureus*	*MIC* = 16 800 ppm [20]
	A, D	*S. marcescens, E. cloace, K. pneumoniae, A. baumani, S. aureus*	7–19 mm (inhibition zone diameter), *MIC* = 1.2–1.6 μL/mL [21]
	D	*Propionobacterium acnes*	*MIC* = 100 μL/mL [22]
camphor	D	*S. aureus, S. epidermidis, P. aeruginosa, E. cloacae, K. pneumoniae, E. coli* (reference: netylmycin *MIC* = $4 \cdot 10^{-3}$–$10 \cdot 10^{-3}$ mg/mL, amfoterycin B MIC = $0.4 \cdot 10^{-3}$–$1 \cdot 10^{-3}$ mg/mL)	*MIC* = 1.33–2.80 μL/mL [15]
	D	*P. aeruginosa, E. coli, S. aureus*	*MIC* = 3000–9800 ppm [20]
	D	*Propionobacterium acnes*	200 μL/mL [22]
limonene	D	*C. xerosis, B. brevis, B. megaterium, B. cereus, M. smegmatis, S. aureus, M. luteus, E. faecalis, P. aeruginosa, K. pneumoniae, K. oxytocica, E. coli*	*MIC* = 1.50–6.00 μL/mL [18]
	A	*A. calcoacetica, A. hydrophila, A. faecalis, B. subtilis, B. natriegens, B. linens, B. thermosphacta, C. freundii, C. sporogenes, E. faecalis, E. aerogenes, E. carotovora, E. coli, F. suaveolens, K. pneumoniae, L. plantarum, L. cremoris, M. luteus, Moraxella sp., P. vulgaris, P. aeruginosa, S. pullorum, S. marcescens, S. aureus, Y. enterocolitica*	6.5 ± 0.1–11.2 ± 0.6 mm (inhibition zone diameter) [19]
	D	*P. aeruginosa, E. coli, S. aureus*	*MIC* = 16 500 ppm [20]
	A	*S. marcescens, E. cloace, K. pneumoniae, A. baumani, S. aureus*	7–17 mm (inhibition zone diameter) [21]
	D	*Propionobacterium acnes*	*MIC* = 50 μL/mL [22]
camphene	D	*C. xerosis, B. brevis, B. megaterium, B. cereus, M. smegmatis, S. aureus, M. luteus, E. faecalis, P. aeruginosa, K. pneumoniae, K. oxytocica, E. coli*	*MIC* = 0.01–0.30 μL/mL [18]
	D	*P. aeruginosa, E. coli, S. aureus*	*MIC* = 9600 ppm [20]
	D	*Propionobacterium acnes*	*MIC* = 25 μL/mL [22]
borneol	A	*A. calcoacetica, A. hydrophila, A. faecalis, B. subtilis, B. natriegens, B. linens, B. thermosphacta, C. freundii, C. sporogenes, E. faecalis, E. aerogenes, E. carotovora, E. coli, F. suaveolens, K. pneumoniae, L. plantarum, L. cremoris, M. luteus, Moraxella sp., P. vulgaris, P. aeruginosa, S. pullorum, S. marcescens, S. aureus, Y. enterocolitica*	5.4 ± 0.6–10.4 ± 0.5 mm (inhibition zone diameter) [19]
	D	*P. aeruginosa, E. coli, S. aureus*	*MIC* = 1500–19 000 ppm [20]
	D	*Propionobacterium acnes*	*MIC* = 200 μL/mL [22]
terpinen-4-ol	A	*A. calcoacetica, A. hydrophila, A. faecalis, B. subtilis, B. natriegens, B. linens, B. thermosphacta, C. freundii, C. sporogenes, E. faecalis, E. aerogenes, E. carotovora, E. coli, F. suaveolens, K. pneumoniae, L. plantarum, L. cremoris, M. luteus, Moraxella sp., P. vulgaris, P. aeruginosa, S. pullorum, S. marcescens, S. aureus, Y. enterocolitica*	7.7 ± 0.1–29.9 ± 0.8 mm (inhibition zone diameter) [19]
	D	*P. aeruginosa, E. coli, S. aureus*	*MIC* = 1900–6200 ppm [20]

T a b l e 3. (*continued*)

eucalyptol	D	*P. aeruginosa*, *E. coli*, *S. aureus*	*MIC* = 9100–18 100 ppm [20]
p-cymene	D	*P. aeruginosa*, *E. coli*, *S. aureus*	*MIC* = 16 900 ppm [20]
	A, D	*S. marcescens*, *E. cloace K. pneumoniae*, *A. baumani*, *S. aureus*	9–11 mm (inhibition zone diameter), *MIC* = 2 μL/mL [21]
pulegone	D	*P. aeruginosa*, *E. coli*, *S. aureus*	*MIC* = 2800–18 400 ppm [20]
fenchol	D	*P. aeruginosa*, *E. coli*, *S. aureus*	*MIC* = 2000–7100 ppm [20]
isoborneol	D	*P. aeruginosa*, *E. coli*, *S. aureus*	*MIC* = 2000–9600 ppm [20]
fenchone	D	*P. aeruginosa*, *E. coli*, *S. aureus*	*MIC* = 4700–18 600 ppm [20]
abietic acid	D	*Propionobacterium acnes*	*MIC* = 25 μL/mL [22]
	D	methicilin-resistant *Staphylococcus aureus* (MRSA) (reference: tetracycline *MIC* = 0.125–128 μg/mL, norfloxacin *MIC* = 0.5––128 μg/mL, erytromycin *MIC* = 0.25–4096 μg/mL, oxacyllin *MIC* = 0.125–512 μg/mL)	*MIC* = 64 μL/mL [24]
communic acid	A	*S. aureus*, *S. epidermidis*, *P. aeruginosa*, *E. cloacae*, *K. pneumoniae*, *E. coli*, *S. mutans*, *S. viridans* (reference: sanguinarine and netilmicin were used as control substances – their inhibition zone varies from 20 to 28 mm)	7–8 mm (inhibition zone diameter) [23]
	D	*Mycobacterium tuberculosis*	*MIC* = 9.38 μL/mL, IC_{50} = 4.5 ± 0.2 μL/mL [25]
pimaric acid	A	*S. aureus*, *S. epidermidis*, *P. aeruginosa*, *E. cloacae*, *K. pneumoniae*, *E. coli*, *S. mutans*, *S. viridans* (reference: sanguinarine and netilmicin were used as control substances – their inhibition zone varies from 20 to 28 mm)	8–12 mm (inhibition zone diameter) [23]
isopimaric acid	D	MRSA (reference: tetracycline *MIC* = 0.125–128 μg/mL, norfloxacin *MIC* = 0.5–128 μg/mL; erytromycin *MIC* = 0.25–4096 μg/mL; oxacyllin *MIC* = 0.125–512 μg/mL)	*MIC* = 32–64 μL/mL [24]

Another method of antioxidative potential assessment is the analysis of linoleic acid peroxidation in the absence and in the presence of a potential antioxidant. Oxidation is induced by the ABAP initiator mentioned before and the amount of hydroperoxides is specified by UV-spectrum, due to their strong UV absorption in sodium dodecyl sulfate (SDS) micelles at 232 nm [12, 13].

The antioxidative properties of succinite components are presented in Table 2 (the most significant ones are underlined). It has been found that *p*-cymene, limonene, terpinen-4-ol and pulegone exhibit antioxidative properties. The first one: *p*-cymene is about half as effective as tocopherol. Other components also show some potential but it is much lower.

ANTIBACTERIAL AND ANTIFUNGAL PROPERTIES

Two methods (in liquid and solid medium) were used to determine the minimal inhibitory concentration (*MIC*). For liquids, series of test-tubes filled with the medium are prepared. Then, a decreasing amount of antimicrobial chemical is added to test-tubes, followed by the same quantity of bacterial or fungi suspension. After incubation at 35 °C for 18 h, the presence of microorganism culture is verified. The lowest concentration of antimicrobial compound without any culture is acknowledged as the *MIC*. For fungi there also exists another index, which is the minimal fungicidal concentration (*MFC*). It indicates the minimal concentration of a chemical that completely inhibits fungi growth. For solids, broth mixed with decreasing amount of the tested antimicrobial chemical is placed on Petri dishes, inoculated and incubated at 35 °C for 18 h. The lowest concentration of antimicrobial compound without any microorganism culture is acknowledged as the *MIC*. Results for potential antimicrobial agents are generally compared with results for commonly used antibiotics.

Another way to determine antimicrobial properties is the agar diffusion test (Kirby-Bauer testing). Microorganism culture is inoculated on agar broth poured into a Petri dish. A filter-paper disc, saturated with a potential antimicrobial agent, is placed on the surface of the agar. After an overnight incubation, the diameter of inhibition zone is measured. The wider the diameter, the more effective is the antimicrobial activity [14–24].

Antibacterial and antifungal properties of succinate components were presented in Tables 3 and 4 (the most significant values were underlined). Pathogenic, or pathogenic and existing on human skin, microorganisms were also indicated. Even the most potent components were not as active as antibiotics, but there were some valuable results, for example abietic acid, α-pinene and camphene are effective anti-acne agents [24]. Also limonene and fenchone totally inhibit the growth of *Rhizoctonia solani*.

T a b l e 4. Antifungal properties of succinite components (pathogenic, or pathogenic and existing on human skin, microorganisms were indicated by the appropriate underlining) determined using agar diffusion test with 60 μL of tested compound (A) and dilution test (D)

Succinite component	Method	Tested microorganisms	Result
camphor	D	*C. albicans*, *C. tropicalis*, *C. glabrata* (reference: amphotericin B *MIC* = $0.4 \cdot 10^{-3}$–$1 \cdot 10^{-3}$ mg/mL)	*MIC* = 3.56–4.85 mg/mL [15]
	D	*C. albicans*	*MIC* = 2000 ppm [20]
limonene	A	*R. solani*	no growth of fungi [16]
	D	*C. albicans*	*MIC* = 16 500 ppm [20]
	A, D	*C. albicans*	16–19 mm (inhibition zone diameter) [21]
fenchone	A	*R. solani*	no growth of fungi [16]
	D	*C. albicans*	*MIC* = 4200 ppm [20]
bornyl acetate	A	*R. solani*	22.2% of inhibition was observed (relative to the control) [16]
terpinen-4-ol	D	*C. albicans*, *C. parapsilosis*, *S. cerevisiae*, *R. rubra*, *Trichosporon sp.*, *E. floccosum*, *M. canis*, *T. interdigitale*, *T. rubrum*, *A. niger*, *A. flavus*, *A. fumigatus*, *Penicillium sp.*	*MIC* = 0.008–0.25 μg/mL, *MFC* = 0.016–0.5 μg/mL [18]
	D	*C. albicans*	*MIC* = 930 ppm [20]
eucalyptol	D	*C. albicans*, *C. parapsilosis*, *S. cerevisiae*, *R. rubra*, *Trichosporon sp.*, *E. floccosum*, *M. canis*, *T. interdigitale*, *T. rubrum*, *A. niger*, *A. flavus*, *A. fumigatus*, *Penicillium sp.*	*MIC* = 0.06–8 μg/mL, *MFC* = 0.5–8 μg/mL [18]
	D	*C. albicans*	*MIC* = 9100 ppm [20]
α-pinene	D	*C. albicans*	*MIC* = 1680–3400 ppm [20]
	A, D	*C. albicans*	22–28 mm (inhibition zone diameter), *MIC* = 0.8–1.6 μL/mL [21]
β-pinene	D	*C. albicans*	*MIC* = 16800 ppm [20]
	A, D	*C. albicans*	15–20 mm (inhibition zone diameter), *MIC* = 1.2–1.6 μL/mL [21]
p-cymene	D	*C. albicans*	*MIC* = 16900 ppm [20]
	A, D	*C. albicans*	17–21.3 mm (inhibition zone diameter), *MIC* = 1.6 μL/mL [21]
pulegone	D	*C. albicans*	*MIC* = 1900 ppm [20]
borneol	D	*C. albicans*	*MIC* = 1000 ppm [20]
camphene	D	*C. albicans*	*MIC* = 9600 ppm [20]
fenchol	D	*C. albicans*	*MIC* = 1000 ppm [20]
isoborneol	D	*C. albicans*	*MIC* = 1000 ppm [20]

NEMATICIDAL PROPERTIES

For determination of nematicidal properties 100 μL of nematode growth medium (NGM), twenty *Caenorhabditis elegans* larvae and *Escherichia coli* solution (as a food source) were placed in each well of a 96-well microtiter plate. The tested compounds were dissolved in 0.5 μL of dimethyl sulfoxide (DMSO) and added to each well to reach the final concentration of 80 μg/mL. Nematodes were incubated at 21 °C and 65 % relative humidity for 24 h. Then, survival of larvae was evaluated by counting of alive individuals [25].

The tested succinite component was abieta-7,13-diene the inhibitory concentration (IC_{50}) (inhibits in 50 % the biological and biochemical functions of organisms) of which was 2 μg/mL whereas the concentration of chloroquine, used as a reference, was 0.15 μg/mL.

ANTIVIRAL PROPERTIES

Two methods were used to determine antiviral properties. The first method consists of covering hands and forearms with the tested compound solution, then with a virus solution, containing approximately 10^4 of 50 % of tissue culture infective dose ($TCID_{50}$). After 7 to 10 min, the virus is eluted from the skin with a plastic vial. The amounts of virus on treated and untreated hands and forearms are compared [26]. The second method consists of infecting monolayers of Vero line cells grown in Eagle's minimum essential medium with *Herpesvirus* (HPV-1). The monolayers are grown on 6-well multi-dishes – they are treated with 1 mL of varying concentrations of the tested compounds. Values of IC_{50} are evaluated two days after infection [27].

T a b l e 5. Antiphlogistic properties of succinite components

Succinite component	Method	Result	Reference
abietic acid	carrageenan induced oedema	paw oedema reduction by 39 % (oral dose – 50 mg/kg of body weight) and 45 % (oral dose – 100 mg/kg of body weight); ear oedema reduction: 46.4 % (topical application – 0.25 mg), 58.6 % (0.5 mg) and 74.8 % (1 mg) [29]	indometacin: paw oedema reduced by 50 % (25 mg/kg), ear oedema by 90 % (0.5 mg/kg)
α-amyrin	% of ear oedema reduction and ID_{50} value	50 % inhibitory dose ID_{50} = 0.2 mg, oedema reduction by 86 % (topical application – 2 mg) [30]	indometacin ID_{50} = 0.3 mg, hydrocortisone ID_{50} = 0.03 mg
β-amyrin	% of ear oedema reduction and ID_{50} value	ID_{50} = 0.4 mg, oedema reduction by 71 % (2 mg) [30]	indometacin ID_{50} = 0.3 mg, hydrocortisone ID_{50} = 0.03 mg
methyl palmitate	% of paw and ear oedema reduction	paw treated with carrageenan (plus methyl palmitate by oral application) showed reduction of oedema by 38 % (75 mg/kg) and 47 % (150 mg/kg) relative to control (no methyl palmitate treatment); ear treated with mixture methyl palmitate and croton oil (70 % w/v) showed reduction of oedema by 50 % relative to control [31]	indomethacin: paw oedema reduction by 60 % (5 mg/kg), ear oedema reduction by 50 % (12.5 % w/v)

Only succinic acid (among succinite components) shows antiviral activity. It is effective against *Rhinovirus* and it decreased the number of viruses in relation to the control sample (a mixture of water and ethanol with 1:1 volume ratio, pH = 3) after 15 min $10^{2.5}$, after 1 h $10^{1.6}$ and after 3 h $10^{1.5}$ [26] and *Herpesvirus* (IC_{50} = 1.3 μg/mL – similar to the antiviral drug foscarnet) [27].

ANTIPHLOGISTIC PROPERTIES

To determine antiphlogistic properties, laboratory mice were usually used. Oedema in various parts of their bodies is induced by carrageenan or croton oil. The tested compound is applied orally or topically, then oedema weight reduction was measured [28–30].

What is significant, every tested succinite component is an effective antiphlogistic agent and can be compared to indomethacin, a non-steroidal anti-inflammatory drug. The detailed results are presented in Table 5.

REPELLENT AND INSECTICIDAL PROPERTIES

Repellent and insecticidal properties were examined using several methods. The leaf disc method consists of covering leaves with the tested chemical solution, placing larvae on them and keeping them in a closed container for 24 h. Then, mortality is evaluated [31, 32]. Modification of the leaf disc method is placing insects on sticky papers covered with varying concentrations of the tested compound – half of maximal lethal dose (LD_{50}) is evaluated [33]. Insects can also be exposed to the tested chemical solution (0.5 mL of 3 % solution for 15 s or 5 % solution for 24 h) in a closed container. Mortality is tested after 1 to 2 h after the exposition [34–36]. Larvicidal activity is verified by adding the tested compound to larvae nourishment. Larvae weight (mg) and growth (mg/day) are calculated relative to the control sample [37]. Two methods are used for determining the repellent activity of insects preying on humans. The first consists of covering forearms with the tested chemicals and placing them in a special box filled with mosquito females – insects landing on the skin are calculated and half maximal repellent dose (RD_{50}) is evaluated (half maximal repellent dose – number of landing mosquitoes is decreased by a half, relative to the control sample) [38]. The second method consists of closing greenflies in a labyrinth-device with two alternate corridors, one of which is ended with the tested compound solution. The numbers of insects choosing each corridor are calculated [39].

Every succinite component shows some repellent potential, but results are not compared with known repellents, so it is not possible to decide whether they are strong or not. Detailed results were presented in Table 6. The most potent succinite compound was *p*-cymene (underlined in the table).

OTHER PROPERTIES

It should be mentioned that succinic acid presents anxiolytic effect and inhibits anaphylaxis. The first of them was tested on mice (3.0, 6.0, and 12.0 mg/kg doses) and allowed them to pass the elevated plus-maze test and the stress-induced hyperthermia test as well as on diazepam-fed mice [40]. The second effect was tested on rats. They were treated firstly with succinic acid solution (in various concentration) and secondly with a 48/80 compound (a mixture of *N*-methyl-*p*-methoxyphenylethylamine and formaldehyde) inducing anaphylactic shock. Mortality of rats treated with succinic acid was decreased by half, as compared to the animals not treated with it [41].

CONCLUSIONS

Basing on the presented literature research, a statement that succinite can be a remedy to many ailments may have some justification. Can we go a step further? Due to the proved properties of succinite components, one might presume all or some of them to be charac-

T a b l e 6. Repellent properties of succinite components

Succinite component	Method	Tested organism	Result
succinic acid	leaf disc method	*Plutella xylostella* larvae	mortality after 24 h: 11.36 %, after 48 h: 20.76 %, after 72 h: 32.36 % [32]
p-cymene	exposition to 0.5 mL of 3 % *p*-cymene solution lasting 15 s; mortality tested after 1 h and 2 h after exposition	*Musca domestica, Periplaneta americana, Blatella germanica, Phlebotomus papatasi, Stomoxys calcitrans, Glossina morsitans, Cimex leticularis, Ctenocephalides felis, Rhodnius prolixus, Triatoma infestans, Culicoides variipennis, Ixodes ricinus, Simulium damnosum, Vespula vulgaris, Tenebrio molitor*	*Phlebotomus papatasi* (1 h: 88 %, 2 h: 100 %), *Culicoides variipennis* (1 h: 86 %, 2 h: 100 %), *Simulium damnosum* (1 h: 78 %, 2 h: 100 %) [33]
	calculation of RD_{50}	*Anopheles gambiae*	$RD_{50} = 1 \cdot 10^{-5}$ mg/cm^2 [39]
camphor	sticky paper method (LD_{50} and LC_{50})	*Liposcelis bostrychophila* (reference: permethrin $LD_{50} = 18.99$ μg/cm^2, dichlorvos $LC_{50} = 1.35 \cdot 10^{-3}$ mg/L)	$LD_{50} = 207.26$ μg/cm^2, $LC_{50} = 1.03$ mg/L [34]
	calculation of RD_{50}	*Anopheles gambiae*	$RD_{50} = 1.40 \cdot 10^{-3}$ mg/cm^2 [39]
	labyrinth method	*Liposcelis bostrychophila* (reference: permethrin $LD_{50} = 18.99$ μg/cm^2, dichlorvos $LC_{50} = 1.35 \cdot 10^{-3}$ mg/L)	8.3 to 12.9 (corridor with sample to corridor with control), 10 mg (sample amount) and 8.3 to 9.5 (corridor with sample to corridor with control), 1 mg (sample amount) [40]
eucalyptol	sticky paper method (LD_{50} and LC_{50})	*Liposcelis bostrychophila* (reference: permethrin $LD_{50} = 18.99$ μg/cm^2, dichlorvos $LC_{50} = 1.35 \cdot 10^{-3}$ mg/L)	$LD_{50} = 1048.74$ μg/cm^2, $LC_{50} = 1.13$ mg/L [34]
	calculation of RD_{50}	*Anopheles gambiae*	$RD_{50} = 1.24 \cdot 10^{-3}$ mg/cm^2 [39]
	labyrinth method	*Liposcelis bostrychophila* (reference: permethrin $LD_{50} = 18.99$ μg/cm^2, dichlorvos $LC_{50} = 1.35 \cdot 10^{-3}$ mg/L)	7.3 to 10.9 (corridor with sample to corridor with control), 10 μL (sample amount) [40]
	contact and non-contact exposition to eucalyptol 3 % solution	*R. speratus*	mortality 20 % (after 48 h – contact condition), 40 % (after 48 h – non-contact condition) [32]
	leaf disc method	*Aedes aegypti, Bemisia argentifolii*	leaf disc method mortality 91.2 % (1000 mg/L), larvae mortality 10 % (25 mg/L) to 100 % (100 mg/L) [33]
camphene	calculation of RD_{50}	*Anopheles gambiae*	$RD_{50} = 2.21 \cdot 10^{-3}$ mg/cm^2 [39]
	labyrinth method	*Myzus persicae*	10.7 to 13.8 (corridor with sample to corridor with control), 10 mg (amount of sample) [40]
	compound added to larvae nourishment	*Choristoneura occidentalis* larvae	growth reduction between 13 and 44 %, weight reduction between 10 and 37 % [38]
limonene	calculation of RD_{50}	*Anopheles gambiae*	$RD_{50} = 1.80 \cdot 10^{-3}$ mg/cm^2 [39]
	labyrinth method	*Myzus persicae*	12.4 to 14.9 (corridor with sample to corridor with control), 10 μL (sample amount) [40]
	mosquitoes exposed on 5 % solution	*Aedes aegypti*	mortality varies from 0 to 57 % after 24 h [37]
α-pinene	calculation of RD_{50}	*Anopheles gambiae*	$RD_{50} = 5.94 \cdot 10^{-3}$ mg/cm^2 [39]
	labyrinth method	*Myzus persicae*	8.6 to 9.0 (corridor with sample to corridor with control), 10 μL (sample amount) [40]
β-pinene	calculation of RD_{50}	*Anopheles gambiae*	$RD_{50} = 1.56 \cdot 10^{-3}$ mg/cm^2 [39]
terpinene-4-ol	calculation of RD_{50}	*Anopheles gambiae*	$RD_{50} = 1.48 \cdot 10^{-3}$ mg/cm^2 [39]
fenchone	calculation of RD_{50}	*Anopheles gambiae*	$RD_{50} = 189 \cdot 10^{-5}$ mg/cm^2 [39]

T a b l e 6. (*continued*)

borneol	calculation of RD_{50}	*Anopheles gambiae*	$RD_{50} = 1.65 \cdot 10^{-3}$ mg/cm^2 [39]
	labyrinth method	*Myzus persicae*	11.2 to 14.6 (corridor with sample to corridor with control), 10 mg (sample amount) [40]
bornyl acetate	labyrinth method	*Liposcelis bostrychophila* (reference: permethrin LD_{50} = 18.99 μg/cm^2, dichlorvos $LC_{50} = 1.35 \cdot 10^{-3}$ mg/L)	7.3 to 9.3 (corridor with sample to corridor with control), 10 μL (sample amount) [40]
	compound added to larvae nourishment	*Choristoneura occidentalis* larvae	growth reduction between 22 and 38 %, weight reduction between 5 and 25 % [38]

teristic also for Baltic amber itself. As a matter of fact, concentrations of the components are various and some synergistic effects are possible.

Antioxidative properties of tested compounds are probably too low to use them as a primary antioxidant, but this property may cause an additional effect in succinite products (*e.g.* cosmetics). Many compounds present in Baltic amber show antimicrobial properties (against bacteria, fungi and viruses). They are probably not as potent as antibiotics, but with the synergy effect, succinite extracts could be used as a main or auxiliary natural preservative. Baltic amber ingredients show rather slight but wide repellent and insecticidal activity. It has been proved that many insect species are vulnerable to succinite compounds. Very promising are antiphlogistic properties. Examined articles bring evidence that several compounds highly reduce different types of oedema.

Cosmetology is the main field of interest for succinite. The question is how to process it without destroying the bioactivity to prepare stable and functional cosmetic ingredients. We have already prepared and preliminarily tested several extracts. They passed skin irritation tests (repeated open application test with 3 % solutions of tested extracts in petrolatum). Moreover, they were successfully induced into cosmetic preparations and showed positive influence on the skin hydration [42–45]. Although several succinite compounds are classified as potentially irritating (camphene, *p*-cymene, borneol, camphor, eucalyptol) [46], due to our skin irritation tests of succinite extracts, they do not show any harmful effect/reaction. Also corneometer, tewameter, sebumeter and chromameter analyses do not show any side effects or harmful reaction.

Literature and our preliminary results are very promising, but our assumptions need more acknowledgements. Firstly, antimicrobial properties should be verified and this would be the subject of the approaching article. There is a great chance to scientifically proof the positive bioactivity of amber and its derivatives what will enable the reasonable application of our Baltic golden resin in cosmetics and pharmacy.

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