Propolis and its bioactive chemical constituents offer a novel and sustainable treatment option for kinetoplastid infections

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Abstract

The kinetoplastids are a group of protozoan parasites characterized by the presence of a unique organelle, called the kinetoplast, that is located inside a single mitochondrion and contains a large amount of DNA called kinetoplast DNA (kDNA). Kinetoplastids of medical and veterinary significance include *Trypanosoma* spp. (the etiological agents of human and animal African Trypanosomiasis, surra, dourine and Chagas disease), and Leishmania spp. (the causative agents of the various forms of leishmaniasis). Millions of people, and their domesticated animals, living in endemic regions across the globe are at risk of these Neglected Tropical Diseases. All of the human and veterinary conditions can be disabling or fatal if not adequately treated, and no vaccines are available. However, drug treatment is hampered by the challenges of drug resistance and toxicity to the mostly very old drugs. We have been investigating propolis (a natural product made by bees from tree resins), and compounds isolated from it, as novel agents against *Trypanosoma* and *Leishmania* species. Our results show that high levels of activity were obtained for all the samples with the levels of activity varying across the sample set. The highest levels of activity were found against *L. mexicana*. Propolis have no *in vitro* growth inhibition against mammalian cells (result not shown), but displayed low EC_{50} against *Trypanosoma* and *Leishmania* species, without a loss of activity against diamidine- and arsenical-resistant or phenanthridine-resistant *T. brucei* strains, or a miltefosine-resistant *L. mexicana* strain. These results provide sufficient scope for further investigations of propolis-derived natural compounds toward the rational development of sustainable drugs against these kinetoplastids.

Method

- Resazurin assay was used to determine the susceptibilities of various kinetoplastids to propolis extracts
- Extracts of 35 propolis samples from various parts of Europe were tested against wild type and resistant strains of the protozoal pathogens *Trypanosoma brucei*, *Trypanosoma congolense* and *Leishmania mexicana*.
- The extracts were also tested against *Crithidia fasciculata* a close relative of *Crithidia mellifcae*, a parasite of bees. *Crithidia, Trypanosoma* and *Leishmania* are all members of the order Kinetoplastida.
- The propolis samples were profled by using liquid chromatography with high resolution mass spectrometry (LC-MS) and principal components analysis (PCA) of the data was done to show (if any) variation in the composition of the propolis samples.

Results

Propolis sample	*T. brucei* 427WT EC_{50}	B48 EC_{50}	R.I.	P value	*T. congolense* IL3000 EC_{50}
Suffolk 4, UK	7.42 ± 0.37	5.7 ± 0.17	0.77	0.013	8.46 ± 1.47
Bulgaria 1	5.20 ± 0.18	3.6 ± 0.52	0.69	0.043	3.69 ± 0.79
Suffolk 2, UK	6.69 ± 0.36	7.7 ± 1.1	1.15	0.423	5.66 ± 1.55
North Yorkshire 1, UK	13.5 ± 0.61	11.0 ± 0.70	0.82	0.058	18.9 ± 1.1
Northamptonshire 1, UK	4.49 ± 0.22	3.0 ± 0.20	0.67	0.007	5.69 ± 1.10
Essex 1, UK	5.97 ± 0.17	4.6 ± 0.26	0.77	0.013	4.40 ± 0.47
Essex 2, UK	14.0 ± 0.13	10.6 ± 1.6	0.75	0.102	17.3 ± 2.4
Norfolk 1, UK	5.23 ± 0.49	3.3 ± 0.31	0.63	0.029	3.08 ± 0.90
Devon 1, UK	8.57 ± 0.26	10.8 ± 1.2	1.26	0.144	11.4 ± 1.8
Leicestershire 1, UK	13.7 ± 1.18	11.6 ± 2.3	0.85	0.448	15.3 ± 3.0
Leicestershire 2, UK	17.8 ± 2.16	22.1 ± 1.4	1.24	0.169	27.6 ± 5.3
Derbyshire, UK	11.8 ± 0.57	9.5 ± 1.49	0.81	0.228	26.4 ± 4.5
Lithuania 1	18.4 ± 1.30	22.1 ± 0.24	1.20	0.049	30.9 ± 2.8
Lithuania 2	16.1 ± 0.93	25.0 ± 1.0	1.56	0.003	23.4 ± 1.4
Suffolk 1, UK	6.82 ± 0.87	4.5 ± 0.23	0.66	0.058	5.12 ± 0.68
Suffolk 3, UK	4.37 ± 0.18	2.9 ± 0.15	0.66	0.003	3.26 ± 1.03
Bulgaria 2	5.80 ± 0.36	4.1 ± 0.41	0.71	0.036	2.06 ± 1.12
Bulgaria 3	6.28 ± 0.69	5.3 ± 0.14	0.84	0.249	1.96 ± 1.01
Cambridgeshire 1, UK	9.79 ± 0.37	8.2 ± 0.32	0.84	0.034	5.65 ± 1.95
Norfolk 2, UK	6.18 ± 0.27	4.2 ± 0.41	0.68	0.015	2.13 ± 0.38
Northamptonshire 2, UK	5.24 ± 0.42	3.4 ± 0.39	0.65	0.030	4.83 ± 1.67
Cambridgeshire 2, UK	12.7 ± 0.09	10.3 ± 1.22	0.81	0.116	7.78 ± 2.15
North Yorkshire 2, UK	18.5 ± 0.48	14.9 ± 0.31	0.81	0.003	16.5 ± 3.1
Northern Ireland, UK	6.30 ± 0.33	6.7 ± 0.34	1.06	0.476	15.2 ± 4.2
North Yorkshire 3, UK	6.97 ± 0.60	5.4 ± 0.72	0.77	0.174	4.90 ± 1.53
North Yorkshire 4, UK	6.79 ± 0.45	4.7 ± 0.31	0.69	0.019	4.99 ± 2.06
North Yorkshire 5, UK	10.0 ± 0.06	9.0 ± 1.3	0.90	0.477	7.41 ± 1.25
North Yorkshire 6, UK	8.75 ± 0.34	7.3 ± 0.41	0.83	0.055	13.6 ± 3.1
Essex 3, UK	6.86 ± 0.71	5.4 ± 0.18	0.79	0.122	35.7 ± 6.5
Berkshire, UK	6.23 ± 0.12	4.2 ± 0.30	0.67	0.003	4.07 ± 1.10
Midlands, UK	5.28 ± 0.51	4.7 ± 0.31	0.89	0.395	6.12 ± 1.82
Devon 2, UK	8.68 ± 0.43	5.6 ± 0.23	0.65	0.003	7.52 ± 1.62
Buckinghamshire, UK	17.4 ± 0.96	13.1 ± 1.5	0.75	0.071	28.4 ± 6.0
Norfolk 3, UK	3.67 ± 0.30	2.5 ± 0.14	0.68	0.028	3.47 ± 0.92
Norfolk 4, UK	4.19 ± 0.21	2.9 ± 0.04	0.69	0.004	3.60 ± 0.99
Pentamidine (μM)	0.0027 ± 3.90E-04	0.6 ± 0.01	222	<0.0001	N.D.
Diminazene (μM)	N.D.	N.D.			0.37 ± 0.12

Table 1. The activity (μg/ml) of 35 European propolis samples against the standard drug-sensitive *T. brucei* 427WT and multi-drug resistant strain *T. brucei* B48, and *T. congolense*. Effective Concentration 50% (EC_{50}) values (μg/ml) are given as averages and SEM of 3 independent experiments for *T. brucei* and 3–4 experiments for *T. congolense*. P value is based on a Student's unpaired t-test, comparing *T. brucei* WT and B48. R. I. is the resistance index, being the ratio of the EC_{50} values for *T. brucei* WT and B48. N.D., not determined.

Propolis	*C. fasciculate* EC_{50} AVG ± SEM	Ratio EC_{50}(Tbb)/ EC_{50}(Cf)	P value
Suffolk 4, UK	6.41 ± 0.22	1.16	0.0798
Bulgaria 1	3.78 ± 0.65	1.37	0.1048
Suffolk 2, UK	2.80 ± 0.47	2.39	0.0029
North Yorkshire 1, UK	8.56 ± 1.19	1.57	0.0215
Northamptonshire 1, UK	3.54 ± 0.20	1.27	0.0324
Essex 1, UK	2.72 ± 0.23	2.20	0.0004
Essex 2, UK	13.4 ± 0.94	1.05	0.5182
Norfolk 1, UK	3.05 ± 0.48	1.71	0.0340
Devon 1, UK	8.11 ± 1.43	1.06	0.7664
Leicestershire 1, UK	9.58 ± 0.25	1.43	0.0269
Leicestershire 2, UK	23.8 ± 1.85	0.75	0.1030
Derbyshire, UK	5.64 ± 0.68	2.09	0.0022
Lithuania 1	5.92 ± 0.03	3.10	0.0007
Lithuania 2	10.1 ± 1.56	1.59	0.0310
Suffolk 1, UK	9.46 ± 1.03	0.72	0.1213
Suffolk 3, UK	7.94 ± 0.70	0.55	0.0077
Bulgaria 2	6.11 ± 0.66	0.95	0.6931
Bulgaria 3	5.55 ± 0.57	1.13	0.4633
Cambridgeshire 1, UK	8.44 ± 0.69	1.16	0.1597
Norfolk 2, UK	5.64 ± 0.93	1.10	0.6068
Northamptonshire 2, UK	4.62 ± 0.56	1.13	0.4258
Cambridgeshire 2, UK	22.7 ± 1.06	0.56	0.0007
North Yorkshire 2, UK	13.7 ± 1.15	1.35	0.0187
Northern Ireland, UK	11.6 ± 0.77	0.54	0.0032
North Yorkshire 3, UK	5.04 ± 0.71	1.38	0.1062
North Yorkshire 4, UK	2.95 ± 0.25	2.30	0.0018
North Yorkshire 5, UK	7.46 ± 1.00	1.34	0.0647
North Yorkshire 6, UK	3.98 ± 0.15	2.20	0.0002
Essex 3, UK	14.0 ± 0.99	0.49	0.0043
Berkshire, UK	5.56 ± 0.70	1.12	0.4015
Midlands, UK	3.27 ± 0.54	1.62	0.0540
Devon 2, UK	2.58 ± 0.43	3.36	0.0006
Buckinghamshire, UK	21.4 ± 1.34	0.81	0.0716
Norfolk 3, UK	4.34 ± 0.35	0.84	0.2208
Norfolk 4, UK	4.21 ± 0.49	1.00	0.9715
PAO[a] (μM)	5.35 ± 4.72	5.44	5.17

Table 2. EC50 values (μg/ml) for European propolis against *C. fasciculata* (n=3). [a]PAO=phenylarsine oxide.

Propolis ID	*L. mexicana* wild type (μg/mL)	*L. mexicana* C12Rx (μg/mL)	Resistance Index	ttest
Suffolk 4, UK	1.04 ± 0.19	0.81 ± 0.15	0.78	0.40
Bulgaria 1	0.35 ± 0.03	0.29 ± 0.04	0.85	0.33
Suffolk 2, UK	0.85 ± 0.14	0.45 ± 0.03	0.53	0.048
North Yorkshire 1, UK	0.90 ± 0.17	0.94 ± 0.15	0.96	0.87
Northamptonshire 1, UK	0.59 ± 0.05	0.28 ± 0.08	0.48	0.029
Essex 1, UK	0.62 ± 0.07	0.37 ± 0.07	0.60	0.073
Essex 2, UK	0.89 ± 0.10	0.42 ± 0.09	0.47	0.027
Norfolk 1, UK	1.94 ± 0.44	0.61 ± 0.003	0.31	0.027
Devon 1, UK	4.97 ± 0.23	0.95 ± 0.16	0.25	0.00014
Leicestershire 1, UK	5.67 ± 0.43	1.33 ± 0.09	0.23	0.00058
Leicestershire 2, UK	4.71 ± 0.33	1.06 ± 0.02	0.23	0.00041
Derbyshire, UK	1.23 ± 0.08	0.50 ± 0.17	0.41	0.016
Lithuania 1	1.51 ± 0.06	1.35 ± 0.02	0.89	0.064
Lithuania 2	0.65 ± 0.12	1.55 ± 0.01	2.38	0.0018
Suffolk 1, UK	0.67 ± 0.05	0.79 ± 0.09	1.17	0.32
Suffolk 3 UK	1.02 ± 0.18	0.50 ± 0.04	0.49	0.048
Bulgaria 2	1.13 ± 0.17	0.69 ± 0.22	0.61	0.19
Bulgaria 3	1.17 ± 0.18	0.78 ± 0.11	0.67	0.14
Cambridgeshire 1, UK	2.38 ± 0.40	1.53 ± 0.21	0.64	0.13
Norfolk 2, UK	0.93 ± 0.06	0.60 ± 0.05	0.65	0.020
Northamptonshire 2, UK	0.65 ± 0.05	0.49 ± 0.002	0.78	0.018
North Yorkshire 2	2.68 ± 0.15	1.36 ± 0.08	0.51	0.003
Northern Ireland	0.61 ± 0.05	0.78 ± 0.17	1.27	0.17
North Yorkshire 4, UK	0.72 ± 0.22	0.67 ± 0.06	0.94	0.75
North Yorkshire 5, UK	0.42 ± 0.12	0.58 ± 0.07	1.38	0.12
Miltefosine APC 12	0.1 ± 0.03	67.0 ± 12.6	670	<0.001
Miltefosine APC 16	2.0 ± 0.20	56 ± 9.7	28	<0.001

Table 3. The activity (μg/ml) of propolis against wild type and miltefosine-APC12 resistant *L. mexicana* (C12Rx). All EC_{50} values are given as average±SEM (n=3). Statistical difference between EC_{50} values of the same sample against two strains was analysed using Student's unpaired t-test.

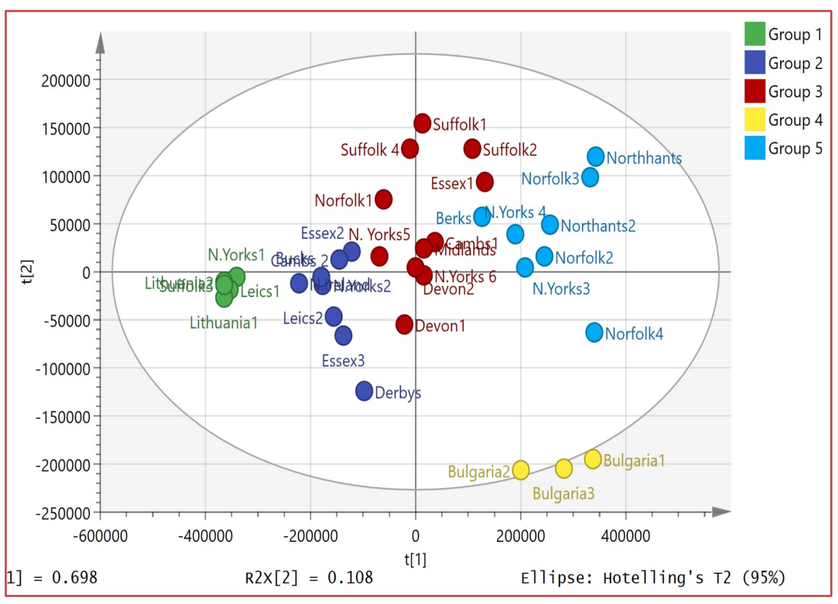

Figure 1. PCA plot showing the variation of propolis composition across 35 European propolis samples (Pareto scaled based on 233 components).

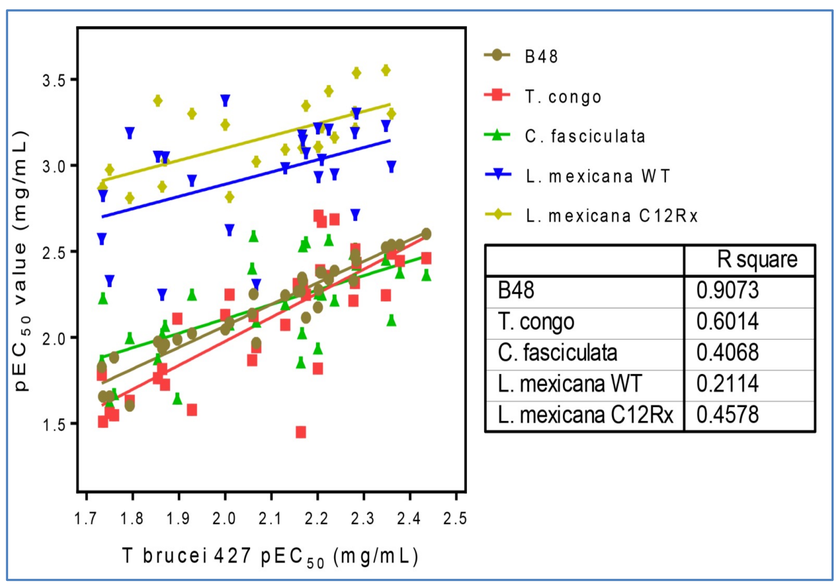

Fig. 2. Correlation between the EC_{50} values of propolis samples against *T. brucei* 427WT and the other parasite strains and species.

Discussion & Conclusion

- ✓ It was previously found that a parasite challenge encouraged bees to collect more propolis and that the propolis envelop improved the immunity of colonies against infection[1].
- ✓ In the current study, regional variations in the antimicrobial properties of propolis have been found to exist.
- ✓ propolis would appear to have broad spectrum activity with individual components in the mixture having activity against different organisms.
- ✓ a good overall correlation between the effects of the various samples against each of the kinetoplastid species. Especially between *T. brucei* and *T. congolense* the correlation is very close, which is important as African animal trypanosomiasis is caused by multiple Trypanosoma species including *T. congolense*, *T. b. brucei* and, in Eastern Africa, *T. b. rhodesiense,* and the disease has now spread far beyond Africa for *T. vivax* and *T. evansi.* Even more important is that the correlation between the drug-resistant and the sensitive strains was very good, with activity against the resistant strains on average better than against the parental strains. These results gives ample scope for further investigations.

Reference:

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[2]Alotaibi, A., Ebiloma, G.U., Williams, R., Alenezi, S., Donachie, A.M., Guillaume, S., Igoli, J.O., Fearnley, J., De Koning, H.P. and Watson, D.G., 2019. European propolis is highly active against trypanosomatids including *Crithidia fasciculata*. *Scientific reports*, *9*(1), pp.1-10.

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