Published in final edited form as:
Fitoterapia. 2007 April ; 78(3): 250–252. doi:10.1016/j.fitote.2006.12.001.

Anti-TB activity of *Evodia elleryana* bark extract

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Abstract

An ethyl acetate extract of bark from *Evodia elleryana* produced significant growth inhibition of *Mycobacterium tuberculosis* at concentrations only minimally inhibitory to human T cells. The crude extract yielded 95% inhibition of TB at 50 μg/ml. The crude extract yielded 29 % growth inhibition of human T-cells in culture at that concentration.

Keywords

Evodia elleryana; TB; Mycobacterium tuberculosis

1. Plant

Evodia elleryana (*Melicope elleryana*) (Rutaceae), locally called sehit, harvested in 2004 from the Kurti region of Manus Island, Papua New Guinea was identified by Dr. Osia Gideon and Mr. Pius Piskaut of the University of Papua New Guinea Herbarium.

2. Use in traditional medicine

People of the Kurti region of Manus commonly use *Evodia elleryana* for cough and fever, especially whooping cough. Women occasionally use the plant to control fertility. A water extract of bark is taken internally for treatment of fever and cough. Previous work has reported a broad spectrum of antimicrobial activity for various plant parts and extracts [1].

3. Previously isolated constituents

Quinoline alkaloid and flavanoids [2–4].

4. Tested material

Soxhlet extracts (hexane, ethyl acetate and methanol in sequence) of bark, stem and leaves were tested.

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5. Test strains

Mycobacterium tuberculosis strain H37Ra grown in vitro (ATCC cat. # H37Ra 25177). CEM-TART cells grown in vitro (reagent obtained through the AIDS Research and Reference Reagent Program, Div. of AIDS, NIAID, NIH: CHEM-TART from Drs. H. Chen, T. Boyle, M. Malim, B Cullen and H.K. Lyerly, cat. # 1944).

6. Studied activity

Inhibition of *M. tuberculosis* growth was quantified using a colorimetric MTT assay [5,6]. Rifampicin and isoniazid (Sigma) were used as positive controls in the TB inhibition assay. Drugs and plant extracts were dissolved in DMSO to produce stock solutions of 10 mg/ml. DMSO was used as the negative control. Other chemical and culture supplies were purchased from commercial sources (Sigma and Becton Dickenson, respectively). *M. tuberculosis* cultures were dispensed into a 96 well culture plate at 50.000 to 100.000 cells per well, in 200 μl of 7H9 medium with 10% ADC (albumin-dextrose-catalase) enrichment. 1 μl of DMSO or DMSO containing drug or extract was added in duplicate wells. After 4 d incubation at 37°C, MTT (5 mg/ml) was added in 10 μl DMSO and incubated overnight. Viable tuberculosis metabolizes the MTT to a purple formazan metabolite which is solubilized by the addition of 50 μl 50%DMF, 5% SDS. After 2 h the 570 nm absorbance was quantified in a microtiter plate reader. The percent inhibition of TB growth was calculated by averaging inhibition of duplicate test wells and comparing the signal to that for the control growth (DMSO only) wells.

Inhibition of Human T cell leukemia growth was also quantified using a colorimetric MTT assay [7,8]. Plant extracts were dissolved in DMSO to produce stock solutions of 10 mg/ml. DMSO was used as the negative control. Other chemical and culture supplies were purchased from commercial sources (Sigma and Fisher Scientific respectively). Modified CCRF CEM cells, (TART cells, obtained from Aids Research and Reagent Program) were dispensed into a 96 well culture plate at 20.000 cells per well, in 200 μl of RPMI medium with 20% fetal bovine serum (Hyclone) and penicillin streptomycin antibiotics (100 U and 100 μg/ml respectively). 1 μl of DMSO or DMSO containing extract was added in duplicate wells. After 3 d incubation at 37°C, MTT (5 mg/ml) was added in 10 μl DMSO and incubated for 3 h. Viable cells metabolize the MTT to a purple formazan metabolite. Cells and formazan were centrifuged into a pellet, the supernatent was aspirated and the pellet was solubilized by the addition of 100 μl DMSO, and after a few minutes the absorbance quantified in a microtiter plate reader at 570 nm. The percent inhibition of T cell growth was calculated by averaging inhibition of duplicate test wells and comparing the signal to that for the control growth (DMSO only) wells.

7. Results

Screening results are presented in Table 1. Repeated experiments show that ethyl acetate extract of *E. elleryana* provided 95% inhibition of TB growth, equivalent to clinically useful drugs rifampacin and isoniazid.

8. Conclusion

E. elleryana, a plant traditionally used in Manus, Papua New Guinea, for cough and fever has demonstrable in vitro activity against H37Ra strain of *M.tuberculosis*. It is reasonable to hypothesize that this activity may be related to antimicrobial activities reported for alkaloid quinoline metabolites of other *Evodia* species [9–11].

Acknowledgments

The authors wish to gratefully acknowledge the assistance of Mr. Pius Piskaut, UPNG Herbarium, for plant identification; NIH support through the ICBG 5UO1TW006671-3, and the collaborating communities of Manus, PNG.

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Table 1

Inhibition % of the *E. elleryana* extracts on *M. tubercolosis* and human T cells growth[a]

Plant extracts [b]	**Human T cells growth inhibition %**[c]		***M. tubercolosis* growth inhibition %**[d]	
	50μg/ml	**5μg/ml**	**50μg/ml**	**5μg/ml**
B-H	A	I	A	I
B-E	I	I	A	I
B-M	A	I	I	I
L-H	A	I	+/-	I
L-E	I	I	I	I
L-M	+/-	I	I	I
S-H	A	I	+/-	I
S-E	+/-	I	I	I
S-M	I	I	I	I

[a] Inhibition of growth: A (≥ 70%), +/− (<70% & ≥30%), I (< 30%)

[b] Plant extracts: M (MeOH), E (EtOAc), H (hexane) obtained from: B (bark), L (leaves), S (stem).

[c] DMSO control growth normalized to 100%.

[d] Rifampicin (5 μg/ml) and isoniazid (50 μg/ml) yielded 99.0% and 96.7% inhibition, respectively.