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Short Communication

Preliminary anti-tuberculosis screening of two Nigerian *Laggera* species (*Laggera pterodonta and Laggera aurita*)

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Crude methanolic extracts of the aerial part of the two *Laggera* species (*Laggera pterodonta* (DC.) Sch. Bip. and *Laggera aurita* (Linn f.) DC.) from the family Asteraceae (Compositea), found in Nigeria were screened against *Mycobacterial bovis* (BCG strains). The two extracts were found to be active at minimum inhibitory concentrations (MIC) of 625 µg/ml. Further fractionation and screening of the fractions against BCG shows moderate activity for some of the fractions. The four compounds (crystals) isolated in the process did not show any activity, but are currently being analyzed by spectroscopy for characterization and identification.

Key words: *Laggera pterodonta*, *Laggera aurita*, anti-tuberculosis.

INTRODUCTION

Laggera pterodonta and *Laggera aurita* are two *Laggera* species found growing as weeds in Nigeria and spread throughout the sub-Saharan Africa (Burkill, 1985). Although both species have been reported to be of significant ethnomedicinal use in Asia country like China, there is little or no ethno-use reported in Africa, except in Cameroon where it has been reported for use in cereal grains preservation and in Nigeria as remedy for pediatric malaria (Njan et al., 2007; Okhale et al., 2010). The Asian researchers have also done a lot of work and have reported various bioactive properties of these plants. Anti-inflammatory, antiviral, antibacterial and hepatoprotective properties have been reported in literature (Zhao et al., 2004; Shi et al., 2007; Wu et al., 2006; Zhao et al., 1997). Also a number of sesquiterpenoids, monoterpenoids and some flavonoids with some bioactivity have been isolated (Shi et al., 2007; Wu et al., 2006; Zhao et al., 1997; Wu et al., 2006; Fraga, 2004; Yang et al., 2006; Xiao et al., 2003). However, no report has been made on anti-tuberculosis activity and no bioactive compound against tuberculosis (TB) has been identified from the plant. This work was aimed at studying the Nigerian specie of the plant on very little work has been reported, for anti-TB properties with the hope of identifying and isolating the bioactive compound in the face of growing organism's resistance to available TB drugs.

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MATERIALS AND METHODS

Both *Laggera* species were collected in Abuja by Professor J.I. Okogun on the 15th of October, 2008 and identified by Mrs. Grace Ugbabe at the NIPRD Herbarium. The plants were air-dried at room

Table 1. Preliminary anti-tuberculosis screening of *L. pterodonta* and *L. aurita*.

LAF	MIC (µ/ml)	LPF	MIC (µ/ml)
CME	625	CME	625
F1	N/A	F1	412
F2	2000	F2	N/A
F3	1000	F3	1000
F4	3000	F4	1000
F5	1250	F5	2500
F6	2000	F6	2000
F7	N/A	F7	N/A
F8	3000	F8	N/A
F9	2000	F9	2500
X 1	N/A	X 1	N/A
X 2	N/A	X 2	N/A
Rifampicin	0.02	Isoniazide	0.02

LAF = *L. aurita* fractions, LPF = *L. pterodonta* fractions, MIC = minimum inhibitory concentration, CME = crude methanol extract, X = crystals, N/A = no activity.

temperature for 2 weeks.

Extraction of plant material

The aerial part of *L. aurita* (250 g) and *L. pterodonta* (150 g) were cut into smaller segments (0.5 - 4cm in length) and pulverized further using mortar and pestle. The pulverized samples were macerated separately with 95% methanol for 24 h. The extraction was repeated once for each plant and the extracts combined and concentrated under reduced pressure. The extraction yield was 5.62 and 5.08% respectively, and a sample was sent for bioassay against mycobacterium bovis (BCG strains).

Chromatography

Five gramme of each plant extract was adsorbed on 8 g of silica gel G 230 - 400 mech. 20 g of the silica gel was suspended in hexane and packed in a chromatographic column of diameter 2 cm. The adsorbed plant extract was introduced into the column and eluted with varying solvents' ratios of hexane, ethylacetate, methanol and water. A total of 32 and 35 fractions were collected for *L. aurita* and *L. pterodonta* extract chromatograms, respectively. These fractions were analyzed on TLC and combined into nine fractions for each plant. The fractions were concentrated under reduced pressure and allowed to dry in the hood. The combined fractions and the isolated crystals were screened against *Mycobacterium bovis* (BCG strain).

Bioassay

The anti-tubercular activity of the plant extract was carried out by minimum inhibitory concentrations (MIC) determination of broth micro-dilution method (BMM) as modified by National Institute for Pharmaceutical Research and Development, Abuja Nigeria, and National Institute for Health, USA, Standard operating procedure No. 1 (2006) and Amsterdam, D. (1996) (Oladosu et al. (2007). 500 µl of test organism, *Mycobacterial bovis* (BCG strain) freshly thawed stock was inoculated into 50 ml of sterile Middle brook 7H9/ADC media from frozen saturated stock solution in 50% glycerol and at 37°C with shaking for 5 - 7 days. The optical density of the resulting culture using a Beckman Coulter's general purpose single cell module uv/vis spectrophotometer model DU 520 was approximately 0.2 at 650 nm. 50 µl of sterile media was introduced into well 2 - 12 of row A-H of a sterile 96 micro well plate. To each well of row 1 was added 100 µl of 10% DMSO in sterile media 7H9 Middle brook containing albumin dextrose complex, 100 µl of 25 µg/ml sterile stock solution of rifampicin and isoniazide, (control drugs, made from a 25 mg/ml solution in DMSO, 10 µl of this solution was added to 10 ml sterile 7H9/ADC media and 1 ml aliquot was frozen as reference standard) and 100 µl of each plant extract (made by dissolving 100 mg of extract in 1 ml DMSO and diluting 50 µl of the DMSO concentrate into 450 µl sterile 7H9/ADC media). Using an 8-channel pipettor, 50 µl was carefully removed from wells 1 and added to wells 2, mixed thoroughly by pipetting up and down four times, expelled excess liquid and drew 50 µl wells 2 and added to wells 3, repeated through micro wells plate to wells 12 and discarded the final 50 µl from well 12. The wells were inoculated as follows: The 5 - 7 days old culture (at OD 0.2) was diluted 1/1000 by adding 25 µl cell culture to 25 ml medium and 50 µl was inoculated to all wells of the plate. The plates were incubated initially for 7 days and recorded the column number of the row at which no apparent growth was seen using a table-top magnifier glass and for another 14 days, observed and recorded the column number of the row at which no apparent growth was seen. The experiment was done in duplicate.

RESULTS

The result of the preliminary anti-tuberculosis screening of *L. pterodonta* and *L. aurita* is given in Table 1.

DISCUSSION AND CONCLUSION

The activity shown by the crude may be a synergistic action of several compounds, or the low activity of the fractions may be due to "loss in column effect". More

work is however, needed to establish this. Two of the fractions from each of the chromatogram gave crystals (m.p. 215 - 222 and 235 - 240 d for the two crystals from *L. aurita,* the other two crystals from *L. pterodonta* is still being purified). The isolated compounds did not show any activity and are currently been subjected to spectroscopic analysis for characterization and identification.

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