SINET: *Ethiop. J. Sci.*, 30(1):49–54, 2007

ISSN: 0379–2897

EXTRACTIVES OF *MILLETTIA FERRUGINEA, TEPHROSIA VOGELLII* AND *TEPHROSIA PENTAPHYLLA* AGAINST THE BEAN BRUCHID, *ZABROTES SUBFACIATUS*

Bekele Jembere [1], Jane Namukobe [2], Bernard T. Kiremire [2] and Ermias Dagne [3]

[1] Department of Biology, Faculty of Science, Addis Ababa University, PO Box 1176 Addis Ababa, Ethiopia. E-mail: bekeleja@yahoo.com or bekeleja@bio.aau.edu.et
[2] Department of Chemistry, Makerere University, PO Box 7062, Kampala, Uganda
[3] Department of Chemistry, Addis Ababa University, PO Box 1176, Addis Ababa, Ethiopia

ABSTRACT: Different extracts from seeds and aerial parts of *Millettia ferruginea, Tephrosia vogellii* and *T. pentaphylla* were tested for insecticidal activity against the bean bruchid, *Zabrotes subfaciatus*, which is a common pest of stored bean. Out of all extracts tested, acetone extracts of *M. ferruginea* and *T. vogellii* seeds were the most active as 100% mortality of the been bruchid was observed. By using medium pressure liquid column chromatography, the active acetone extract of *M. ferruginea* seeds was further fractionated and the different fractions were tested for their toxicity against the bruchid. When the most toxic fraction was subjected to chromatographic and spectroscopic methods, it resulted in the identification of rotenone as the most active compound against this pest.

Key words/phrases: ***Millettia ferruginea*, rotenone, *Tephrosia pentaphylla, Tephrosia vogellii, Zabrotes subfaciatus***

INTRODUCTION

The bean bruchid, *Zabrotes subfaciatus* (Boheman) is one of the most common pests of stored beans in Africa (Slim, 1993). It is a major primary pest of common bean (*Phaseolus vulgaris*), cow pea (*Vigna unguiculata*) and lima bean (*Phaseolus lunatium*) (Hill, 1990). In Ethiopia, *Zabrotes subfaciatus* is one of the major pests of stored beans (Tsedeke Abate, 1995). A loss of 12% of the available proteins has been reported due to the infestation of this pest (Mc Farlance and Taylor, 1994). Beans are one of the most common foods as well as a source of income for people in rural parts of Africa. Because rural farmers cannot afford expensive synthetic pesticides, there is need, therefore, to utilize on a wider scale, naturally occurring pesticides derived from plants.

Millettia ferruginea (Hochst.) Bak. is a large shady tree endemic to Ethiopia and widely distributed in the country (Thulin, 1983). The powder from the seeds of this plant is commonly used for fish poisoning (Clark, 1943; Agbon *et al.*, 2004). Chemical studies of this plant led to the isolation of several flavonoids and rotenoids (Ermias Dagne *et al.*, 1989). The crude extracts from the seeds of this plant were found to be toxic to *Sitophilus zeamais* (Bekele Jembere, 2002) and a recent study also revealed that *Millettia ferruginea* in combination with other botanicals were effective against the stem-boring moth *Chilo partellus* (Sabiiti and Bekele Jembere, 2005).

The genus *Tephrosia* is well known for elaborating flavonoids and isoflavonoids (Gomes *et al.*, 1981). *Tephrosia vogelii* Hook. is a shrubby plant indigenous to Africa, but distributed to other parts in the tropics like in the US where it is used as a fish-poison and pesticide (Ibrahim *et al.*, 2000) and the principal active ingredient has been reported to be rotenone (Msonthi, 1985). In Botswana, Zambia and Tanzania, this plant has been introduced to farmers for use as a pesticide where the leaves are used extensively (Amelie, 1994). *Tephrosia pentaphylla* (Roxv.) G. Don is an annual perennial plant known to occur in many parts of East Africa, Arabia and south India (Thulin, 1983). Flavonoids and Isoflavonoids including rotenone have been isolated from its aerial parts (Ermias Dagne *et al.*, 1989).

This study was undertaken primarily to screen the various extracts of *M. ferruginea, T. vogellii, T. pentaphylla* against the bean weevil, *Zabrotes subfaciatus* in order to find a natural pesticide against this common pest.

MATERIALS AND METHODS

Materials

Millettia ferruginea was collected from private garden near the Faculty of Science, Addis Ababa University, Arat Kilo, *Tephrosia vogellii* was collected from the Herbal Garden near Meta Brewery, Sebeta and *Tephrosia pentaphylla* from the Blue Nile Gorge 199 km North of Addis Ababa. Identity of the plants was confirmed at the National Herbarium of Addis Ababa University with voucher numbers S1112 for *Tephrosia vogellii*, 140 for *Tephrosia pentaphylla* and S1113 for *Millettia ferruginea*.

The test insect, bean bruchid (*Zabrotes subfaciatus*) was obtained from the Entomology Laboratory of the Department of Biology, Addis Ababa University. The bruchids were sieved out of the beans containing them and discarded. Newly emerged and three day old bruchids were used as test insects.

Methods

Extraction

The dried seeds of *M. ferruginea* were ground to fine powder and soaked in each of water, acetone, chloroform and petrol at the rate of 100 g per 200 ml. The extracts were then filtered using a filter paper and concentrated to dryness using rotary evaporator. For *T. vogellii* and *T. pentaphylla*, extraction was done using acetone alone.

Bioassay of seed powder and its extracts

Different treatments were made by dissolving 150 mg of the different extracts, obtained through the above procedure, in 3 ml of the respective solvents used for their extraction. For each treatment, three Petri dishes containing Whatman No 1, 9 cm diameter filter papers were prepared considering each Petri dish as a replicate. The treatment was then uniformly applied onto the filter papers at the rate of 1 ml per filter paper (50 mg per filter paper). After application, treated filter papers in the Petri dishes were exposed to air for about 30 minutes to allow evaporation of the organic solvents. After evaporation of the organic solvents, distilled water (1 ml) was added to each filter paper to facilitate the transfer of the compounds from the filter papers to the insect body.

Similarly, seed powder of *M. ferruginea* was uniformly applied onto each filter paper at the rate of 200 mg per filter paper and replicated three times. Then, ten insects were introduced to each Petri dish containing the treated filter paper and mortality observation was made after 24 h.

Bioassay guided isolation of active compound

After observing the acetone extract of seeds of *M. ferruginea* as the most active extract among the tested extracts, the extract was further fractionated using medium pressure liquid column chromatography (MPLC). The extract was loaded on a column packed with silica gel, eluted with dichloromethane and petrol with increasing polarity. Five fractions were collected as a result. Each of the fractions was then applied onto a filter paper at the rate of 10 mg/filter paper for each replication and each treatment was replicated three times. As described above, ten test insects were introduced to each Petri dish containing the treated filter papers after evaporating solvents. Mortality count was done 24 h after introduction of the test insects into the treated filter papers.

Chemical analysis

Chemical analysis of fraction No 3, the most active fraction, was done using both chromatographic and spectroscopic methods guided by bioactivity assay of each band. Co TLC, ^{1}H NMR and ^{13}C NMR were used to confirm the structure of the active compound.

Data analysis

The data obtained for the mortality in the different treatments was analyzed using the SPSS computer software (1989). One-way analysis of variance (ANOVA) was used to compare the effects of the treatments, which were laid down in CRD design. Comparison of mean mortality was done using Student-Newman-Keuls (SNK) method at 5% level of significance.

RESULTS AND DISCUSSION

Toxicity of Millettia ferruginea extracts and seed powder

From the dried leaf and seed powder extracts tested the seed powder extracts showed significant toxicity. Among the crude extracts of the seed powder, the acetone extract was the most active causing 100% mortality of the tested insects. This is consistent with the findings of Bekele Jembere (2002) who reported that acetone extract was the most toxic extract to the maize weevil. The seed powder caused 100% mortality while the chloroform and petrol extracts caused 93% and

70% mortality, respectively. There was however, no mortality recorded for the water extract within 24 h (Table 1). These results indicate that acetone is efficient in extracting the active compound against the bean weevil. The lack of mortality for the water extract showed that the active compound is less or not soluble in water.

Table 1. Mean % mortality of the bean bruchids due to *Millettia ferruginea* seed powder and its different extracts.

Mean % mortality of the different extracts	
Treatment	Mean% mortality
Petrol extract	70 ± 5.7 [b]
Chloroform extract	93.3 ± 6.7 [a]
Acetone extract	100 ± 0.0 [a]
Methanol extract	20 ± 0.0 [c]
Water extract	0 ± 0.0 [d]
Seed powder	100±0.0 [a]
Control of all solvents	0 ± 0.0 [d]

The means followed by the same letter showed no significant difference from each other [Student-Newman-Keuls (SNK), α <0.05].

Toxicity test of the different fractions obtained from the acetone extract during isolation of the active compound showed that fraction 3 was the most active (Fig. 1).

Toxicity of T. vogellii *and* T. pentaphylla

The seed powder and leaf acetone extracts of *T. vogellii* showed 100% and 96% mortality, respectively. The extract from the leaves of *T. pentaphylla* showed 63% mortality of the insects (Table 2). These results indicate that the seed and leaf of *T. vogellii* have more of the active compounds than *T. pentaphylla*.

Table 2. Mean % mortality of the bean bruchid due to acetone extracts of leaf and seed powders of *T. vogellii* and *T. pentaphylla*.

Treatment	Mean% of mortality
T. vogellii seed	100 ± 0.0 [a]
T. vogellii leaf	96.6 ± 3.3 [a]
T. pentaphylla leaf	63.3 ± 3.3 [b]

The means followed by the same letter showed no significant difference from each other (SNK, α <0.05).

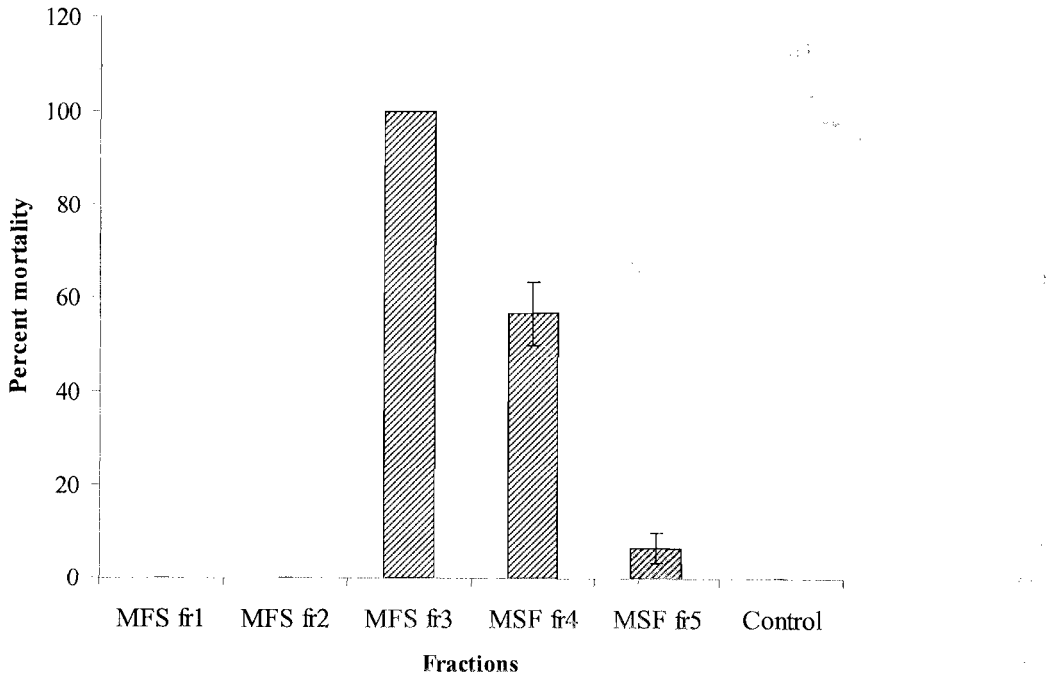

Fig. 1. Mean % mortality of the bean bruchids due to *Millettia ferruginea* seed powder acetone extract fractions. (MFS = *Millettia ferruginea* seed, fr =fraction)

Chemical analysis of the active compound

TLC analysis of the active fractions showed that the pure compound in the most active fraction (fraction No 3) was the only active compound and that the activity of the other fractions was due to the presence of this compound in those fractions. ^{1}H NMR and ^{13}C NMR analysis revealed identity of the compound with rotenone (Table 3; Fig. 2). This finding is in line with Gabor *et al.* (1989), Msonthi (1985) and Ermias Dagne (1989). Co TLC of the active compound with an authentic rotenone confirmed that the active compound against the bean bruchid, *Zabrotes subfaciatus,* was rotenone. Rotenone is a well-known botanical insecticide with a rat oral LD_{50} =132–1500 mg kg^{-1} through contact and stomach poisoning (BCPC, 1979; George, 1980). Its mode of action was reported by Rockstein (1978) to be blocking oxidation of $NADH_2$ in electron transport system.

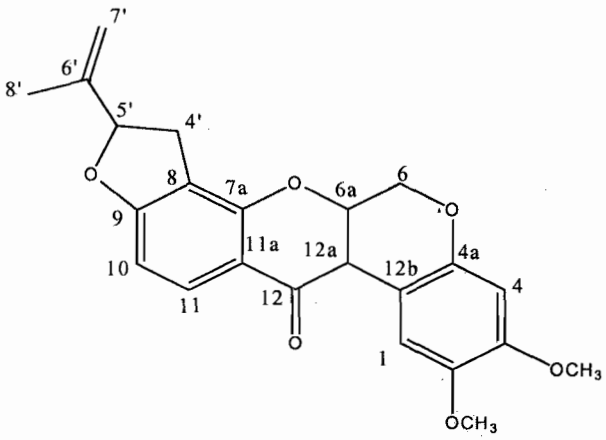

Fig. 2. Structure of the active compound (rotenone).

Table 3. ^{1}H and ^{13}C NMR data of the active compound.

C	δ ^{13}C/ppm	δ ^{13}C/ppm (lit -Gabor 1989)	δ ^{1}H/ppm	δ ^{1}H/ppm (lit -Gabor 1989)
1	110.9 (CH)	110.06	6.67(s)	6.71(s)
2	144.3 (C)	143.61		
3	149.9 (C)	149.23		
4	101.4 (CH)	101.70	6.34(s)	6.38(s)
4a	147.8(C)	147.19		
4′	31.7 (CH_2)	31.12	3.20(dd)	3.30(dd)
5′	88.2 (CH)	87.66	5.12(t)	5.21(t)
6	66.6 (CH_2)	66.10	4.52 (dd), 4.08 (bd)	4.61 (dd), 4.17 (bd)
6a	72.6 (C)	72.04		
6′	143.5 (C)	142.83		
7a	158.3 (C)	157.73		
7′	111.8 (CH_2)	112.40	5.18(s), 4.96(s)	5.01(s), 4.88(s)
8	112.9 (CH_3)	112.80	1.66(s)	1.70(s)
8′	17.5 (C)	17.00		
9	167.7 (C)	167.14		
10	105.2 (CH)	104.68	6.38(d)	6.43(d)
11	130.2 (CH)	129.77	7.72(d)	7.76(d)
11a	113.1 (C)	113.14		
12	189.3 (C)	188.73		
12a	45.3 (CH)	44.41	3.87(d)	3.76(d)
12b	105.3 (C)	104.63		
OCH_3	56.8 (CH_3)	56.13	3.69(s)	3.73(s)
OCH_3	56.4 (CH_3)	55.69	3.66(s)	3.70(s)

δ= Chemical shift, (s) =singlet, (d) = doublet, (dd) = double doublet, (Lit) =literature

CONCLUSION

This study revealed that the acetone extract was very toxic to the bean bruchid, *Zabrotes subfaciatus*, compared to other tested solvents. The lack of mortality for the water extract within 24 h period reflected the low solubility of rotenone in water. Because rotenone is less soluble in water, the water extract is active only if it is not filtered by filter paper. However it is active if it is filtered using cheese cloth only. Similarly, *Millettia* seed powder was observed to be as toxic as the acetone extract indicating that the seed contains the active compound, but needs carrier to convey it to the body of the test insect. Therefore, the small-scale farmers can use the seed powder directly without extraction, but need a medium or carrier like water to transfer the active plant material to the body of the insect.

ACKNOWLEDGEMENTS

We would like to acknowledge International Program in Chemical Sciences (IPICS-Sweden) for supporting the research through a grant to the African Laboratory for Natural Products (ALNAP) based at Chemistry Department, Addis Ababa University and Jane Namukobe is grateful to NUFU Basic Science Project for covering the travel support to come to Addis Ababa.

REFERENCES

1. Agbon, A., Ofojekwu, C. and Ezenwaka, I. (2004). Acute toxicity of water extract of *Tephrosia vogellii* Hook to species relevant in aquaculture ponds: rotifer, cyclops, mosquito larva and fish. *Journal of Applied Ichthyology* **20**:521–524.

2. Amelie, B. (1994). Using natural pesticides: Current and Future Perspectives: A report for the Plant Protection Improvement Programme in Botswana, Zambia and Tanzania. Department of Entomology, Swedish University of Agricultural Sciences.

3. BCPC (1979). *The Pesticide Manual: A world Compendium.* Philadelphia. British Crop Protection Council (BCPC), Glasshouse Crop Research Institute, PA.

4. Bekele Jembere (2002). Evaluation of the toxicity of *Millettia ferruginea* (Hochest) Baker against *Sitophilus zeamais* (Motsch.). *International Journal of Pest Management* **48**:29–32.

5. Clark, E.P. (1943). The occurrence of rotenone and related substances in the seeds of the Berbera tree. A procedure for the separation of deguelin and tephrosin. *Journal of American Chemical Society.* **65**:27.

6. Ermias Dagne, Abiy Yenesew and Waterman, P.G. (1989). Flavonoids and isoflavonoids from *Tephrosia fulvinervis* and *Tephrosia pentaphylla. Phytochemistry* **28**:3207–3210.

7. Gabor, B., Hui-Ling, S., John, M.P. and Geoffrey, A.C. (1989). ^{13}C-NMR spectral assignment and evaluation of the cytotoxic potential of rotenone. *Journal of Natural Products* **52**:1363–1366.

8. George, W.W. (1980). Complete *Guide to Pest Control With and Without Chemicals.* Thomson Publications, New York.

9. Gomes, C.M.R., Gottlieb, O.R., Marini Bettolo, G.B., Monache, F.D. and Polhill, R.M. (1981). Systematic significance of flavonoids in *Derris* and *Lonchocarpus. Biochemical Systematics and Ecology* **9**:129.

10. Hill, D.S. (1990). Pests of stored products and their control. *Belhaven Press*, London, 274 pp.

11. Ibrahim, B., Mbatchi, B., Mounzeo, H., Bourobou H.P.B. and Posso, P. (2000). Effect of *Tephrosia vogellii* and *Justicia extensa* on *Tilapia nilotica* in vivo. *Journal of Ethnopharmacol.* **69**:99–104.

12. Msonthi, J.D. (1985). Naphthoquinones, Rotenoids, Anthrones and Bianthrones from Medicinal Plants of Malawi. **In:** *NAPRECA Proceedings of the International Symposium*, August 20–24, 1985, pp. 148–152. Addis Ababa, Ethiopia.

13. Mc Farlance, J.A. and Taylor, R.W.D. (1994). *Insect Control.* **In:** *Grain Storage Techniques. Evolution and Trends in Developing Countries. FAO Agricultural Service Bulletin*, Vol. 109, pp. 189–227, (Proctor, D.L., ed.), Rome.

14. Rockstein, M. (1978). *Biochemistry of Insects.* Academic Press Inc, New York, 535 pp.

15. Sabiiti, S.M. and Bekele Jembere (2005). Effects of botanical, a synthetic insecticide and an insect growth regulator on survival and development of the parasitoid wasp *Cotesia flavipes* parasitizing the stem-boring moth *Chilo partellus. International Journal of Pest Management* **51**:25–30.

16. Slim, M.N. (1993). Studies of the control of the bean bruchids *Acanthoscelides obtectus* (Say) and *Zabrotes subfaciatus* (Boheman) Bruchidae: Coleoptera in Eastern Africa. **In:** *Proceedings of third Multidisciplinary Workshop on Bean Research*

in Africa, workshop series. No. 28, (Smithson, J.B., ed.) , CIAT, Kampala, Uganda.

17. Thulin, M. (1983). Leguminosae of Ethiopia. *Opera Botanica* **68**:71

18. Tsedeke Abate (1995). Pest management in lowland pulses: progress and prospect. In: *Proceeding of the 25th Anniversary of Nazareth Research Center,* 25 years of experience in lowland crop research, 20-33, September 1995, pp. 181-194, (Habtu Assefa, ed.), Nazareth Agricultural research Centre, Nazareth, Ethiopia.