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Spectrophotmetric evaluation of rotenone extraction from leaves and seeds of mature *Tephrosia vogelii* plant

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The percentage yield of rotenone extracted from *Tephrosia vogelii* leaves and seeds was investigated. Ten samples of well ground leaves and seeds were each subjected to extraction in soxhlet extraction system using trichloromethane, ethanol and methanol solvents for 24 h at room temperature. The maximum absorption wavelength of rotenone was determined using Ultra violet-visible spectrophotometer. Different solvent extracts were quantified using high performance liquid chromatography instruments fitted with Ultra Violet detector and their yield expressed as percentage rotenone. Trichloromethane recorded the highest rotenone yield from both leaves (8.3 and seeds 2.7%) compared to the other two solvents. Ethanol was second with 5.9% in leaves and 1.9% in seeds while methanol had 4.8% in leaves and 1.6% in seeds. In general, the leaves extracts were found to have a higher rotenone percentage yield by an average factor of 3 compared to seeds. Rotenone can be commercially extracted from *T. Vogelii* using ethanol that is locally available to complement natural pyrethrum insecticide industry.

Key words: Rotenone, *Tephrosia vogelii,* solvents, seeds, leaves.

INTRODUCTION

The legume *Tephrosia Vogelii* (*T. vogelii*) Hook. f. (Fabaceae) is a shrubby plant distributed to many parts in the tropics where it is used as shelter, cover crop, fish poison and as a pesticide. It was identified in Kenya in 1930 in Shimba Hills, Kwale County at the Coast region, 1932 at Mt.Elgon in the western part and in Kiambu County in 1934 in the central part of the country. This plant has over the years been known to have insecticidal and acaricidal activities though it has not been commercially exploited locally. It is worth noting that Pyrethrum, *Chrysanthemum cinerariaefolium*, was introduced in Kenya in early 1930s by the colonial farmers and a processing plant constructed in Nakuru Town in 1935. Pyrethrum is now grown in the four East Africa countries namely, Kenya, Rwanda, Uganda and Tanzania, by small scale farmers for sale to three processing plants in the region.

In Kenya, pyrethrum is grown in more than 15 counties,

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with the highest yielding crop (3.0%) pyrethrins, found in the highlands of West Pokot County (PBK, 1996).

In East and Southern Africa, *T. Vogelii* is grown in small plantations by small scale farmers for their own use in crop protection, both in the field plantation against moles and in storage against insects (Belmain et al., 2012). Crop production in Kenya is constrained, where the small scale farmers loose almost 30% of their yield to pest in the field and in post harvest storage (Midega et al., 2016). There has been a renewed interest in botanical pesticides because of several distinct advantages that include; i) pesticidal plants are generally safer than conventional synthetic ones due to their degradation rate and metabolites, ii) Plants have more than one molecule as active principle ingredients responsible for biochemical properties hence, insects are highly unlikely to develop resistance. Pyrethrum has six pyrethrins different molecules and has been in use as an insecticide in the last 70 years (Casida and Quistad, 1995).

The insecticidal activity of *T. Vogelii* plant is attributed to, rotenone, tephrosine, deguelin and toxicarol compounds collectively referred to as rotenoids. Rotenone show stronger insecticidal activity than tephrosine and degueline while toxicarol is toxic to fish (Stevenson and Belman, 2016; Watt and Brandwijk, 1988).

Rotenoids are respiratory enzyme inhibitors, acting between a coenzyme involved in oxidation and reduction in metabolic pathways and a respiratory enzyme responsible for carrying electrons in some electron transport chain (Hassanali et al., 1989). Rotenone is unstable and decomposes rapidly in light and air with a half life ($t_{1/2}$) of 6 h (Kariuki et al., 2014; Mkenda et al., 2015). *T. vogelii* is also found in other parts of the world and was introduced to the United States of America in the sixties for the purpose of commercial production of Rotenone with much success than *Derris* and *Lonchocarpus* spp. which hitherto were the main sources of the pesticide (Yenesew et al., 2004). The plant's extract is not phytotoxic and only moderately toxic to mammals by inhalation than by ingestion. Skin irritation and inflammation of mucous membranes may result from skin contact (Sola et al., 2014; Tavershimal et al., 2015).

T. Vogelii extracts have been shown to effectively synergize the insecticidal activities of Pyrethrum by a Co-toxicity Coefficient (CC) factor of 4, when combined at a Pyrethrum:*T. vogelii* ratio of 57:2, against cockroach (*Americana periplaneta*) (Kariuki et al., 2014). This makes *T. vogelii* an important and effective ingredient to support natural derived insecticides that will not only substitute for insect resistive overused synthetic molecules, but will also sustain the environment in addition to increasing income revenues to small scale farmers in Kenya. The aim of this study was to evaluate *T. vogelii* as an additional commercial source of sustainable natural insecticide that is effective and environmentally friendly, capable of enhancing the activity and hence the demand of pyrethrum uses, to farmers and general public. The successful positive evaluation will not only introduce a new cash crop to farmers, but also promote growing of *T. vogelii* and Pyrethrum in Kenya.

MATERIALS AND METHODS

Sampling

T. vogelii leaves and seeds samples were collected from a farm in Kikuyu area, from 2 year old mature plants. The leaves were harvested, put into polythene bags and brought to the Laboratory in the Department of Chemistry, University of Nairobi for further preparations prior to extraction.

Sample preparation

The leaves and seeds were spread on laboratory benches and dried at room temperature for two weeks. They were then ground into powder using a high speed blender. 250 g of the ground plant material was weighed in triplicate and stored in paper bags in the lockers away from light.

Extraction

Each 250 g sample was then transferred into a thimble and assembled the soxhlet extraction system and extracted using either of the solvents viz; 95% trichloromethane, 98% methanol and 95% ethanol. The extraction experiments were repeated with each of the other two solvents separately to give a set of three extracts. The extracts were then evaporated to near dryness on a rotary evaporator at reduced pressure and 40°C to give a dark to light brown oily trichloromethane, methanol or ethanol crude extract. The weight of each extract obtained was determined and then stored in a refrigerator at 0-4°C for analysis for analysis.

Ultra violet- visible absorption wavelengths of rotenone

One gram of rotenone standard reference material and trichloromethane extract from *T. vogelii* was each separately dissolved in 100 cm^3 of acetone. This experiment was repeated with each of the other two solvent *T. vogelii* extracts. The absorption wavelength of rotenone standard reference material and the rotenoids extracts were determined while acetone was used as the blank. The maximum absorption wavelength of rotenone was used to monitor the separations in the detector in the HPLC analysis.

High performance liquid chromatography analysis of rotenoids

The analysis of rotenone was quantitatively analyzed using high performance liquid chromatography fitted with an ultra violet detector. A stationary phase (PR C18, 250 x 4.6 mm) column was used and a mobile phase system comprising of acetonitrile and water at a ratio of 85:15, flowing at a rate of 2 cm^3/min and a chart speed of 1 cm/min. The liquid chromatography system was switched on and allowed to stabilize for 5 minwith the mobile phase before injecting the analyte. The injection volume in each case was20 µl. The analysis was performed at the maximum absorption wavelength on injecting an appropriate amount of standard solution

Table 1. UV/Visible absorption wavelengths of standard Rotenone and *T. Vogelii* extracts.

S/N	Sample	Maximum absorption wavelength (λnm)
1	Rotenone [95%]	340
2	*Tephrosia vogelii* Extracts	340,570,695,665,666

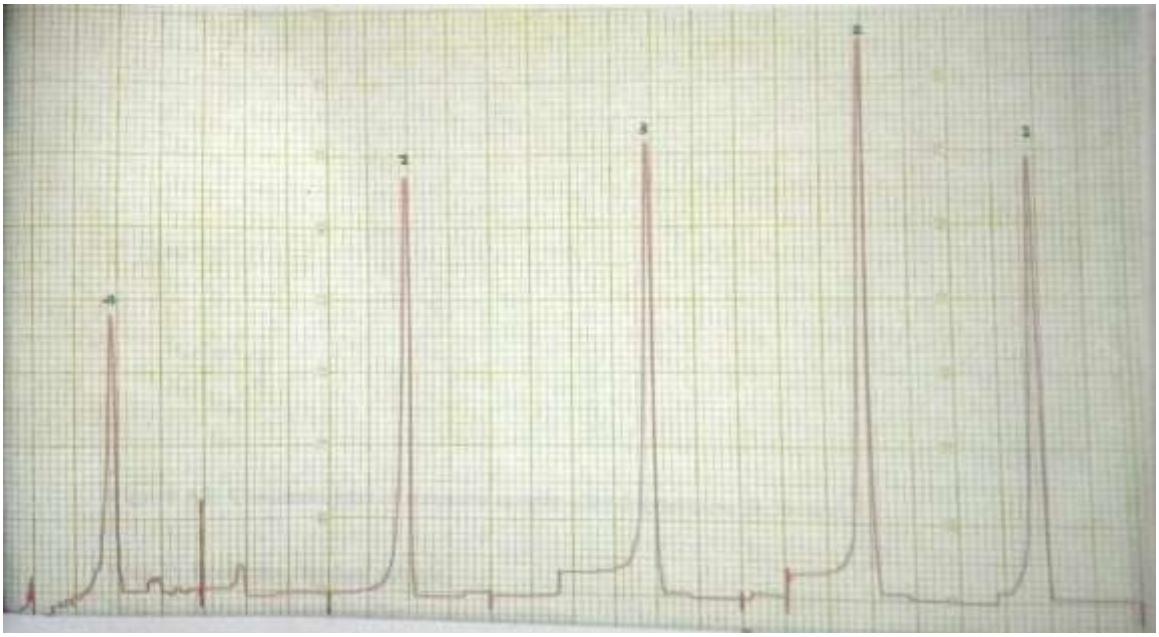

Figure 1. HPLC *Tephrosia Vogelii* Leaves Extracts Chromatogram. Peak 1, rotenone standard sample; Peak 2, trichloromethane extract; Peak 3, ethanol extract; Peak 4, methanol extract.

of 1 mg/cm^3. The quantification of the analyte was based on peak areas corrected with the rotenone standard reference material.

Determination of percentage yield of rotenone

A stock solution of *T. vogelii* trichloromethane extract was prepared by dissolving 1 g of extract with 100 cm^3 of acetone. 1 cm^3 aliquot of the stock solution was measured and then homogenised with a mobile phase solution. The sample was then injected into the HPLC for analysis. Similar procedure was repeated for methanol and ethanol extracts and each experiment was replicated four times. Concentrations of various extracts and standard deviations were then established. The yield of rotenone in leaves and seeds was then expressed as the percentage concentration of the initial density of the original extract.

RESULTS AND DISCUSSION

Maximum absorption wavelength of rotenone in ultra violet-visible spectrophotometer

The establishment of absorption wavelength of Rotenone standard reference material and rotenoids from extract had several peaks in the scan from the ultra violet to the visible region of the electromagnetic spectrum.

The maximum absorption wavelength of Rotenone standard reference material was established at 340 nm. The *T. vogelii* extracts exhibited five absorption peaks between 340 and 666 nm (Table 1). At 340 nm, the peak was slightly broader compared to the one realized in the case of Rotenone standard reference material. The five peaks were realized from all the three extracting solvents but at varying proportion, thereby signifying extraction efficiencies.

High performance liquid chromatography (hplc) of *tephrosia vogelii* extracts

The concentration of the *T. vogelii* extracts of the three solvents was analysed using HPLC. The chromatogram in Figure 1 shows the varying peaks of the extracts.

The percentage yield of the solvents' extracts was established on the basis of rotenone in *T.vogelii* leaves and seeds.

Table 2. Mean densities and concentration of rotenone in leaves and seeds extracts.

S/N	Solvent	Plant material	Weight of dry material	Concentration of Rotenone, g/kg (sd)	Yield % (sd)
1	Trichloromethane	Leaves	124.1	10.3 ±0.84	8.3±0.71
		Seeds	40.7	4.8 ±0.054	2.7±0.15
2	Methanol	Leaves	85.4	4.1 ±0.08	4.8±0.22
		Seeds	112.5	1.8±0.03	1.6±0.08
3	Ethanol	Leaves	61.0	3.6±0.04	5.9±0.24
		Seeds	78.9	1.5±0.02	1.9±0.1

Percentage yield of rotenone extracts in leaves and seeds

The three solvents were separately used to extract rotenone from two different parts of a mature plant. Table 2 shows the percentage yields of leaves and seeds extracted with three solvents.

The trichloromethane extracts recorded the highest concentration of rotenone per kilogram of dry material extracted in both leaves and seeds extracts while the least concentration was recorded in methanol extracts. The percentage yield of rotenone in trichloromethane leaves and seeds extracts were 8.3 and 2.7% respectively. All the solvents recorded a higher percentage yield in leaves compared to the seeds.

Conclusion

Rotenone is therefore highly concentrated in the mature *T. vogelii* plant's leaves than in the seeds by a factor of 3. The extraction efficiency of solvents used (described on the basis of the actual content of rotenone present in one kilogram of dry material seeds or leaves extracted) showed that trichloromethane (8.3%) yield in leaves is about three times the highest percentage yield of 3% w/w pyrethrins, achieved from highest yielding *Chrysanthemum cinararaefolium* flowers in Kenya. Moreover, the amount of *T. vogelii* required to synergise Pyrethrum extract is 28.5 times lower; to achieve an average of 4 times pyrethrins efficacy. In conclusion growing of *T. vogelii* may be commercialized to effectively extract rotenoids from its leaves using trichloromethane or ethanol. Trichloromethane is a relatively harsh solvent, expensive and given that ethanol is locally manufactured, it would well be used for commercial extraction of Rotenoids from the leaves of *T. vogelii*.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests

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