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Quantitative and Fourier Transform Infrared Analysis of Saponins from Three Kenyan *Ruellia* Species: *Ruellia prostrata*, *Ruellia lineari-bracteolata* and *Ruellia bignoniiflora*

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Abstract*—Ruellia* (syn. *Dipteracanthus*) species are wild perennial creepers belonging to the Acanthaceae family. These species are reported to possess anti-inflammatory, analgesic, antioxidant, gastroprotective, anticancer, and immuno-stimulant properties. Phytochemical screening of both aqueous and methanolic extracts of *Ruellia* species revealed the presence of saponins. Saponins have been reported to possess anti-inflammatory, antioxidant, immuno-stimulant, antihepatotoxic, antibacterial, anticarcinogenic, and antiulcerogenic activities. The objective of this study was to quantify and analyze the Fourier transform infrared (FTIR) spectra of saponins in crude extracts of three Kenyan *Ruellia* species namely *Ruellia prostrata* (RPM), *Ruellia lineari-bracteolata* (RLB) and *Ruellia bignoniiflora* (RBK). Sequential organic extraction of the ground whole plant material was done using petroleum ether (PE), chloroform, ethyl acetate (EtOAc), and absolute methanol by cold maceration, while aqueous extraction was by hot maceration. The plant powders and extracts were mixed with spectroscopic grade KBr and compressed into a pellet. The infrared spectra were recorded using a Shimadzu FTIR spectrophotometer of 8000 series in the range of 3500 cm^{-1} - 500 cm^{-1}. Quantitative determination of the saponins was done using standard procedures. Quantitative analysis of saponins showed that RPM had the highest quantity of crude saponins (2.05% ± 0.03), followed by RLB (1.4% ± 0.15) and RBK (1.25% ± 0.11), respectively. FTIR spectra revealed the spectral peaks characteristic for saponins in RPM, RLB, and RBK plant powders, aqueous and methanol extracts; O-H absorption (3265 - 3393 cm^{-1}), C-H absorption ranging from 2851 to 2924 cm^{-1}, C=C absorbance (1628 - 1655 cm^{-1}), oligosaccharide linkage (C-O-C) absorption due to sapogenins (1036 - 1042 cm^{-1}). The crude saponins from RPM, RLB and RBK showed similar peaks to their respective extracts. The presence of the saponins in extracts of RPM, RLB and RBK may be responsible for some of the biological activities reported in the *Ruellia* species.

Keywords*—Ruellia bignoniiflora*, *Ruellia lineari-bracteolata*, *Ruellia prostrata*, Saponins.

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I. Introduction

PLANTS have been used as medicines since the beginning of civilization. The reported healing powers of plants indicate that they have medicinal values where they have played a significant role as alternative therapeutic tools for prophylaxis and treatment of both communicable and non-communicable diseases [1].

It was reported that 80% of the world population had used complementary medication [2], [4]. The discovery of drugs from medicinal plants started from the era when the isolation of drugs such as digitoxin, quinine, cocaine, morphine, and codeine began. These drugs came from an enormous reservoir of biologically active molecules (secondary metabolites), although only a small fraction of these products with medicinal value has been assayed [5], [6].

Ruellia (syn. *Dipteracanthus*) genus belongs to the family of Acanthaceae. It is a genus of flowering plants commonly known as *Ruellias* or wild petunias. These species are distributed in tropical and temperate regions of both the hemispheres; Indonesia, Malaysia, Africa, Brazil, and Central America are the leading producers [7]-[9]. *Ruellia* species are also known for their many medicinal properties. One of the species, *R. tuberosa,* has been extensively used as a diuretic, antidiabetic, antipyretic, analgesic, antioxidant [10], antihypertensive, gastroprotective [11], and for treatment of gonorrhea [12]. The phytochemical properties of *R. tuberosa* have been investigated revealing the presence of alkaloids, triterpenoids, saponins, sterols, and flavonoids [13]. *Ruellia asperula* has been used in management of bronchitis, asthma, flu, fever, and uterus inflammation [14]. *RPM* leaf is used in the treatment of chronic rheumatism, eczema, facial paralysis, cephalgia, and hemiplegia. The leaf juice is an efficient remedy of colic in children [15]. Antioxidant activity of *R. prostrata* has been reported [16], [17]. Anti-inflammatory activity of aqueous and alcoholic extracts of *R. prostrata* was also reported in [18]-[20]. RLB and RBK species have been reported to possess antioxidant activities [21]. The fresh leaf of *R. patula* is pounded and then soaked in water until the concoction turns black. It is decanted and the solution applied

to the ear [22]. Anti-inflammatory activity of *R. patula* has also been reported [23].

Although there are about 300 species of *Ruellia,* only a few have been investigated for their biologic or pharmacologic activities. *Ruellia* is widespread around the world, and many of its species have been used in folkloric medicine. Phytochemical screening of *R. prostrata, R. linearibracteolata,* and *R. bignoniiflora* species revealed the presence of saponins, flavonoids, phenols, terpenoids, tannins, and glycosides [16], [17], [20]. Saponins are secondary metabolites, from many plants, which belong to either steroidal or triterpenoid glycosides. Several biological activities have been attributed to the presence of saponins in such plants. Such activities include; anti-inflammatory, antibacterial, immunostimulant, anticarcinogenic, *inter alia* [24]-[26].

The aim of this study was to screen crude extracts from three Kenyan *Ruellia* species namely *R. prostrata* (RPM), *R. lineari-bracteolata* (RLB) and *R. bignoniiflora* (RBK) for the presence of saponins, quantify the saponins and analyze their FTIR spectra.

II. Materials and Methods

A. Collection and Identification of Plant Specimens

Plant samples of RPM, RLB and RBK were collected from Muthetheni (Machakos County), Isiolo (Isiolo County) and Kibwezi (Makueni County), in Kenya, respectively. The plants were taxonomized and Voucher specimens; UoN/ 2010/598 of 16/12/2010 (RPM); UoN/2013/811 of 15/3/2013 (RBK), and UoN/2015/003 of 3/7/2015 (RLB) were deposited at the Department of Botany Herbarium, University of Nairobi, Kenya. The collected plant materials were dried in shade at ambient temperature, ground and milled to coarse powder by use of an electrical grinder made from Mechanical Engineering Department of Jomo Kenyatta University of Agriculture and Technology.

B. Preparation of Plant Extracts

1. Aqueous Extraction

The crude aqueous extracts of RPM, RLB and RBK were prepared according to standard techniques as previously described [6].

Briefly, 50 gm of plant powder was mixed with 500 mL of distilled water in 1L flask and heated on a water bath at 60°C for 6 hours. The contents were left overnight. Next day, the extracts were filtered in a Buchner funnel using Whatman No.1 filter paper. The extracts were then evaporated to dryness by use of a freeze dryer (Christ Alpha 1-4 LD) and stored at 4°C.

2. Organic Extraction

Organic extraction was done by soaking 50 gm of plant powder of *Ruellia* species in 500 mL of; PE, chloroform, EtOAc and absolute methanol, sequentially in order of increasing polarity. The contents were shaken for 6 hours on a mechanical shaker, left overnight, and filtered through a Buchner funnel using Whatman filter paper No. 1. Concentration of the extracts was done using a rotary evaporator (BUCHI R-200) at 40-60°C (PE), 61°C (chloroform), 77°C (EtOAc), and 65°C (absolute methanol). The extracts were stored at 4°C. A schematic presentation of the organic extraction is shown in Fig. 1.

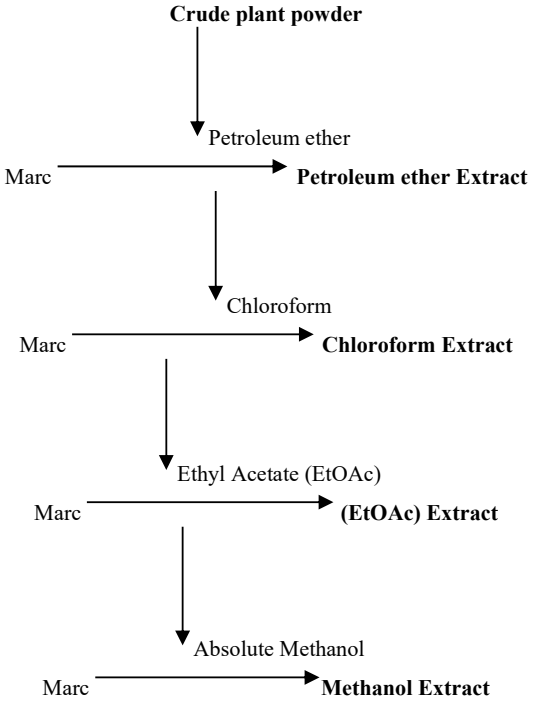

Fig. 1 A schematic presentation of the organic extraction of RPM, RLB and RBK powders

C. Phytochemical Screening

1. Tests for Saponins

Saponin tests were carried out on the plant powders of RPM, RLB, and RBK using standard procedures of plant constituents' identification as described by [6], [27], [28]. Briefly, 3 gm of each dry plant powder was weighed and extracted with 300 ml of hot distilled water in a beaker. After filtration, 5 mL of the filtrate was placed into a test tube and diluted with 5 mL of distilled water. The mixture was shaken vigorously for two minutes to examine the appearance of any frothing. Later, 3 drops of olive oil were added and the contents shaken again to examine the appearance of an emulsion.

D. Quantitative Analysis of Saponins

The method of Obadoni and Ochuko was used [29]. Briefly, 20 gm of each crude powder of RPM, RLB and RBK was put into a conical flask. Subsequently, 200 mL of 20% aqueous ethanol was added. The sample was heated over a hot water bath at 55°C for 4 h with continuous stirring. The mixture was filtered and the residue re-extracted with another 200 mL of 20% ethanol. Combined extracts were reduced to 40 mL over water bath at 90°C. The concentrate was transferred into a 250

mL separatory funnel and 20 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the diethyl ether layer was discarded. Purification process was done with 60 mL n-butanol and contents shaken together. The process was repeated with another 60 mL of n-butanol. The combined n-butanol filtrates were washed twice with 10 mL of 5% w/v aqueous sodium chloride. The washed n-butanol filtrates were pooled together and heated on a water bath at 90°C. After evaporation, the samples were dried in the oven at 90°C to a constant weight. Three determinations were performed and the average taken. The saponin content was then calculated as percentage using the following formula:

$$\% \text{ yield} = \frac{\text{weight of saponins extract}}{\text{weight of plant sample}}$$

2. Infrared Spectra

An amount of 10 mg of crude plant material of RPM, RLB and RBK and both aqueous and methanol extracts were mixed with spectroscopic grade KBr in a crucible and compressed into a pellet. Crude saponins from RPM, RLB and RBK were also compressed with spectroscopic grade KBr in a crucible and compressed into a pellet. The pellets from crude plant material, extracts and crude saponins were subjected to FTIR spectroscopy. The infra-red spectra were recorded using a Shimadzu FTIR spectrophotometer of 8000 series in the range of 3500 cm^{-1} - 500$cm^{-1.}$ Absorbances/ transmittances obtained were recorded in Tables IV-VII.

III. Results

Solvent extraction yields of RBK, RPM and RLB are shown in Table I.

TABLE I
SOLVENT EXTRACTIVE (N=3) VALUES (% MEAN ± SD) OF *RUELLIA* SPECIES

Solvent	Plant solvent extractive value (Mean ± SD)		
	RBK	RPM	RLB
1. Methanol	4.38± 0.13	4.4± 0.55	2.3± 0.19
2. Chloroform	1.43 ± 0.02	1.3± 0.03	0.5± 0.05
3. Petroleum Ether	0.52 ± 0.05	0.35± 0.03	0.08± 0.03
4. Ethyl Acetate	1.10 ± 0.17	0.8± 0.01	0.4± 0.04
5. Water	37.60 ±1.94	27.4± 0.69	20.7± 0.69

A. Phytochemical Screening

Results obtained from the phytochemicals screening of extracts of *Ruellia* species are presented in Table II.

TABLE II
PHYTOCHEMICAL SCREENING FROM EXTRACTS OF *RUELLIA* SPECIES

Samples	Aqueous	Methanol
RBK	+	+
RPM	+	+
RLB	+	+

+ indicates the presence of saponins

Frothing was obtained on shaking the aqueous solutions of RPM, RLB, and RBK plant powders.

Later, an emulsion was formed after addition of 3 drops of olive oil to each aqueous extract and the contents shaken together.

B. Quantitative Analysis of Saponins

The phytochemical content of crude saponins from RPM, RLB, and FTIR spectra of plant powders, aqueous and methanol extracts of RPM, RLB, and RBK showed the following characteristic infrared absorbance of saponins; the hydroxyl group (-OH) ranging from 3429 cm^{-1} to 3316 cm^{-1}; C-H ranging from 2922 cm^{-1} to 2929 cm^{-1}; C = C absorbance ranging from 1619 cm^{-1} to 1651 cm^{-1}; C=O ranging from 1740 cm^{-1} to 1736 cm^{-1}. Oligosaccharide linkage absorptions to sapogenins, that is C-O-C, were evident between 1034 cm^{-1} to 1072 cm^{-1}. The results are shown in Tables IV-VII.

TABLE III
SUMMARY OF SAPONIN CONTENTS

Samples	Phytochemical Content (%w/w)
	Saponins
RBK	1.25± 0.11
RPM	2.05±0.03
RLB	1.4±0.15

TABLE IV
FTIR SPECTRA OF RBK, RPM AND RLB PLANT POWDERS (KBR DISC), CM^{-1}

RBK	*RPM*	*RLB* *
3433	3438	3258
2925	2927	2945
1733	1733	2870
1651	1652	1626
1423	1429	1407
1041	1037	1001

*No C=O peak observed

TABLE V
FTIR SPECTRA OF RBK, RPM AND RLB METHANOL EXTRACTS (KBR DISC), CM^{-1}

RBK *	*RPM* *	*RLB* *
3421	3404	3258
2937	2935	2958
2133	2131	2870
1629	1627	1628
1402	1402	1470
1055	1060	1069

* No C=O peak observed.

TABLE VI
FTIR SPECTRA OF RBK, RPM AND RLB AQUEOUS EXTRACTS (KBR DISC), CM^{-1}

RBK *	*RPM* *	*RLB*
3286	3400	3425
2939	2945	2923
1716	1996	1732
1647	1651	1651
1612	1451	1560
1413	1415	1424
1056	1049	1038

*No C=O peak observed

IV. Discussion

Despite differences in their geographical locations, phytochemical analyses of RPM, RLB and RBK species gave

positive results for saponins. RPM showed highest amount of crude saponins (2.05 %± 0.03) compared to RLB (1.4% ± 0.15) and RBK (1.25% ± 0.11). This difference is not surprising since bioactive compounds in plants are determined by several factors including soil composition of plants, geographical locations, seasonal variation, intraspecies variation, *inter alia* [35]. Infrared absorptions spectroscopy confirmed these results. The hydroxyl group (-OH) ranging from 3429 cm^{-1} to 3316 cm^{-1} (Crude RPM, RLB and RBK; MeOH RPM, RLB and RBK; Aqueous RPM and RLB; Crude saponins RPM); C-H ranging from 2922 cm-1 to 2929 cm^{-1} (Aqueous RPM and MeOH RPM); C = C absorbance ranging from 1619 cm^{-1} to 1651 cm^{-1} (Aqueous, MeOH, crude powders and crude saponins of RPM, RLB and RBK), C=O ranging from 1740 cm^{-1} to 1736 cm^{-1} (Crude RPM and RBK; Aqueous RLB). Oligosaccharide linkage absorptions to sapogenins; C-O-C, were evident between 1034 cm^{-1} to 1072 cm^{-1} (Aqueous RPM, RLB and RBK; MeOH RPM, RLB and RBK; Crude saponin RPM; Crude powder RPM and RBK).

TABLE VII
FTIR SPECTRA OF RBK, RPM AND RLB CRUDE SAPONINS (KBR DISC), CM^{-1}

RBK *	*RPM*	*RLB* *
3483	3419	3482
3406	2920	2978
2090	1728	2380
1639	1649	1641
1393	1458	
1037	1045	

* No C=O peak observed
No C-O-C peak observed in RLB

The infrared functional group absorptions were characteristic of saponins. The –OH, C-H, C=C, C=O and C-O-C found in RPM, RLB and RBK were cited in literature [3], [33]. Oleanane-type triterpenoid saponins are characterized by the C=O infrared absorbance due to oleanolic acid/ester. In this study, crude RPM and RBK powders and aqueous RLB showed the C=O absorbance, suggestive of oleanane triterpenoid saponins [31], [32]. Such triterpenoid saponins are also likely to be bidesmosides [31], [33], since they have two attachments of glycones (thus, glycosidic and ester groups) to the sapogenins [34]. In this study, saponins detected in RPM and RBK, were likely to be bidesmosidic, oleanane-type triterpenoids. RLB showed the C=O bond only in aqueous solution. Crude saponins from RPM, RLB and RBK did not show the C=O bond, although the crude powders showed the C=O bond. IR functional group absorptions reported for pure isolated saponins were identical to those reported in this study [30], [33]. Entada leptostachya solvent extracts and plant powders showed similar FTIR results to the ones in this study [34].

Further analysis of the saponins present in RPM, RLB, and RBK by other spectroscopic techniques including High Performance Liquid Chromatography-Mass Spectroscopy, and Nuclear Magnetic Resonance are needed to elucidate their structures and determine their molecular weights. It is worth noting that crude plant powders of RPM, RBK and RLB exhibited almost similar absorbances to those of their aqueous and organic extracts. Presence of saponins in the plants of this study may thus partially justify their ethnomedical use in management of various ailments

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