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Cytotoxicity and antiretroviral activity of the leaf and stem bark extracts of *Croton macrostachyus*

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The Pharmaceutical Society of Kenya (PSK) is a representative organization that was formed enabling Pharmacists' to employ their professional expertise in the care of patients.

Established in 1964, PSK has its roots in the Pharmaceutical Society of East Africa, which was registered in 1950. Since its formation, PSK continues to promote a common standard for professional conduct and code of ethics for its members, as well as advocate for the welfare of Pharmacists.

EDITORIAL

CHALLENGES IN PHARMACEUTICAL REGULATION

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The emergence of a wide range of pharmaceutical products with various uses calls for the tightening of the regulatory process by the local National Medicines Regulatory Authority (NMRA). In Kenya, the NMRA is the Pharmacy and Poisons Board (PPB). The regulatory process requires that all pharmaceutical products have their pharmacological efficacies, safety and toxicological profiles clearly established before being registered for distribution and consumption in the market. Since all drugs are poisons, proper regulation and registration is to ensure end-user and patient safety.

Challenges still face the regulatory process as the market is infiltrated by unscrupulous business people and quacks who sell products to consumers with total disregard to the consequences that follow thereafter. The matter worsens as drugs such as antibiotics that should be prescription-only medicines find their way along the roadside, in shops, and are being hawked in buses and stages to unsuspecting clients. The sellers of such unregulated products have mastered the art of convincing their customers and tapping into their desperation by selling them hope through their harmful products. It is not uncommon to find products that are advertised to enhance sexual performance, treat infertility and other gynecological problems, or cure HIV and cancer, being the best sellers. Most of these are supposedly herbal remedies, though a huge number are products of, or related to, conventional medicines.

The field of cosmetics is yet another area that requires strict regulation because many products that purport to lighten skin, enhance certain body parts, and promote weight loss, among other claims, are readily found in the mainstream market. Some of these products are laced with harmful ingredients such as mercury and hydroquinone in quantities that are beyond those recommended for dermatological use. Furthermore, these products are not necessarily subjected to quality control processes, and they do not meet the labelling requirements of pharmaceutical products, nor do they bear the relevant warnings. The use of such unregulated products found in the market results in the end-users experiencing harmful effects of the products.

The PPB must enhance their market regulation to restore sanity and ensure consumer safety, whether through increased juridical enforcement or review of the existing legal framework. Other relevant stakeholders such as regulatory affairs pharmacists of the different Pharmaceutical companies should ensure that the products they submit for registration consideration meet the set requirements and their quality are assured. Outlets in the retail sector should have qualified personnel practicing ethically, stocking and dealing only in those products duly registered by the PPB. This fight is a tough one and is expected to be long drawn, but all the relevant stakeholders must be ready and willing to join in to win.

Anti-Inflammatory Activity of Whole Plant Extracts of selected Kenyan *Ruellia* Species

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Abstract

Ruellia species (Fam. Acanthaceae) is a perennial creeper with many reported biological activities. Studies on extracts of *Ruellia* species revealed *in vivo* analgesic activity in mice. The objective of this study was to determine the anti-inflammatory activity of aqueous and methanolic whole plant extracts of selected Kenyan *Ruellia* species (viz. *R. prostrata, R. bignoniiflora* and *R. lineari-bracteolata*) in Wistar albino rats. Anti-inflammatory activity of species was evaluated by determining the percentage inhibition of carrageenan-induced rat paw swelling. The aqueous and methanolic *Ruellia* extracts (250, 500, 1000 and 1500mg/kg) and positive controls (diclofenac sodium 20mg/kg and ibuprofen 200mg/kg) were administered orally prior to carrageenan injection. Determination of swelling was done every hour for six hours following carrageenan injection using a digital water plethysmometer. Both aqueous and methanolic *Ruellia* extracts exhibited dose-dependent anti-inflammatory activity. The aqueous extract of *R. prostrata* was the most potent. The highest percentage inhibition of swelling was exhibited at the 6-hour point by 1500mg/kg of the aqueous extract of R. prostrata (79.12%), followed by *R. bignoniiflora* (70.83%) and *R. lineari-bracteolata* (68.82%). At the same time point, diclofenac sodium (20mg/kg) and ibuprofen (200mg/kg) displayed percentage inhibition in swelling of 67.87% and 60.53%, respectively. Aqueous extracts were consistently more potent than the corresponding methanolic extracts. All test groups showed significantly increased activity ($p \leq 0.05$) compared to the negative (untreated) control. The aqueous extracts of whole plant parts of Kenyan *Ruellia* species possess significant anti-inflammatory activity, and there is need to investigate these species further for the management of inflammatory conditions such as rheumatoid arthritis. This study also reported anti-inflammatory activity of *R. bignoniiflora* and *R. lineari-bracteolata* for the first time.

Keywords: *Ruellia, R. prostrata, R. bignoniiflora, R. lineari-bracteolata, anti-inflammatory, carrageenan.*

Introduction

Ruellia (Fam. Acanthaceae), is a genus of flowering plants commonly known as *Ruellias* or wild petunias. Acanthaceae Family contains about 300 genera and 2500 species. They are distributed in Indonesia and Malaysia, Africa, Brazil, Central America, and Pakistan, with only a few species distributed in temperate regions [1, 2].

Some of these species have medicinal and ornamental value. Many species of the genus have reported antinociceptive, analgesic, antioxidant, antiulcer, antidiabetic, antispasmodic, anti-inflammatory properties. The genus has been traditionally used for the treatment of joint disorders of varied aetiology [3]; painful conditions, high blood pressure, eczema, flu, bronchitis, asthma, fever, ulcer, and diabetes [2, 4].

Several studies have reported on the anti-inflammatory activities of other *Ruellia* species such as *R. prostrata* [5], *R. tuberosa* [6] and *R. patula* [7]. Aqueous and methanolic extracts of *R. prostrata*, have shown antioxidant activity [8]; [9]. *R. bignoniiflora* and *R. lineari-bracteolata* aqueous and methanolic extracts showed antioxidant activity [10]. These findings reveal a possible trend between *Ruellia* species and anti-inflammatory activity.

Previous studies revealed the analgesic and antinociceptive activities of the aqueous and methanolic extracts of *R. prostrata, R. bignoniiflora* and *R. lineari-bracteolata* [11]. Since these Kenyan *Ruellia* species displayed high analgesic activity in both acute pain and progressive pain models, the current study aimed to investigate these Kenyan *Ruellia* species for *in vivo* anti-inflammatory activity using Wistar albino rats.

Methodology

Study sites

Plant materials were collected from their natural habitats i.e., *R. prostrata* (Muthetheni and Kavoo areas) in Machakos

County, *R. bignoniiflora* (Kibwezi) in Makueni County, and *R. lineari-bracteolata* (Isiolo) in Isiolo County. Animal experiments were carried out from the Small Animal Facility for Research and Innovation animal house in Jomo Kenyatta University of Agriculture and Technology.

Sample collection, preparation and extraction

Upon identification by a plant taxonomist, the species were collected from their natural habitats and Voucher specimens, (UoN/ 2010/598 of 16/12/2010 for *R. prostrata*; UoN/2013/811 of 15/3/2013 for *R. bignoniiflora*, and UoN/2015/003 of 3/7/2015 for *R. lineari-bracteolata*) were deposited at the University of Nairobi Herbarium.

The plant materials were dried under a shade for 2-3 months, and ground into a fine powder using an electric grinder. Organic extraction was done by cold maceration for 6 hours using absolute methanol, whereas aqueous extraction was done using hot maceration also for 6 hours. The organic extracts were concentrated *in vacuo* using a rotary evaporator (BUCHI R-200, Labortechnik Switzerland), whereas aqueous extracts were obtained by freeze drying (Alpha 1-4 LD, Christ, Germany). The extracts were stored in refrigerator at 4°C until required.

Laboratory Animals

Wistar albino rats of either sex (6-8 weeks old) of weight 150–250g were used for the study. All animals were purchased from the Small Animal Facility for Research and Innovation (SAFARI) located in JKUAT. Standard laboratory conditions of ambient temperature 22°C±3°C, humidity 50-60% and artificial lighting of a 12-hour light and 12-hour dark cycle was maintained. Conventional laboratory diets were used, and an unlimited supply of drinking water was provided. Approval for the use of laboratory animals was sought from Animal Care and Use Committee (ACUC) of the Kenya Medical Research Institute (KEMRI).

Evaluation of the anti-inflammatory activity

Anti-inflammatory activity of the extracts of the various *Ruellia* species was evaluated by determining their ability to inhibit the swelling of the rat hind paw induced by injection of the inflammatory agent carrageenan [12]. Rats were randomly assigned into groups of 6 animals each. The two positive control groups received either diclofenac sodium 20mg/kg or ibuprofen 200mg/kg. The treatment groups were treated with one of the plant extracts at a dose of either 250mg/kg, 500mg/kg, 1000mg/kg or 1500mg/kg. One group of rats remained untreated (negative control). The extracts were administered by the oral route 1 hour before carrageenan injection, whereas the positive control groups were treated orally 30 minutes before carrageenan injection.

Following injection of 0.1mL of λ-carrageenan (1% w/v in normal saline) into the sub plantar region of the left rat hind paw, the extent of the induced swelling was determined by volume displacement using a digital water plethysmometer (LE 7500, Panlab Harvard Apparatus, Spain). Paw volume measurements were taken every hour for 6 hours.

Data analysis

The data was analyzed using Minitab Statistical Software Version 18.0 (Pennsylvania State University). Average paw volumes were calculated at each time point for each group of rats and expressed as mean ± standard error of the mean (SEM). The percentage inhibition of rat paw swelling was then calculated at each time point for each treatment group relative to the untreated (negative control) group. One-way Analysis of Variance (ANOVA) was used to analyze for significant differences in percentage inhibition of rat paw swelling between the different treatment groups. Significant differences in percentage inhibition between the different treatment groups identified by one-way ANOVA were further explored using the Tukey's *post hoc* multiple comparison test. A value of $p \leq 0.05$ was considered significant.

Results

The findings for the percentage inhibition of swelling in the rat hind paw by the aqueous extracts R. prostrata, R. bignoniiflora and R. lineari-bracteolata are presented in Table 1.

Table 1. Percentage inhibition of swelling in the rat hind paw by the aqueous extracts of *R. prostrata, R. bignoniiflora* and *R. lineari-bracteolata*.

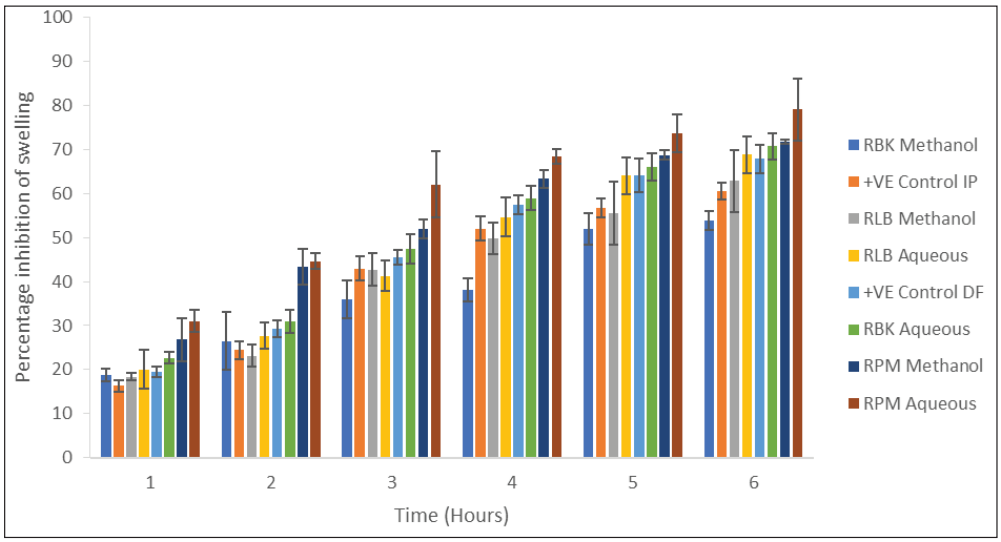
Dose (mg/kg)	Type of extract	Time in hours					
		1	2	3	4	5	6
		Percentage inhibition of rat hind paw swelling (n=6)					
	Untreated Control	0.00±0.00^{d}	0.0±0.00^{b}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{b}
	IP	16.31±1.36^{c}	24.42±2.10^{b}	43.00±2.73^{a}	52.00±2.72^{a}	56.82±2.18^{a,b}	60.53±1.84^{a}
	DF	19.46±1.21^{b,c}	29.19±1.92^{a,b}	145.51±1.62^{a}	57.38±2.16^{a}	64.07±3.82^{a,b}	67.87±3.27^{a}
250	RPM RBK RLB	21.83±4.22^{a,b,c} 14.77±3.15^{a} 13.37±2.41^{b}	29.24±3.93^{a,b} 18.38±4.81^{a} 19.14±2.79^{a}	37.32±7.38^{a} 29.55±5.45^{a} 27.99±2.70^{a}	43.28±7.29^{b} 37.28±5.74^{b} 37.76±4.39^{a}	45.2±10.7^{b} 43.31±8.89^{b} 45.71±3.42^{a}	54.20±8.26^{b} 46.55±3.48^{b} 47.71±5.14^{a}
500	RPM RBK RLB	24.05±2.72^{a,b,c} 19.15±1.24^{a} 17.97±1.46^{b}	35.65±6.85^{a,b} 25.96±1.53^{a} 21.64±5.09^{a}	47.57±5.67^{a} 39.14±2.15^{a} 35.90±5.48^{a}	58.58±4.25^{a,b} 43.18±2.97^{a,b} 44.45±5.11^{a}	64.51±5.39^{a,b} 49.41±6.55^{a,b} 51.95±7.80^{a}	67.73±1.69^{a} 57.63±5.19^{a,b} 53.85±3.46^{a}
1000	RPM RBK RLB	27.96±1.55^{a,b} 20.22±1.18^{a} 18.61±4.63^{b}	38.42±3.06^{a,b} 28.22±1.64^{a} 25.26±2.39^{a}	57.66±7.19^{a} 42.45±1.43^{a} 38.58±1.92^{a}	65.57±3.54^{a,b} 51.78±1.96^{a,b} 50.03±2.08^{a}	68.23±5.74^{a,b} 62.13±2.27^{a,b} 57.02±2.51^{a}	66.89±4.48^{a} 67.61±1.87^{a} 63.53±1.96^{a}
1500	RPM RBK RLB	31.05±2.53^{a} 22.64±0.99^{a} 20.07±4.45^{b}	44.60±1.76^{a} 31.02±2.63^{a} 27.63±2.96^{a}	62.08±7.51^{a} 47.36±3.39^{a} 41.32±3.39^{a}	68.52±1.70^{a} 59.00±2.71^{a} 54.65±4.47^{a}	73.71±4.30^{a} 66.01±3.17^{a} 64.04±4.24^{a}	79.12±7.11^{a} 70.83±2.99a 68.82±4.08a

Key: IP-Ibuprofen 200mg/kg; DF-Diclofenac sodium (20mg/kg) standard; RPM-*Ruellia prostrata*; RBK-*R. bignoniiflora*; RLB-*R. lineari-bracteolata* Values are expressed as Mean ±SEM. Values with the same superscript down the column are not significantly different (p>0.05).

The percentage inhibition in rat hind paw swelling by *R. prostrata, R. bignoniiflora* and *R. lineari-bracteolata* aqueous extracts was dose-dependent. All test groups showed significant inhibition of rat hind paw swelling compared to the untreated control. The highest percentage inhibition of swelling was exhibited by aqueous extract of *R. prostrata* (79.12%), followed by *R. bignoniiflora* (70.83%) and *R. lineari-bracteolata* (68.82%) at the 6-hour point and doses of 1500mg/kg. This percentage inhibition of the *Ruellia* extracts was higher than the 67.87% inhibition observed for diclofenac sodium at 20mg/kg and the 60.53% inhibition observed for ibuprofen at 200mg/kg at the same time point. There was no significant difference in percentage inhibition of swelling in the rat hind paw ($p>0.05$) between *R. prostrata, R. bignoniiflora* and *R. lineari-bracteolata* at doses of 1000mg/kg and 1500mg/kg.

Comparable findings were observed for the methanolic extracts of *R. prostrata, R. bignoniiflora* and *R. lineari-bracteolata* (Table 2). The highest percentage inhibition of swelling exhibited by the methanolic extracts were *R. prostrata* (71.87%) followed by *R. lineari-bracteolata* (68.82%) and R. bignoniiflora (53.33%) at the 6-hour point and doses of 1500mg/kg.

However, the methanolic extracts of the three Ruellia species consistently displayed lower percentage inhibition of swelling than their corresponding aqueous extracts. A comparison of the effects of 1500mg/kg doses of the aqueous and methanolic extracts of *R. prostrata, R. bignoniiflora* and *R. lineari-bracteolata* on the percentage inhibition of swelling in the rat hind paw versus time is shown in Figure 1.



Key: IP-Ibuprofen 200mg/kg; DF-Diclofenac sodium (20mg/kg) standards; *RPM-Ruellia prostrata;* RBK-*R. bignoniiflora;* RLB-*R. lineari-bracteolata*.

Figure 1. Comparison of % inhibition of rat paw swelling of 1500mg/kg doses of the aqueous and methanolic extracts of *R. prostrata, R. bignoniiflora* and *R. lineari-bracteolata*.

Table 2: Percentage inhibition of swelling in the rat hind paw by the methanolic extracts of *R. prostrata, R. bignoniiflora* and *R. lineari-bracteolata.*

Conc. (mg/kg)	Type of extract	Time in hours					
		1	2	3	4	5	6
		Percentage inhibition of rat hind paw swelling (n=6)					
	Untreated Control	0.0±0.00^{d}	0.0±0.00^{b}	0.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{b}	
	IP	16.31±1.36c	24.42±2.10b	43.00±2.73a	52.00±2.72a	56.82±2.18a,b	60.53±1.84a
	DF	19.46±1.21b,c	29.19±1.92a,b	45.51±1.62a	57.38±2.16a	64.07±3.82a,b	67.87±3.27a
250	RPM RBK RLB	16.95+3.24^{a} 14.00+3.59^{a} 9.15±2.67^{a}	21.33+4.18^{b} 15.55+2.42^{a} 12.15±5.06^{a}	32.97+6.89^{a} 17.88+4.27^{c} 27.26±1.95^{a}	37.19+3.63^{b} 18.22+2.05^{d} 32.90±4.75^{b}	40.84+6.85^{b} 34.75+2.52^{b} 37.06±2.70^{b}	49.59+8.95^{b} 43.26+3.54^{b} 44.86±4.00^{b}
500	RPM RBK RLB	19.15+1.02^{a} 16.84+1.28^{a} 13.71±3.78^{a}	26.06+2.5^{a,b} 20.55+2.60^{a} 16.29±4.35^{a}	42.39+2.62^{a} 24.32+1.43^{b,c} 32.92±5.01^{a}	52.48+2.13^{a,b} 30.72+1.78^{c,d} 41.96±2.41^{a,b}	57.59+2.7^{a,b} 44.59+6.75^{a,b} 47.58±2.34^{a,b}	60.71+1.99^{a} 45.75+3.32^{b} 50.26±2.81^{a,b}
1000	RPM RBK RLB	22.67+1.54^{a} 17.458+0.83^{a} 16.42±2.05^{a}	35.99+4.77^{a,b} 24.77+4.32^{a} 20.52±2.89^{a}	48.38+2.88^{a} 32.94+4.68^{a,b,c} 36.46±2.06^{a}	60.00+2.97^{a} 35.43+4.37^{b,c,d} 46.83±2.43^{a,b}	63.57+3.57^{a} 47.54+3.62^{a,b} 52.73±2.52^{a,b}	65.47+4.63^{a} 50.11+2.05^{a,b} 58.12±2.61^{a,b}
1500	RPM RBK RLB	26.79+4.96^{a} 18.72+1.45^{a} 18.36±0.808^{a}	43.41+4.12^{a} 25.84+2.63^{a} 23.17±2.60^{a}	51.93+2.21^{a} 37.43+2.53^{a,b,c} 42.76±3.70^{a}	63.33+2.04^{a} 43.39+4.15^{a,b,c} 49.81±3.68^{a,b}	68.78+1.13^{a} 50.564.24^{a,b} 55.62±7.21^{a,b}	71.87+0.45^{a} 53.33+5.42^{a,b} 62.88±7.04^{a,b}

Key: IP-Ibuprofen 200mg/kg; DF-Diclofenac sodium (20mg/kg) standard; RPM-*Ruellia prostrata*; RBK-*R. bignoniiflora*; RLB-*R. lineari-bracteolata*; Conc.- concentration. Values are expressed as Mean ±SEM. Values with the same superscript down the column are not significantly different ($p>0.05$).

Discussion

Carrageenan-induced oedema is considered a multi-mediated process that liberates a diversity of inflammatory mediators such as prostaglandins and leukotrienes which are released through the cyclooxygenase pathway and lipoxygenase pathways [13]. It is believed to be biphasic, with the first phase (≤1 hr.) involving the release of 5-hydroxytrypramine (5-HT) and histamine, while the second phase (>1 hr.) is mediated by prostaglandins, the cyclooxygenase products. The transition between the two phases is provided by kinins [14-16]. A correlation between the development

of oedema induced by carrageenan and early exudative stage of inflammation has also been reported [15, 16]. It can, therefore, be argued that suppression of the first phase of inflammation is due to inhibition of release of early mediators, whereas inhibition of the second phase of inflammation is due to the inhibition of the cyclooxygenase pathway.

The present study showed that the aqueous extract of whole plant parts of the Kenyan *Ruellia* species possessed a significant anti-oedematogenic effect on rat hind paw oedema induced by carrageenan. Both the *Ruellia* species and reference drugs in the present study displayed the highest anti-inflammatory activity at the 6-hour point. Therefore, the inhibitory effect of the extracts of the *Ruellia* species in the present study on carrageenan-induced oedema over a period of 6 hours was similar to the effect of non-steroidal anti-inflammatory drugs of inhibiting the second phase of the inflammatory response. This suggests that the extracts acted by inhibiting the second phase of inflammation mediated by arachidonic acid metabolites which produce oedema [17]. It has also been reported that the carrageenan-induced oedema is a significant predictive test for anti-inflammatory agents acting by the inhibition of mediators of acute inflammation [18]. The anti-inflammatory activity of extracts of *R. prostrata, R. bignoniiflora* and *R. lineari-bracteolata* species in the present study, may be attributed to the presence of flavonoids, polyphenols and saponins in the plant [19].

Conclusion

The aqueous extracts of whole plant parts of Kenyan *Ruellia* species possess significant anti-inflammatory activity. Both the aqueous and methanol extracts of *R. prostrata, R. bignoniiflora* and *R. lineari-bracteolata* showed significant dose-dependent anti-inflammatory activity compared to the negative (untreated) control. Aqueous extracts were consistently more potent than the corresponding methanolic extracts. Overall, the aqueous extract of *R. prostrata* showed the most promising analgesic and anti-inflammatory activities, followed by the aqueous extract of *R. bignoniiflora*. Anti-inflammatory activity of the aqueous extract of *R. prostrata* (1500 mg/kg) was superior to that of diclofenac sodium (20 mg/kg) and ibuprofen (200 mg/kg) standards. To the best of the researcher's knowledge, this is the first ever report of the anti-inflammatory activities of *R. bignoniiflora* and *R. lineari-bracteolata*.

Recommendations

Bioactive phytocompounds from the Kenyan *Ruellia* species should be isolated, identified and characterized using standard spectroscopic technique such as liquid chromatography-mass spectrometry, ^{1}H proton and ^{13}Carbon Nuclear magnetic resonance, 2-dimensional COSY nuclear magnetic resonance. This could be accompanied by in silico studies that could predict the targets of drug action, to establish the most probable mechanism of action. The bioactive compounds present in *R. prostrata* and *R. bignoniiflora* should also undergo in vivo toxicity evaluation. Promising findings could justify subsequent formulation and clinical trials of these compounds as potential anti-inflammatory and anti-arthritic drugs.

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Conclict of Interests

Authors have declared no conflict of interest.

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Phytochemical, Antioxidant and Antimicrobial activities of the Hydroalcoholic Extract of the Roots of *Adenodolichos paniculatus* against selected Pathogenic Microorganisms

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Abstract

Drugs derived from natural sources present a significant alternative therapy in the management of infectious diseases. The increasing resistance of pathogenic microorganisms to orthodox medicine has necessitated the search for alternative means of treatment. This study aimed at evaluating the phytochemicals, antioxidant and antimicrobial activities of the hydroalcoholic extract of the roots of *Adenodolichos paniculatus* against selected pathogenic microorganisms. Evaluation of phytochemical constituents was done using standard procedures and the antioxidant activity was evaluated using 2,2-diphenyl-2-picryl-hydrazyl. Antimicrobial activity was investigated using fifteen pathogenic organisms, which included five gram-positive and seven gram-negative bacterial strains and three fungal strains using Agar diffusion method. The zones of inhibition of the extract against the test organisms, their minimum inhibitory concentrations (MIC) as well as the minimum bactericidal/fungicidal concentrations (MBC/MFC) were determined. The extract was found to contain steroids, flavonoids, alkaloids and saponins. The total phenols, flavonoids, alkaloids and saponins were found to be 1.427±0.02 mg/g gallic acid, 0.4239±0.19 mg/g quercetin, 5% and 75.23% respectively. The antioxidant activity at concentrations of 7.53 and 15.51 µg/mL were not statistically different from that of vitamin C ($P<0.05$) and the IC50 (Half-maximal inhibitory concentration) found to be 4.906 µg/mL. The zones of inhibition ranged from 22 – 47 mm while the MIC and MBC/MFC was observed to be 5 to 10 mg/mL, and 10 to 40 mg/mL respectively. The availability of phytochemicals with remarkable antioxidant activities and the demonstration of antimicrobial activity against gram-positive, gram-negative bacteria and fungi strains show that *A. paniculata* root is a potential source for the production of antioxidants as well as antimicrobial drugs.

Key Words: *Adenodolichos paniculatus, antimicrobial activity, antioxidant activity, Phytochemical, zone of inhibition, hydroalcoholic extract.*

Introduction

The search for new drugs from natural sources is attracting increasing scientific interest around the world, especially because of the growing incidence of drug-resistant strains of micro-organisms which limit the use of currently available anti-microbial agents and the wide margin of safety associated with plant-derived drugs. Plants contain a variety of secondary metabolites, such as tannins, alkaloids, quinones, simple phenols and flavonoids, which have been found *in-vitro* to have antimicrobial properties, therefore, making them viable sources of new arsenal of antimicrobial drugs [1,2]. This thus sets the stage for further search of more readily available and accessible natural sources of antimicrobial drugs from plants.

Adenodolichos paniculatus (HUA) (Leguminosae) is a

perennial woody multi-purpose shrub that grows up to 4m high in savanna region of Northern Nigeria, Guinea and across to Sudan. The stems are flattened with a velvety red-brown colour when young, and smooth when mature. The leaves are foliate with 3-5 leaflets. The lower surface of leaflets is glandular [3]. *A paniculatus* is commonly called "wáákénwuta" or "fire bean" in Hausa [4]. The root –decoction is used for blennorrhoea and liver problems in Central Africa Republic [5]. The leaf is applied topically with butter oil for dressing burns in Nigeria and also for toothache in Ubangi [5]. The methanolic leaf extract of *A. paniculatus* have been demonstrated to possess both anti-inflammatory and analgesic activities [6]. Similarly, the methanolic leaf extract of the plant has been reported to have no harmful effect on the liver in sub-chronic toxicity test [6, 7].

Following the plant anti-inflammatory and analgesic activities which have been reported, and the series of its traditional uses, the present study is aimed at evaluating the phytochemicals, antioxidant and antimicrobial activities of the hydroalcoholic extract of the roots of A*denodolichos paniculatus* against the selected pathogenic microorganisms in the effort to discover new and effective antimicrobial agents.

Methodology

Chemicals and Equipment

Methanol, gallic acid, aluminium chloride, Folin-Ciocalteu's phenol reagent, quercetin, sodium carbonate, sodium chloride, 1,1-diphenyl-2-picrylhydrazyl (DPPH), potassium ferricyanide, iron (III) chloride, tannic acid, sulphuric acid, acetic anhydride, sodium hydroxide, hydrochloric acid, magnesium ribbon, chloroform, glacial acetic acid, Mayer's and Wagner's reagents were from Sigma Aldrich (Gillingham, UK). Other reagents used include Mueller Hinton agar, Sabouraud's dextrose agar and nutrient agar were obtained from Oxoid (Basingstoke, UK).

Equipment used were Electro-Heating Standing Temperature Air Dry Oven, water bath (Fisher Scientific Company, USA), UV-visible Spectrophotometer (Hewlett Packard), analytical weighing balance (Ohaus, USA), Autoclave (Cole Parmer, England) and Incubator (Microfield, SM9052, England).

Collection and Preparation of Plant Sample

The root of *A. paniculata* was collected in Jos in July 2012. It was authenticated at the department of plant science and technology, University of Jos. The roots were dried under shed for two weeks, pulverized and transferred into an extraction bottle. The extraction was carried out using known standard procedures [8]. The pulverized plant material (580g) was macerated using 70% methanol in distilled water for 3 days. The extract was filtered using Whatman filter paper (No. 1) and concentrated in vacuo at 40ºC. The hydroalcoholic extract yielded a solid residue weighing 41.13g (7.1% w/w). The extract was then kept in sterile bottles, under refrigerated conditions, until further use. The crude hydroalcoholic extract was used for further investigation for potential of antimicrobial properties.

Qualitative Phytochemical Analysis of hydroalcoholic root extracts

The hydroalcoholic root extracts was subjected to preliminary phytochemical screening to identify the chemical constituents using the standard procedures with some modifications [8, 9]. A stock solution of the extract (10 mg/mL) was prepared and tested for phenols and tannins, steroids, saponins, flavonoids, cardiac glycosides, terpenoids and alkaloids.

Quantitative Phytochemical Analysis

Determination of total phenolics

Total phenolic contents of the hydroalcoholic root extracts were evaluated on each extract with Folin-Ciocalteu's phenol reagent [10, 11]. The extract solution in methanol (2.0 mg/mL) was mixed with 2.0 mL Folin-Ciocalteu reagent previously diluted with water (1:9 v/v). After 5 minutes, 1.6 mL of 7% Na2CO3 solution was added with mixing. The tubes were shaken for 5 seconds and allowed to stand for 30 min at 40°C in an oven for color development. Absorbance was then measured at 765 nm using UV-Vis spectrophotometer. Samples of extract were evaluated at a final concentration of 0.01 mg/mL. Gallic acid at different concentrations of 0.01 to 0.07 mg/mL was used as standard. All tests were performed in triplicates. Total phenolic content was expressed as mg/g Gallic acid equivalent using the following equation based on the calibration curve: $y = 1.6232x$, $R^2 = 0.6658$, where y was the absorbance and x was the concentration.

Determination of total flavonoids

Colorimetric aluminum chloride method was used for flavonoid determination of each extract according to the methods of Nabavi et al. [11] with some modifications. 3.0 mL solution of each extract dissolved in methanol was separately mixed with 3.0 mL of 2.0% aluminum chloride. After one hour at room temperature, the absorbance was measured at 420 nm. Extracts were evaluated at a final concentration of 0.1 mg/mL. Quercetin was used as a standard. All tests were performed in triplicates. Total flavonoid content was calculated as quercetin equivalents (mg/g) using the following equation based on the calibration curve: $y = 0.0.23x$, $R^2 = 0.9424$, where y was the absorbance and x was the concentration.

Alkaloids determination

The method of Obadoni and Ochuko [12] was used in which 3 g of the powdered sample was weighed into 150 mL of 20 % acetic acid in ethanol and allowed for 4 h. The mixture was filtered and the filtrate concentrated using a water bath at 55°C to one-quarter of its original volume. Concentrated NH_4OH was added drop wise into the extract until precipitation was complete. The whole solution was allowed to settle and then filtered. The precipitate which is the crude alkaloid was washed with dilute NH_4OH solution. The crude alkaloid was weighed and calculated according to the equation:

$$\% \text{ amount of alkaloid (mg/g)} = \frac{\text{weight of precipitate}}{\text{weight ofsample}} X100$$

Saponin determination

The saponin content in the plant extracts was estimated as described in literature [13]. Ten grams of the powdered sample was placed in 200 mL of 20% ethanol in water. The suspension was heated in a water bath at 55°C for 4 hours with continuous stirring. The mixture was filtered and the residue was re-extracted as above. The combined extracts were reduced to 40 mL over a water bath at 90°C. The concentrate was transferred into a 250 mL separatory funnel and 20 mL diethyl ether was added and shaken vigorously. The ether layer was discarded, while the purification process was repeated three times. 60 mL of n-butanol was added and the extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the sample was dried at 90 oC in the oven to a constant weight. The saponin content was calculated according to the equation:

$$\% \text{ amount of alkaloid (mg/g)} = \frac{\text{weight of residue}}{\text{weight of sample}} X100$$

Antioxidant activity: DPPH scavenging activity

The DPPH radical-scavenging activity of the test extracts was examined as described by Kumarasamy et al. [14] with some modifications. Different concentrations (0.03- 0.1µg/ml) of each extract were separately added, to an equal volume of hydroalcoholic solution of DPPH (100 µM). The mixture was allowed to react at room temperature in the dark for 30 minutes. Vitamin C was used as standard control while a mixture without the extract was taken as blank. After 30 minutes, the absorbance (A) was measured at 518 nm. Each test was carried out in duplicates converted into the percentage antioxidant activity using the following equation:

$$\% \text{ scavenged} = \frac{\text{Absorbance (DPPH) - Absorbance (Extract}}{\text{Absorbance (DPPH)}} X100$$

The IC_{50} values of each fraction were calculated by nonlinear regression using graph pad prism. Where the abscissa represented the concentration of fractionated extract and the ordinate represented the average percent of scavenging capacity from the triplicates.

Antimicrobial activities

Test Microorganisms and Culture Media

The test microorganisms used were collected from the department of Pharmaceutical Microbiology and biotechnology, University of Ilorin. The bacteria isolates used were: Methicillin resistant *Stapylococcus aureus* (MRSA), *Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis, Corynebacterium ulcerans, Escherichia coli, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhii, Shigella dysentariae, Klebsiella pneumonia.* The fungi test micro-organisms used are: C*andida albicans, Candida krusei, and Candida tropicalis*. The bacterial strains were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C, whereas the fungal strains were grown in Sabouraud dextrose agar (SDA) and Potato dextrose agar (PDA) media, respectively at 28°C. The stock cultures were maintained at 4°C.

Determination of Zone of Inhibition

The antimicrobial activities of crude hydroalcoholic extract were determined against bacterial and fungal isolates. About 0.4 g of the AP extract was dissolved in 10ml DMSO to obtain a concentration of 40 mg/ml of the extract; this was the initial concentration of the extracts used to check the antimicrobial activities of the extracts.

Mueller Hinton agar was selected as the growth medium to the microbes. The medium was prepared according to the manufacturers' instruction, sterilized at 121°C for 15 minutes; the sterilized medium was then poured into sterile petri dishes and was allowed to cool and solidify. Agar diffusion method was the method used to screen the extracts according to the standard procedures [15]. The sterile Mueller Hinton agar plates were seeded with 0.1 ml of standardized inoculum of the test microorganisms, the inoculum was spread evenly over the surface of the medium by the use of a sterile swab.

By the use of a standard cork borer of 6 mm in diameters, a well was cut at the center of each inoculated medium and base sealed with molten agar. 0.1 ml of the extract at different concentrations was then introduced into each well on the inoculated medium. Dimethyl sulfoxide was used as negative control while ciprofloxacin and Fluconazole were used as positive controls for bacterial and fungi respectively. The inoculated medium were then incubated at 37°C for 24 h (bacteria) and 48 h (fungi) respectively, after which each plate of medium was observed for the zones of inhibition of growth.

Determination of Minimum Inhibition Concentration (MIC)

The minimum inhibitory concentration of the extract was carried out using the macro broth dilution method [16, 17]. Two-fold serial dilutions of the extract in the broth was prepared and 2mls aliquots of different concentrations of the solutions were added to 18ml of sterile molten nutrient agar and Saboraud dextrose agar for bacteria and fungi respectively to obtain the following concentrations of the plant extract at 40, 20, 10, 5 and 2.5 mg/ml. The initial concentration of the plant was obtained by dissolving 0.4g of the extract in 10 mL of the sterile broth. Having obtained the different concentrations of the extract in the broth, 0.1 ml of the standardized inoculum was inoculated into the different concentrations of the extract in the broth; the incubation was made at 37°C for 24hrs. for bacterial strains and 28°C for 48hrs. for the fungal strains. Thereafter each test-tube was observed for turbidity (presence or absence of growth); the lowest concentration of the extract in the broth

which showed no turbidity (growth) was recorded as the minimum inhibition concentration. A test tube with sterile nutrient broth/ potato dextrose media and 1mL suspension of test organisms each (for bacteria and fungi) without the extract and another tube with nutrient broth and 1mL of the extract without the test organisms were provided as positive and negative controls.

Determination of Minimum Bactericidal / Fungicidal Concentration (MBC/MFC)

Minimum Bactericidal/ Fungicidal Concentration was carried out to check whether the test organisms were killed or only their growth was inhibited. Mueller Hinton agar and Saboraud dextrose agar were prepared, sterilized and poured into sterile petri dishes, the plates were allowed to cool and solidify. The contents of the MIC in the serial dilution were then sub-cultured onto the prepared media, plates were then incubated at 37°C for 24hours for bacteria and at 28°C for 48hrs after which the plates were observed for colony growth. The MBC/MFC were the plates with lowest concentrations of the extract without colony growth [16, 17].

Statistical Analysis

The data were presented as mean ± standard error of mean and the IC_{50} analysed using Graph Pad prism 6 (Graph Pad prism 6 software. Inc, USA). Differences of $P<0.05$ was taken as statistically significant

Results

Qualitative Phytochemistry

Phytochemical constituents present in the hydroalcoholic extract of the roots of *A. paniculatus* were steroids, flavonoids, alkaloids, tannins and saponins as shown in table 1.

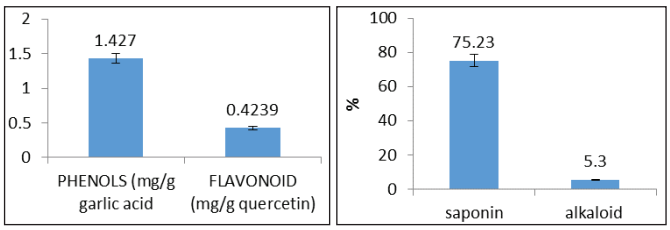
Table 1. Phytochemical constituents present in the hydroalcoholic extract of the roots of *A. paniculatus*

Phytochemical constituent	Test	hydroalcoholic extract
Alkaloids	Wagners'Test	+
Cardiac glycosides	Keller-kilaniTest	-
Flavonoids	NaOH, Shinoda	+
Saponins	Frothing Test	+
Steroids	Acetic anhydride + Conc. H_2SO_4	+
Tannins	$FeCl_3$	-
Terpenoids	$CHCl_3 + H2SO_4$	-

+ (presence of phytochemical) - (Absence of phytochemical)

Quantitative phytochemistry

The level of these phenolic and flavonoid compounds in the methanol extract of the roots of *A. paniculatus* was considerable as shown in Figure 1. The total phenolic and flavonoid contents of the extract were 1.427±0.02 mg/g gallic acid and 0.4239 ± 0.19 mg/g quercetin respectively (figure1a). The alkaloids and saponins contents were 5 % and 75.23 % respectively as shown in Figure 1b.



a b

Figure 1. Total phenolic and flavonoid constituents (a) and Percentage saponins and alkaloids (b) in methanol extract of *A. paniculatus*

Antioxidant Activity

The scavenging activity was expressed as percentage inhibition of DPPH free radical (Figure 2). The results of the DPPH assay also showed a dose dependent scavenging activity. As shown in Figure 3, the DPPH radical-scavenging activity at 15.51 and 7.53 µg/mL was not significantly different ($P<0.05$) from that of vitamin C (5µg/mL). The IC_{50} was found to be 4.906 µg/ml

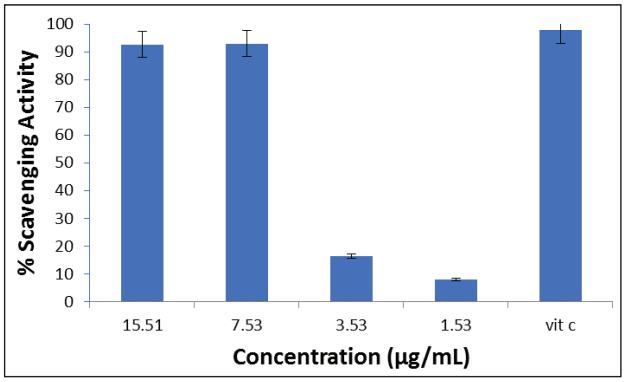


Figure 2. DPPH Scavenging activity of *A. paniculatus* at different concentrations

Antimicrobial activity

The antimicrobial activity of the hydroalcoholic extract of *A. paniculatus* was studied at different concentrations (40, 20, 10, 5 and 2.5 mg/ml) against twelve pathogenic bacterial strains: five gram-positive (Methicillin resistant *Staphylococcus aureus; Staphylococcus aureus; Streptococcus pyogenes; Bacillus subtilis, Corynebacterium ulcerans*), seven Gram-negative (*Escherichia coli; Proteus mirabilis; Proteus vulgaris; Pseudomonas aeruginosa; Salmonella typhii; Shigella dysentariae; Klebsiella pneumonia*), and three fungal strains (*Candida albicans; Candida krusei* and *Candida tropicalis*). The results of the antibacterial and antifungal activities are presented in Table 2. The antimicrobial profile of the hydroalcoholic root extract of *A. paniculatus* are presented in Tables 3 to 5. (***See tables overleaf***)

Table 2. Zones of inhibition of the hydroalcoholic extract and standard drug against the Test Microorganisms

Zones of inhibition (mm)			
Test Organisms	**Hydroalcoholic extract(200 mg/mL)**	***Ciprofloxacin (5 µg/mL)**	***Fluconazole (5 µg/mL)**
MRSA	0.0	0.0	
S. aureus	25.0	42.0	
S. pyogenes	27.0	47.0	
B. subtilis	27.0	47.0	
C. ulcerans	0.0	37.0	
E. coli	0.0	34.0	
P. mirabilis	23.0	30.0	
P. vulgaris	27.0	24.0	
P. aeruginosa	22.0	0.0	
S. typhii	0.0	24.0	
S. dysentariae	0.0	27.0	
K. pnuemoniae	28.0	30.0	
C. albicans	0.0		32.0
C. krusei	24.0		30.0
C. tropicalis	0.0		29.0

Table 3. The Antimicrobial profile of *A. paniculata* plant extract.

Test Organisms	Gram +ve or –ve	Plant Extract	Ciprofloxacin	Fluconazole
MRSA	+ve	R	R	R
S. aureus	+ve	S	S	R
S. pyogenes	+ve	S	S	R
B. subtilis	+ve	S	S	R
C. ulcerans	+ve	R	S	R
E. coli	-ve	R	S	R
P. mirabilis	-ve	S	S	R
P. vulgaris	-ve	S	S	R
P. aeruginosa	-ve	S	R	R
S. typhii	-ve	R	S	R
S. dysentariae	-ve	R	S	R
K. pnuemoniae	-ve	S	S	R
C. albicans	Fungus	R	R	S
C. krusei	Fungus	S	R	S
C. tropicalis	Fungus	R	R	S

Key: +ve = Gram positive, -ve = Gram negative, R = Resistant strain, S = Susceptible strain.

Table 4. Minimum inhibitory concentration (MIC) of the extract against the micro-organisms.

Test Organisms	40 mg/ mL	20 mg/ mL	10 mg/ mL	5 mg/mL	2.5 mg/ mL
S. aureus	-	-	**	+	+
S. pyogenes	-	-	**	+	+
B. subtilis	-	-	-	**	+
P. mirabilis	-	-	**	+	++
P. vulgaris	-	-	-	**	+
P. aeruginosa	-	-	**	+	++
K. pnuemoniae	-	-	-	**	+
C. krusei	-	-	**	+	++

Key: - = No turbidity (no growth), ** = Minimum inhibitory concentration, + = Turbid (light growth), ++ = Moderate turbidity, +++ = High turbidity

Table 5. Minimum bactericidal/fungicidal concentration of the extract against the micro-organisms

Test Organisms	40 mg/ mL	20 mg/ mL	10 mg/ mL	5 mg/ mL	2.5 mg/ mL
S. aureus	-	**	+	++	+++
S. pyogenes	-	-	**	+	++
B. subtilis	-	**	+	++	+++
P. mirabilis	**	+	++	+++	++++
P. vulgaris	-	-	**	+	++
P. aeruginosa	**	+	++	+++	++++
K. pnuemoniae	-	-	**	+	++
C. krusei	-	**	+	++	+++

Key: - = No colony growth, ** = Minimum bactericidal/ fungicidal concentration, + = Scanty colony growth, ++ = Moderate colony growth, +++ = Heavy colony growth

Discusion

The results in the present study revealed the presence of low level of hydroalcoholic soluble phytochemicals as extractive values (7.1% w/w). From the phytochemistry, the extract contained mostly flavonoids, alkaloids and saponins. The results also revealed a considerable high level of the phenolics. Reports on phytochemistry on the leaves show the presence of flavonoids, tannins, steroids, carbohydrates, glycosides, alkaloids, anthraquinones and cardiac glycosides [6].

Attention of antioxidants derived from natural sources has increased and efforts in screening and characterization have been put into identifying antioxidant compounds suitable to replace synthetic ones [18]. Thus, there is continued search for phytochemicals with potent antioxidant for remedies against free radical mediated diseases prevention of oxidative stress, oxidative reaction in food, protection against DNA damage and carcinogenesis [19, 20].

Flavonoids is one of the several categories of phenolic compounds which have potent antioxidant activities [21]. Flavonoids reduce free radicals by chelating, up-regulating and quenching radical intermediate compounds [22]. DPPH is used to measure the electron donation ability of extracts through decolorization of DPPH solution by antioxidant or a radical species [21]. Free radical scavenging activity of the extract is indicated by decrease in the absorbance of the reaction mixture between DPPH and the extract [23]. In this study, the hydroalcoholic extract of *A. paniculata* showed highest antioxidant activity at 15.51 and 7.53 µg/mL which was statistically significant ($P<0.05$) compared to that of vitamin C. The high presence of flavonoids in this extract justifies its high antioxidant activity. The antioxidant and antimicrobial activities of other members in this family as well as this plant species is scarce in the literature.

All the tested microorganisms were susceptible to ciprofloxacin except for MRSA (gram +ve) and *P. aeruginosa* (gram -ve) that demonstrated resistant strains toward ciprofloxacin. In like manner, two-gram positive (MRSA and *C. ulcerans*) and three- gram negative (*P. aeruginosa, E. coli and S. dysentariae*) strains demonstrated resistant to the

plant extract. Also, the three fungi strains used were susceptible to fluconazole while only *C. krusei* was susceptible to the plant extract.

For the minimum inhibitory concentration (MIC) experiment, only the microorganisms which were susceptible to the plant extract were selected and the plant extract at 5mg/mL was able to inhibit the growth of *B. subtilis, P. vulgaris* and *K. pnuemoniae* while the growth of *S. aureus, S. pyogenes, P. mirabilis, P. aeruginosa* and *C. krusei* were inhibited at higher concentration of the plant extract (10mg/mL). No inhibition was observed when 2.5mg/mL of the plant extract was used. However, all the test micro-organisms had no growth at 20mg/mL and 40mg/mL of the plant extract. This result shows that the inhibitory activities of the plant extract was concentration dependent with higher potency observed on *B. subtilis, P. vulgaris* and *K. pnuemoniae*.

The bactericidal and fungicidal activities result of the plant extract showed that the plant extract had a bactericidal effect on *B. subtilis, P. vulgaris* and *K. pnuemoniae* at 10mg/mL while the bactericidal effect was at 20mg/mL for *S. aureus* and *S. pyogenes*. Only at a higher concentration of 40mg/mL was bactericidal effect of the plant extract observed for *P. mirabilis* and *P. aeruginosa*. The plant extract also demonstrated fungicidal activity on *C. krusei* at 20mg/mL.

Flavonoid and its derivatives have shown a wide range of antimicrobial, antiviral, anti-inflammatory, as well as anticancer activities [24]. Antifungal activity of saponins have also been reported [25]. Results from these studies show a good level of antimicrobial activity of the extract as the same was observed to inhibit the growth of three out of the four (75%) gram positive organisms tested and four out of seven (57.14%) of the gram-negative organisms tested with pronounced activity against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. These two-gram negative organisms are known pathogenic organisms which are mostly resistant to antibiotics commonly prescribed. Aqueous and hydroalcoholic extracts have been reported to be more effective against gram positive than gram negative bacteria [26]. However, the antifungal properties are not as broad as the antibacterial properties as 33% of the fungal isolates were observed to be susceptible to the extract while the standard antifungal drug gave 100% inhibition of the isolates. The presence of flavonoids and saponins could therefore have accounted for the broad-spectrum antimicrobial activity of the hydroalcoholic extract of *A. paniculata*.

Limitation of the study

The limitation of this study is the utilization of "one-dimensional" character of methods used to evaluate antioxidant activity. Also, the percentage yield was too low and prevented further investigations.

Conclusion

A. paniculata contains phenolic compounds as well as alkaloids with antioxidant and wide spectrum antimicrobial activity which serve as scientific basis for the use of this plant in traditional medicine.

Conflict of Interest

There is no conflict of interest with the data contained in this manuscript.

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ORIGINAL RESEARCH

Cytotoxicity and antiretroviral activity of the leaf and stem bark extracts of *Croton macrostachyus*

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Abstract

Croton macrostachyus (C. macrostachyus) is one of the most widely used plants in traditional medicine in Africa for treatment of amoebiasis, constipation, malaria, cancer, ringworm, sexually transmitted diseases including HIV and hepatitis. Several studies have been conducted to validate antimicrobial activity of the plant. However, there are no previous reports regarding the anti-HIV activity of *C. macrostachyus*. In this study we report the anti-HIV activity of solvent fractions of *C. macrostachyus*.

Stem bark and leaves of *C. macrostachyus* were first extracted separately with 1:1 v/v dichloromethane and methanol followed by partitioning using different solvents. The solvent fractions obtained were then tested for their inhibition of laboratory adapted strains HIV-1_{IIIB} replication activity in Human T-lymphocytic MT-4 cell line. The solvent fractions were further evaluated for their cytotoxicity in MT-4 cells using MTT assay.

The hexane fraction of the leaf displayed promising anti-HIV effect by inhibiting 82.78% of the viral induced cytopathic effect at an inhibitory concentration (IC_{50}) of 0.02 ±0.01µg/mL, maximum nontoxic concentration (MNTC) of 142.2 ± 0.4 µg/mL and selectivity index (SI) of 9752.7 indicating the effectiveness of the fraction. Similarly, the ethyl acetate fraction of the leaves inhibited 83.81% viral induced cytopathic effect (IC_{50}= 0.05 ±0.03µg/mL; MNTC=100.9 ± 0.51µg/mL; SI of 3186.7).

Based on our findings, the hexane and ethyl acetate fractions of the leaves, illustrated higher anti-HIV activity by inhibiting more than 80% of viral induced cytopathic effects. Further studies aiming at evaluating the efficacy of isolated pure compounds from *C. macrostachyus* against viral proteins should be conducted to support the development of newer antiretroviral compounds through bioassay studies.

Keywords: *HIV, MT-4 cells, Croton macrostachyus, Cytotoxicity, Antiviral activity.*

Introduction

Acquired immunodeficiency syndrome (AIDS) is a clinical syndrome that is the result of infection with the Human immunodeficiency virus (HIV), which causes profound immunosuppression. Currently, available anti-HIV drugs are chemically synthesized and are often limited by side effects and emergence of drug resistance. On top of this, still over 5 million people do not have access to the treatment. This calls for a need to identify local alternative less expensive and less toxic drugs for treatment of HIV.

As plants continue to be the major source of drugs, the search for new antiretroviral compounds can start from herbal medicines used by traditional healers as remedy for Human Immunodeficiency Virus (HIV) and related opportunistic infections. Phytochemicals isolated from natural products can be important sources of lead compounds for the treatment of HIV/AIDS and other viral diseases.

Croton plants have wide traditional use including treatment of constipation, malaria, anthrax, jaundice, hepatitis, sexually transmitted infections, and as cardioprotective [1–4]. The *Croton* genus has gained attention of many researchers for their potential source of bioactive compounds against HIV [5]. Alkaloids isolated from *Croton echinocarpus* leaves have exhibited significant *in vitro* anti-HIV activity by inhibiting HIV-1 reverse transcriptase enzyme [6]. In this study we explored the antiretroviral activity of *C. macrostachyus* against Human immunodeficiency virus type 1.

Croton macrostachyus, is a medium sized tall tree or shrub that grows up to 30 meters [7,8]. It is commonly known as "broad-leaved Croton" or "rush foil". It is also known by various local/vernacular names including, "*bisana*" in Ethiopia, "*msinduzi, mutundu*" in East Africa [8–13]. Previous ethnopharmacological surveys have confirmed the traditional use of *C. macrostachyus* in the treatment of a number of infectious diseases. *C. macrostachyus* is used for

treatment of sexually transmitted infections, hepatitis, amoebiasis, epilepsy, anthrax, jaundice, leprosy, malaria, cancer, ringworm, skin diseases [10,12–19].

In Kenya, *C. macrostachyus* bark juice, leaf, and root decoction are used as remedy for backache, bleeding, cancer, colds, cough, diarrhea, dysmenorrhea, east coast fever, malaria, measles, obesity, pneumonia, ringworm, skin diseases, typhoid, warts, and wounds [20–23]. The leaves of *C. macrostachyus* are used by farmers in Kenya as biological pest control when mixed with tobacco (*Nicotiana tobacuum* L.) and boiled overnight [16]. The resultant mixture is used as a biological pesticide for the control of maize stalk borers and aphids.

In addition, the leaf and root decoction of *C. macrostachyus* is used by many patients including HIV infected patients as cure for cough, back pain, bleeding, skin diseases, warts, pneumonia and wounds [20,22–24]. Bark decoction of *C. macrostachyus* mixed with leaves of *Juniperus procera,* stems of *Eragrostis tef* (Zucc.) and roots of *Cyphostemma cyphopetalum* is used as herbal medicine for rabies in Ethiopia [11]. Similarly, root decoctions are taken orally to treat hepatitis [25]. Based on this information, we evaluated the cytotoxicity and anti-HIV activities of solvent fractions of *C. macrostachyus* against laboratory adapted strains of HIV (HIV-1_{IIIB}) in Human T-lymphocytic MT-4 cells

Different phytochemicals including phytosterol [26], triterpenoids [11, 27, 28], diterpenoids [29] and phenolic compounds [26] have been isolated from *C. macrostachyus* as depicted in Table 1.

Table 1. Phytochemicals isolated from *Croton macrostachyus* [14]

Ser. No.	Name of compound	Part of the plant	Reference
Phytosterols			
1.	β-Sitosterol palmitate	Twigs	[26]
2.	Beta sitosterol	Stem bark and twigs	[11,26]
3.	Stigmasterol	Stem bark and twigs	[26,27]
Triterpenoids			
4.	Betulin	Stem bark and twigs	[11,26–28]
5.	Lupeol	Stem bark and twigs	[11,26–28]
6.	3β-Acetoxy taraxer-14-en-28-oic acid	Roots	[11,26–29]
7.	Lupenone	Twigs	[26]
8.	Betulinic acid	Twigs	
9.	Zeroin	Twigs	
10.	28-O-Acetylbetulin	Twigs	
11.	Lupeol acetate	Twigs	
Diterpenoids			
Clerodane diterpenoids			
12.	Neoclerodan-5,10-en-19,6β;20,12-diolide	Roots	[29]
13.	[+] Hardwickiic acid	Stem bark and twigs	[29,31]
14.	12-Oxo-hardwickiic acid	Stem bark and twigs	
15.	Crotomacrine	Fruits	[32]
16.	Floridolide A	Stem bark	
Pentacyclic diterpenoids			
17.	Trachyloban-19-oic acid	Roots	[29]
18.	Trachyloban-18-oic acid	Roots	
Labdane diterpenoid			
19.	Crotomachlin	Stem bark and twigs	[31]
Cyclohexane Diepoxide			
20.	Crotepoxide	fruits	[33,34]
Phenolic compounds			
21.	Lichexanthone	Twigs	[26]
22.	Methyl 2,4-dihydroxy-3,6-dimethyl-benzoate	Twigs	
23.	Methyl gallate	Twigs	
24	Benzoic acid	Twigs	

Methodology

Preparation of solvent factions

The leaves and stem bark of *Croton macrostachyus* were collected from United States International University – Africa (USIU-Africa) botanical garden, Nairobi, Kenya in June 2020. The collection of these medicinal plants was done after obtaining the required ethical approval from the Kenyatta National Hospital-University of Nairobi Ethics and Research Committee (KNH-UON ERC), approval number P992/12/2019. Taxonomic identification was done by Ms. Lucy Wambui (botanist) and voucher specimen TEREFE E./045 was deposited at the USIU-Africa herbarium for future reference.

The leaf and stem bark of *C. macrostachyus* were first air dried and ground to a fine powder before being extracted separately using methanol and dichloromethane (DCM) in 1:1 ratio using cold maceration technique. Then the dried crude extract of the leaf and stem bark were dissolved separately in distilled water (200 mL) and successively partitioned using different solvents of increasing polarity (n-hexane, dichloromethane, ethyl acetate and methanol) using a separator funnel as shown schematically in Fig. 1. The different solvent fractions were concentrated under reduced pressure using rotary evaporator and the resulting product dried in an oven at 30°C. The dried fractions were then transferred into separate vials and stored in a desiccator for further use.

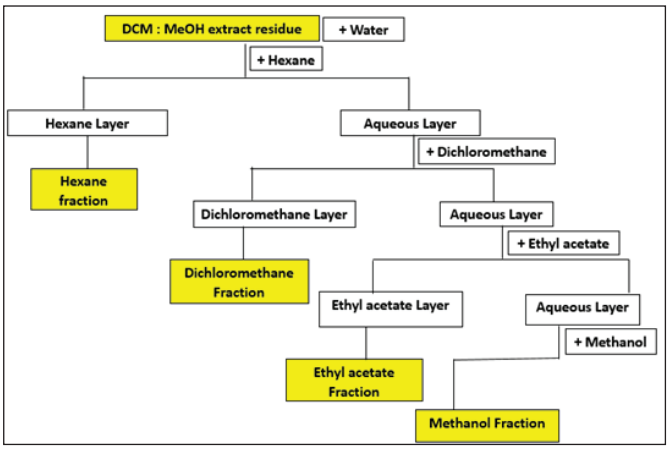


Figure 1. Schematic representation of solvent partitioning process.

Phytochemical screening

The solvent fractions of the leaf and stem bark were screened for the presence of active principles such as alkaloids, anthraquinones, flavonoids, glycosides, phenolic compounds, saponins, sterols, tannins and terpenoids, using standard procedures [35, 36].

Sample preparation

The concentrated dried extracts were dissolved in 0.5-1% dimethylsulphoxide (DMSO) solution and then diluted with RPMI 1640 medium. Each fraction was reconstituted to prepare stock of 4 mg/ml. The stock solutions were filtered through 0.22 µm membrane filter and stored at 4°C until further use [37].

Cytotoxicity test

The cytotoxicity test was carried out in a 96-well, flat bottomed microtitre plates and 200 µl of MT-4 cells (1×10^5) cells/ml in growth media were added to each well. The plates were pre-incubated for 24h at 37°C to allow stabilizations. Then fifty microliters (50µl) of various concentrations (800 – 8.192×10^5 µg/mL) of the test compounds and the positive control (zidovudine, tenofovir, abacavir and nevirapine), were added to the wells in triplicate. The negative control (NC) wells contained 50 µl of MT-4 cells in 0.5 % DMSO [24]. The plates were incubated at 37°C in a humidified atmosphere of 5% CO_2 for 5 days. Twenty (20) µl of MTT reagent (5 mg/ml MTT in phosphate-buffered saline) was then added to each well. After 4 hours of incubation, 100µl of DMSO was added to dissolve the purple colored formazan crystals from surviving cells [39]. The resulting optical density (OD) readings were measured relative to the controls on ELISA plate reader at 540 nm with a reference wavelength of 620 nm [40]. A dose-response curve was plotted to enable the calculation of the concentrations that reduced the number of viable cells by 50% (CC_{50}). The 50% cellular cytotoxicity concentration (CC_{50}) was defined as the concentration of test compound that reduces the viability (absorbance) of the negative control by 50%. The concentration that determined cell viability above 80% was chosen as the maximum non-toxic concentration (MNTC).

Anti-HIV activity test

The effect of the test compounds in preventing cytopathic effect which occurs as a result of HIV-1 replication was evaluated by MTT colorimetric assay described above. The HIV-infected MT-4 cells were seeded in 96-well flat-bottomed microtitre culture plates with 50 µl of different concentrations (800 – 8.192×10^5 µg/mL) of the test compounds. The test compounds were dissolved in 0.5% DMSO and assayed at varying concentrations as described above. Each dilution was tested in triplicates. The microtitre plates were incubated at 37°C in a 5% CO_2 incubator for 5 days. Two negative controls, untreated HIV infected cells and uninfected untreated (mock) cells, and the positive control (zidovudine, tenofovir, abacavir and nevirapine) were also included. After 5 days of incubation, cell viability was determined by MTT assay [41,42]. The antiviral activity was calculated as percent cell protection or inhibition % for viral induced cytopathic effect (CPE inhibition %) as described by [43–46]. The effective inhibitory concentration at 50% (IC_{50}) values was calculated from the percent cell protection results by non-linear regression analysis. The selectivity index (SI) of the test compounds were calculated as the ratio of 50% cytotoxic concentration (CC_{50}) to 50% effective concentration (IC_{50}).

Statistical analysis

The CC_{50} and IC_{50} values were calculated with GraphPad Prism v9, using the equation for sigmoidal dose-response (variable slope). Statistical significances in comparison between control drugs and fractions cytotoxicity and antiviral activity parameters (CC_{50}, $Emax_C$, IC_{50} and $Emax_{AV}$) were determined by one-way ANOVA followed by Dunnett's post-hoc tests, A difference was considered significant when $p<0.05$.

Results

Phytochemical screening

Phytochemical screening of the solvent fractions of crude (1:1 Dichloromethane:Methanol) extracts of *Croton macrostachyus* revealed the presence of different secondary metabolites (Table 2).

Table 2. Phytochemical analysis for the solvent fractions of *Croton macrostachyus*

	Hexane		Dichloro-methane bark		Ethyl acetate		Methanol	
	Leaf	Bark	Leaf	Bark	Leaf	Bark	Leaf	Bark
Alkaloids	+	+	+	+	+	+	+	-
Anthraquinones	-	-	-	-	+	-	-	-
Flavonoids	+	+	+	+	+	+	+	+
Glycoside	+	+	+	+	+	+	-	-
Saponins	+	+	-	-	+	+	+	+
Steroids	+	+	+	+	+	+	+	+
Phenolic compounds	+	+	+	+	+	+	+	+
Tannins	+	+	-	+	+	+	-	+
Terpenes	+	+	+	+	+	+	+	+

Cytotoxicity assay

The methanol (AM) and hexane (AH) soluble fraction of the bark were found to be cytotoxic at CC_{50} value of 0.25 + 0.07 µg/mL and 0.58 + 0.05 µg/mL respectively inhibiting cell growth ($Emax_C$) by more than 49%. The ethyl acetate fraction (AE) of the bark showed high cytotoxicity with MNTC and CC_{50} values at 141.9 + 0.25 and 171.8 + 0.1 µg/mL respectively and significantly ($P<0.05$) reduced cell viability by 63%, which is the highest as compared to the other fractions tested (Table 3). The dichloromethane fraction of the bark (AD) displayed less cytotoxicity by ensuring viability of more than 70% of the cells ($Emax_C = 29.33\%$).

The hexane soluble fraction of the leaves (ALH) showed the lowest cytotoxicity as compared with the other leave fractions ($Emax_C = 39.44\%$) with CC_{50} value of 173.5 µg/mL followed by the ethyl acetate fraction of the leaves (ALE) which displayed CC_{50} value of 165.9 µg/mL. The methanol fraction of the leaves (ALM) displayed the highest cytotoxicity with CC_{50} of 6.28 ± 0.51µg/mL and inhibiting cell viability by 54.47%.

Among the FDA approved antiretroviral drugs, zidovudine (AZT) and nevirapine (NVP) have higher cytotoxicity with CC_{50} value of 0.53 ± 0.29 and 0.82 ± 0.0 µg/mL respectively as compared with tenofovir (TDF) and abacavir (ABC).

Regarding the cytotoxic effect of the solvent fractions and the control drugs on MT4 cell line according to MTT assay, it was found that fractions AE, AM and ALM showed significantly higher cytotoxicity (P <0.01) as compared with TDF and ABC (Figure 2). On the other hand, most of the solvent fractions demonstrated higher maximum cytotoxic effect ($Emax_C$) when compared to the control drugs, indicating their possible higher toxic effects with increasing concentration level.

Table 3. Cytotoxicity and anti-HIV-1 activities of control drugs and solvent fractions using MT4 cell line.

Tested substanceh	Cytotoxicity			Antiviral activity		SI
	MNTC (µg/mL)	CC_{50} (µg/mL)	$Emax_C$	IC_{50} (µg/mL)	$Emax_{AV}$	
AZT	0.38 ± 0.19	0.53 ± 0.29	36.28 ± 0.83	0.002 ± 0.00	83.5 ± 0.57	279.4
TDF	4.92 ± 0.71	6.73 ± 0.24	13.17 ± 0.43	0.04 ± 0.01	80.55 ± 0.46	176.5
ABC	0.18 ± 0.03	0.26 ± 0.00	17.83 ± 0.57	0.05 ± 0.031	58.67 ± 0.43	5.0
NVP	0.57 ± 0.0	0.82 ± 0.0	39.13 ± 0.65	0.24 ± 0.089	72.53 ± 0.47	3.5
AH	0.18 ± 0.09	0.25 ± 0.07	49.11 ± 0.16	8.09 ± 0.55	90.8 ± 0.88	0.03
AD	0.99 ± 0.26	1.13 ± 0.16	29.33 ± 0.18	0.04 ± 0.00	87.61 ± 0.56	26.2
AE	141.9 ± 0.25	171.8 ± 0.1	63.05 ± 0.32	3.69 ± 0.11	86.53 ± 0.71	46.6
AM	0.08 ± 0.07	0.58 ± 0.05	52.74 ± 0.44	1.15 ± 0.03	67.91 ± 0.55	0.5
ALH	142.2 ± 0.4	173.5 ± 0.8	39.44 ± 0.65	0.02 ± 0.01	82.78 ± 0.67	9752.7
ALD	1.328 ± 0.66	2.47 ± 1.37	29.64 ± 0.54	5.37 ± 0.13	76.45 ± 0.14	0.5
ALE	165.9 ± 0.65	165.9 ± 0.65	42.89 ± 0.84	0.05 ± 0.03	83.81 ± 0.01	3186.7
ALM	6.28 ± 0.51	6.28 ± 0.51	54.47 ± 0.06	0.05 ± 0.02	77.04 ± 0.37	138.6

(MNTC) maximum non-toxic concentration, (CC_{50}) 50% cytotoxic concentration, ($Emax_C$) Maximum cytotoxic effect %, (IC_{50}) 50% antiviral effect concentrations, ($Emax_{AV}$) maximum antiviral effect %, (SI) selective index, AZT= Zidovudine; TDF= Tenofovir; ABC= Abacavir; NVP= Nevirapine; AH= hexane fraction of the bark; AD= dichloromethane fraction of the bark; AM= methanol fraction of the bark; AE= Ethyl acetate fraction of the bark; ALH= hexane fraction of the leaves; ALD= Dichloromethane fraction of the leaves; ALE= ethyl acetate fraction of the leaves; ALM= methanol fraction of the leaves. Results are shown as means ± S.E.M.

Anti-HIV assay

The dichloromethane fraction of the bark showed better anti-HIV activity by inhibiting virus induced cytopathic effects (CPE) by 87.61% with an IC_{50} value (0.04 ± 0.00 µg/mL). This is lower than the MNTC (0.99 ± 0.26 µg/mL) giving selectivity index (SI) of 26.2. Similarly, the ethyl acetate fraction of the bark (AE) reduced the cytopathic effect (CPE) by 86.53% with IC_{50} value of 3.69 ± 0.11 µg/mL which is much lower than the MNTC (141.9 ± 0.25 µg/mL), giving SI of 46.6. Despite being less efficacious with IC_{50}= 8.09 ± 0.55 µg/mL, the hexane fraction of the bark (AH) inhibited 90.8 % of cell death that occurred due to CPE. The methanol fraction (AM) showed less anti-HIV activity ($Emax_{AV}$= 67.91%; SI= 0.5) (Table 3).

The hexane and ethyl acetate fraction of the leaves displayed higher anti-HIV activity with IC_{50} values of 0.02 ± 0.01 and 0.05 ± 0.03 µg/mL respectively, inhibiting more than 80 % of virus induced CPE. The hexane fraction of the leaves (ALH) displayed the highest SI of 9752, which is attributed to the low IC_{50} values. The dichloromethane fraction of the leaves showed lower efficacy to inhibit the viral replication with IC_{50}= 5.37 ± 0.13 µg/mL and SI of 0.5.

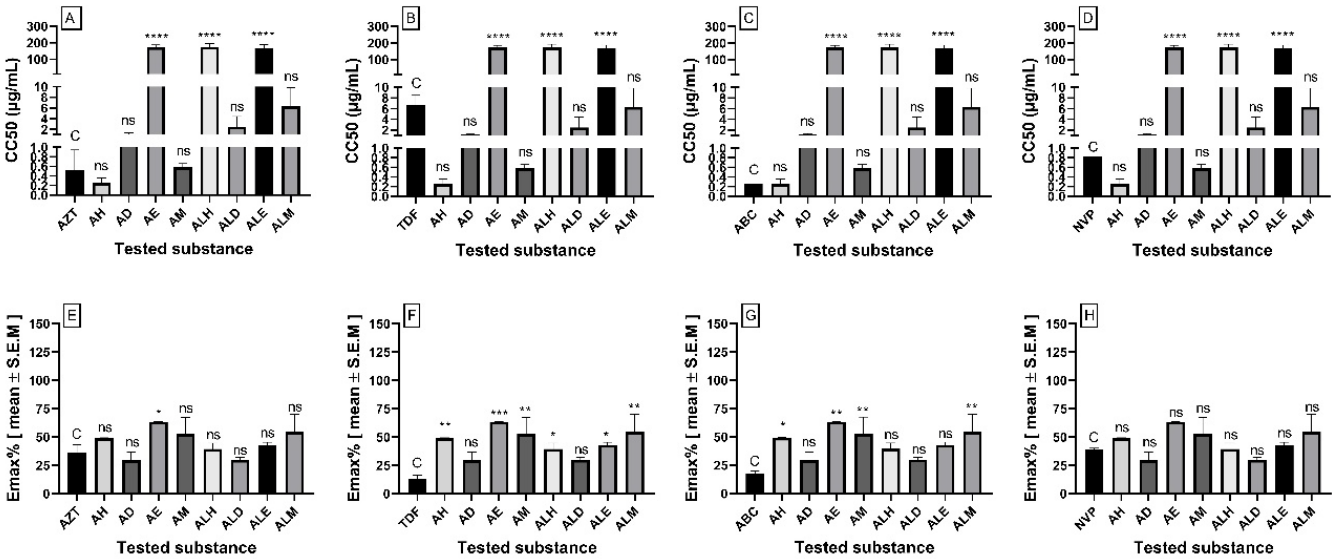


Figure 2. CC_{50} (A, B, C and D) and Emax (E, F, G and H) values of the control drugs and the solvent fractions cytotoxic activity. Results expressed are mean of three independent experiments ± S.E.M. C; control, ns; not significant, *; P <0.05 and **; P <0.01, ***; P <0.001, ****; P <0.0001. AZT= Zidovudine; TDF= Tenofovir; ABC= Abacavir; NVP= Nevirapine; AH= hexane fraction of the bark; AD= dichloromethane fraction of the bark; AM= methanol fraction of the bark; AE= Ethyl acetate fraction of the bark; ALH= hexane fraction of the leaves; ALD= Dichloromethane fraction of the leaves; ALE= ethyl acetate fraction of the leaves; ALM= methanol fraction of the leaves.

Among the FDA approved antiretroviral drugs, zidovudine showed the highest anti-HIV activity with IC_{50} values of 0.002 ± 0.00 µg/mL inhibiting 83.5 % of CPE induced by the virus (SI= 279.4), followed by tenofovir with IC_{50} = 0.04 ± 0.01 giving SI of 176.5.

The antiviral activity of the tested fractions showed that both the control drugs and the tested fractions had approximately similar antiviral efficacy as they showed non significantly different $Emax_{AV}$ values when comparison was carried between them (Figure 3). In addition, most of the fractions showed IC_{50} value that is not significantly different from the control drugs indicating their comparable potency, except for fractions AH and ALD which demonstrated lower potency shown by the significantly (P <0.01) higher IC_{50} value. Figure 4 depicts the concentration-response curve for the cell viability % and the inhibition % of the viral induced cytopathic effect associated with the tested substances.

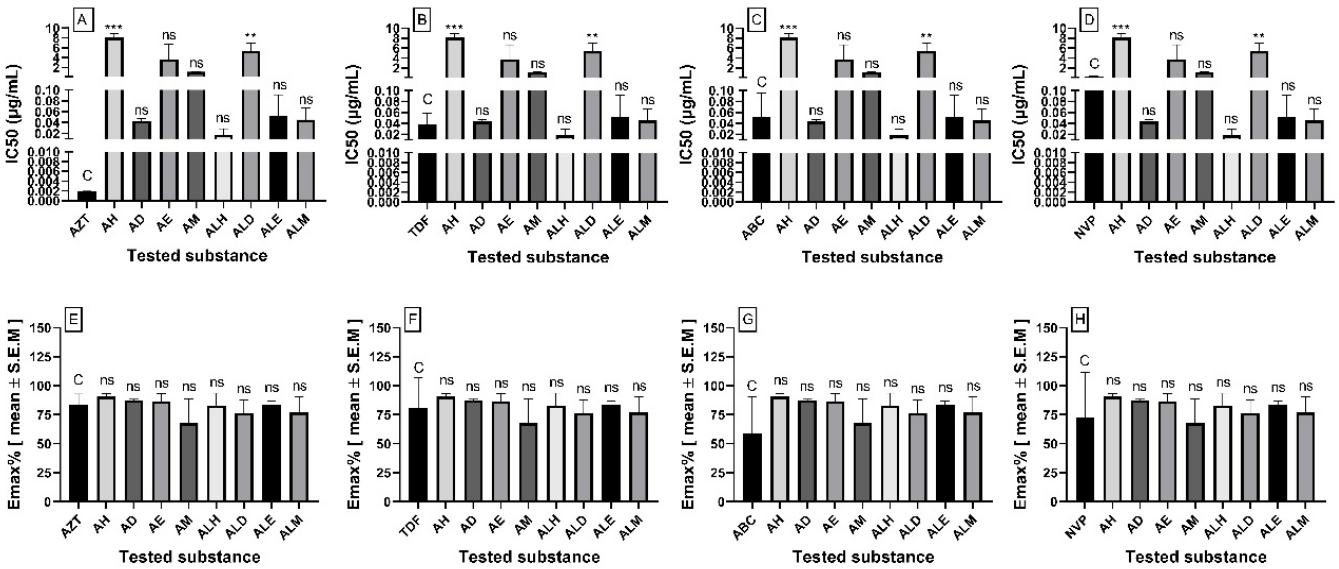
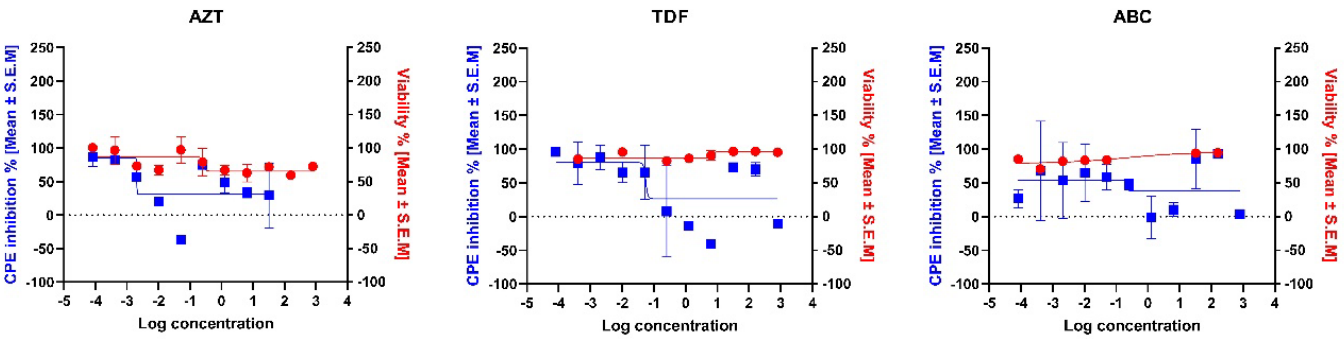


Figure 3. IC_{50} (A, B, C and D) and Emax (E, F, G and H) values of the control drugs and the tested fractions antiviral activity. Results expressed are mean of three independent experiments ± S.E.M. C; control, ns; not significant, **; P <0.01, ***; P <0.001. AZT= Zidovudine; TDF= Tenofovir; ABC= Abacavir; NVP= Nevirapine; AH= hexane fraction of the bark; AD= dichloromethane fraction of the bark; AM= methanol fraction of the bark; AE= Ethyl acetate fraction of the bark; ALH= hexane fraction of the leaves; ALD= Dichloromethane fraction of the leaves; ALE= ethyl acetate fraction of the leaves; ALM= methanol fraction of the leaves.



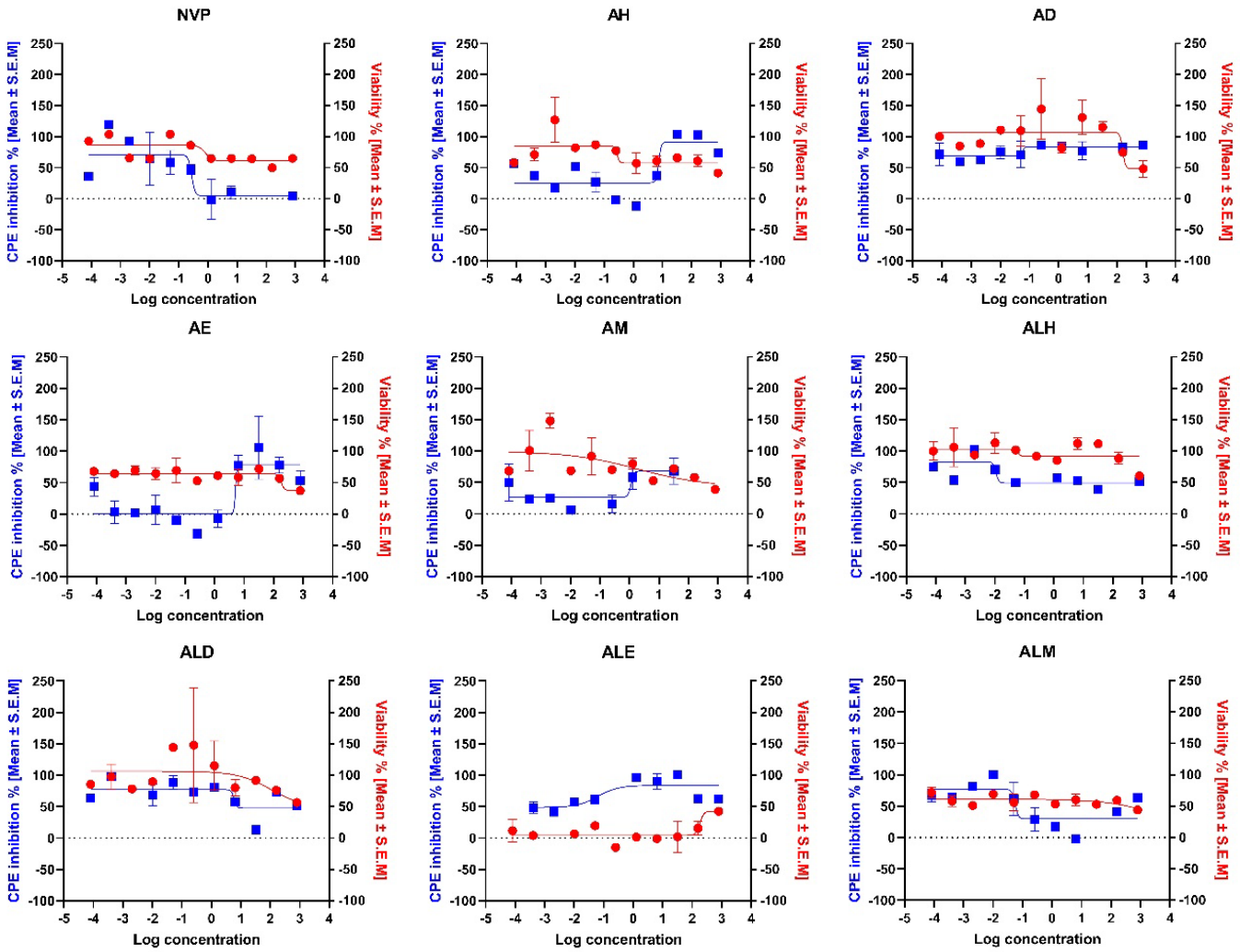


Figure 4. Concentration – response curve analysis for the cell viability % (red line), and the inhibition % of the viral induced cytopathic effect (blue line) associated with control drugs and the tested fractions at concentration level (800 – 8.192×10^5 µg/m 4. L). results presented in the curves are means ± S.E.M of three independent experiments. AZT= Zidovudine; TDF= Tenofovir; ABC= Abacavir; NVP= Nevirapine; AH= hexane fraction of the bark; AD= dichloromethane fraction of the bark; AM= methanol fraction of the bark; AE= Ethyl acetate fraction of the bark; ALH= hexane fraction of the leaves; ALD= Dichloromethane fraction of the leaves; ALE= ethyl acetate fraction of the leaves; ALM= methanol fraction of the leaves.

Discussion

The efficacy of the hexane and ethyl acetate fraction of the leaves to produce high antiviral activity ($Emax_{AV}$ > 80.8%) could be attributed to the pentacyclic triterpenoids, which are a promising class of HIV-1 inhibitors [47]. As depicted in Table 1, different triterpenoids have been isolated from *C. macrostachyus*, including betulin, betulinic acid, lupeol and lupeol acetate. It has been previously reported that Lupeol acetate inhibits the viral entry to CD4 cells [48] and that other pentacyclic triterpenoic derivatives, such as Betulinc acid, inhibit the HIV-1 RT-associated activities [49]. A recent report by Chaniad *et al* [50] explained the efficacy of Betulin as potent anti-HIV compound with IC_{50} value 17.7 ± 0.6 µM. Similarly Esposito et al [49] reported that Lupeol acetate and Lupeol inhibited the HIV-1 RT-associated RNase H function with IC_{50} values of 63 and 11.6 µM, respectively. The activities of the hexane and ethyl acetate fraction of the leaves of *C. macrostachyus* could be due to the individual or synergistic effect of the phytochemical compounds.

Considering the wide medicinal use of these plants by HIV-positive patients, this research evaluated the effect of the solvent fractions of *C. macrostachyus* in inhibiting viral replication. The findings of this study could be used as a basis for further studies aiming at isolation and characterization of potential anti-HIV compounds from the leaves of *C. macrostachyus* and also support the ongoing efforts in development of new antiviral agents that are less toxic and more efficacious.

Conclusion

In this study we evaluated anti-HIV activity of leaves and stem bark of *Croton macrostachyus*. Based on our finding, the hexane and ethyl acetate soluble fraction of the leaves could be considered the most promising among the tested fractions considering both their cytotoxic and antiviral

activity profile. These fractions have higher CC_{50} value compared to the control drugs while having statistically comparable IC_{50} value to those of the control drugs and the wide difference between the MNTC and the IC_{50} value indicating their safety. Hence, further studies should be conducted focusing on characterizing the bioactive compounds and evaluating the efficacy of the fractions in different retroviral enzymes.

Ethical Approval- The research was approved by Kenyatta National Hospital-University of Nairobi Ethics and Research Committee (KNH-UON ERC), approval number P992/12/2019.

Acknowledgments

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Conflict of Interest

The authors declare that they do not have any conflicts of interest.

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Assessment of Prescribing Practice at Thika Level 5 Hospital

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Abstract

To deliver quality healthcare services and ensure optimum utilization of resources, rational drug use is essential. Irrational prescribing is a major cause of irrational drug use. Irrational prescribing of drugs has serious consequences for patients in terms of poor health outcomes and waste of scarce health resources resulting in a weak healthcare system. This study aimed to assess the prescribing practices at Thika level V hospital using the World Health Organization (WHO) core prescribing indicators.

A quantitative retrospective cross-sectional study was conducted. A total of 525 prescriptions written from February 9, 2021 to March 5, 2021 were collected from the outpatient pharmacy department and analyzed according to the WHO prescribing indicators. The indicators included the average number of drugs per encounter, the percentage of encounters with an antibiotic prescribed, the percentage of encounters with an injection prescribed and the percentage of drugs prescribed by a generic name. Ethical approval to carry out the study was obtained from Thika Level V – County Government of Kiambu.

Based on the findings, the average number of drugs per encounter was 3.18 (optimal value <2). Percentage of encounters with an injection prescribed was 9.17% (optimal value <20%). Percentage of drugs prescribed by a generic name was 58.9% (optimal value = 100%) while percentage of encounters with an antibiotic prescribed was 52.4% (optimal value <30%).

This study showed that irrational prescribing practices were common and prescribers were not compliant with the WHO prescribing indicators. There was practice of polypharmacy, irrational prescribing of antibiotics, and increased prescribing of drugs using the brand name, instead of the generic name, in the hospital. The results indicated that there is scope for improvement and the hospital can offer better healthcare services with compliance to the national guidelines and consistent monitoring of the prescribing practices. The evidence-based information generated from this study can be used to determine the quality of service offered to the patients and can also assist decision-makers to develop policies that would enable rational prescribing and use of drugs.

Keywords: *Rational Drug Use, Irrational Drug Use, WHO core drug prescribing indicators, Prescribing pattern, Quality healthcare.*

Introduction

Rational drug use is essential to deliver quality healthcare services and optimum utilization of resources. Drugs are the most used therapeutic resources and thus practices aiming at rational use of drugs should be promoted. Rational drug use refers to the prescription of the right drug for the right indication in the right dosage and dosing frequency for the correct duration [1]. The irrational prescribing practices include prescription of too many medicines per patient (polypharmacy), over-prescription of antibiotics, failure to prescribe in accordance with the clinical guidelines, prescribing using brand names, and overuse of injectables when oral formulations are available and are appropriate [2]. Such irrational use of drugs has serious consequences for patients in terms of poor health outcomes and waste of scarce health resources resulting in a weak health care system. The impacts of irrational drug use have rarely been evaluated. This may be due to the lack of evidence about the gravity of the problem of rational drug use [3]. The assessment of prescribing patterns has not been regularly conducted in Kenya and there is little published data available on the same. A national survey conducted in Kenya in 2013 found that there was practice of polypharmacy, irrational prescribing of antibiotics, and prescription using the brand name instead of the generic name in the public health facilities[4].

In the late nineties, the World Health Organization collaborated with the International Network for Rational Use of Drugs (INRUD) to develop and standardize five core prescribing indicators that can be utilized to evaluate the prescribing patterns of drugs in health care settings [4]. These indicators provide a measure of optimal use and prescription of drugs [1]. WHO has proposed standard reference values for each indicator that can be used to assess the prescribing practice in any country, region, or health

facility [5]. The prescribing indicators include the average number of drugs per encounter, the percentage of encounters with an injection prescribed, the percentage of drugs prescribed by a generic name, the percentage of encounters with an antibiotic prescribed, and the percentage of drugs prescribed from the essential drug list. This study focused on assessing the prescribing practice at the outpatient pharmacy of Thika level V hospital using the core prescribing indicators proposed by the WHO.

Methodology

A quantitative retrospective cross-sectional study was conducted. Data was collected from the outpatient pharmacy department of the hospital according to the WHO prescribing indicators. The study population consisted of the prescriptions in the outpatient pharmacy of the hospital between February 9, 2021, and March 5, 2021. Data from 525 prescriptions were collected throughout this study. The data was statistically analyzed and presented using Microsoft Excel Software. Percentages and averages were calculated for statistical analysis.

Study location

The study was conducted at Thika Level V Hospital which is situated in Thika sub-county, Kiambu County, Kenya. Thika Level V is a government hospital that offers both inpatient and outpatient services.

Study Design, population, and sampling size

A retrospective hospital-based cross-sectional study design was adopted. A structured checklist for prescribing indicators was used to collect data from prescription records. The study population consisted of the prescriptions received in the outpatient pharmacy between February 9, 2021, and March 5, 2021, which were processed in the outpatient pharmacy. The sample size was determined by using the Fischer formula [6].

$$N = \frac{Z^2 P(1-P)}{d^2}$$

Where;
N = the estimated sample size when the population is more than 10,000
Z = Statistic corresponding to the level of confidence (1.96)
P = the expected prevalence from similar studies conducted by researchers. P was estimated to be 50.0%, this was based on the proportion of encounters with an antibiotic prescribes from a study conducted in Rift Valley Provincial General Hospital that showed the prevalence of antibiotics prescribing to be 54.7%. [7]
q = 1-P = (0.5)
d = margin of the error (set at 5%)
Therefore, n= (1.96^2 x 0.5 (1 – 0.5)) / 0.05^2 = 384.16
Applying this formula, a minimum sample size of 384.16 was found.
10% of 384 = 38.42
10% was added to the sample size to reduce the margin of error in the study.
384 + 38.42 = 423
Sample size = 423

A sample size of 525 was used to increase the level of precision and accuracy of the study.

Sampling Technique and Data Collection Methods

A systematic random sampling technique was employed to select outpatient prescriptions from the hospital to prevent data collection bias. The sampling of the prescription was distributed across the month. Data was collected using a pre-designed structured checklist. Data was collected from the prescriptions as they were processed in the pharmacy in accordance with the core WHO indicators. Ethical approval to carry out the study was obtained from Thika Level V – County Government of Kiambu.

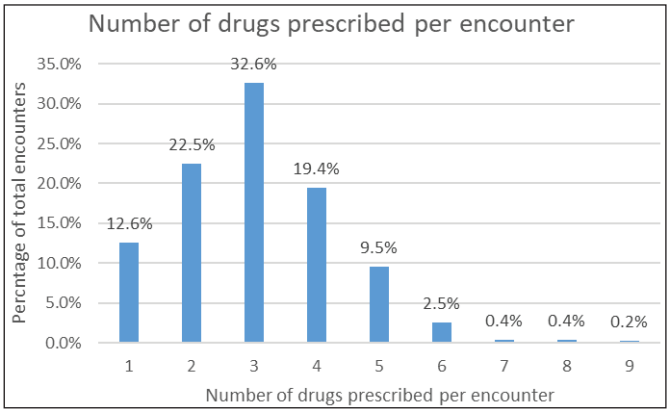
Data processing and analysis

Microsoft Excel was utilized for data processing and analysis. The quantitative variables were presented in percentages and standards.

Results and Discussion

Average number of drugs per encounter

Out of the 525 encounters studied, only 184 encounters had less than 2 drugs prescribed and 341 encounters had at least 3 drugs prescribed. More than half of the prescriptions (65%) had 3 or more drugs prescribed as shown in figure 1. The practice of polypharmacy was common with the range of 3 to 9 drugs per prescription. Most of the prescriptions (up to 33%) had 3 drugs prescribed. These values do not comply with the standard set by WHO which is less than 2.0 drugs per encounter. The results of this study reflect the practice of polypharmacy in the hospital.



Figre 1. Number of drugs prescribed per encounter.

In studies done in other facilities in Kenya, the average number of drugs prescribed in a prescription was 2.9 to 3.85 which was higher than the recommended range set by WHO [8] [9] [10] [11]. In the majority of the developing countries, this value was between 1.76 and 3.5 [12] [13] [14] [15] [16]. Most of the countries had an average value of around 3, and thus were practicing polypharmacy.

Practicing polypharmacy leads to an increased risk of prescription of potentially inappropriate medications (PIMs). This may further cause increased drug-drug interaction and adverse effects in the patients which further results in excess health care utilization. A study done on the factors associated with polypharmacy indicated that the prescription of multiple medicines increases the risk of exposure to potentially inappropriate medications [17]. Other than that, polypharmacy leads to poor compliance to the drug regimens as the patient experiences a pill burden. This will further lead to clinical outcomes not being achieved. The patient may also incur unnecessary drug expenses.

This practice of polypharmacy in the hospital may lead to an increase in the risk of adverse effects especially in patients with comorbidities, geriatric patients, or patients who are immunocompromised. However, in chronic conditions such as hypertension and diabetes, patients require more drugs and thus polypharmacy may be accepted. Fixed-dose combinations can be used to reduce the pill burden on the patients. This will further improve medication adherence as well.

Percentage of encounters with an antibiotic prescribed

As shown in figure 2, the percentage of encounters with an antibiotic was 52%. The recommended value by WHO is less than 30%. The finding of this study was above the recommended values and thus indicated that there is a practice of irrational antibiotics prescribing. Irrational prescribing of antibiotics involves the use of too many antibiotics, using antibiotics at inadequate dosages and durations, and using them inappropriately for non-microbial infections [7]. This majorly contributes to the emerging problem of antimicrobial resistance.

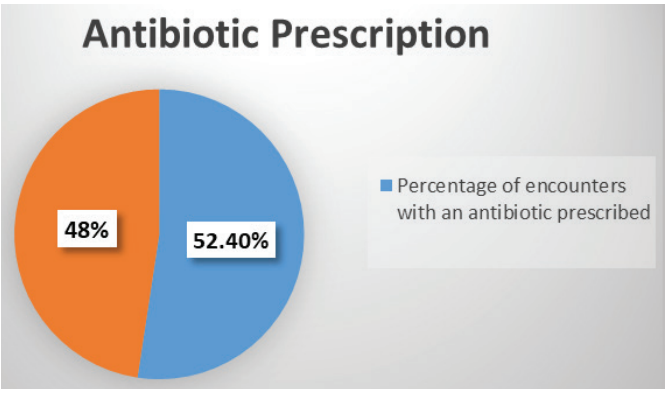


Figure 2. Percentage of encounters with an antibiotic prescribed.

In comparison to other studies done in Kenya, the percentage of encounters with an antibiotic prescribed ranged from 61.8% to 84.8% [8] [9] [10]. Studies done in various other countries in Africa and around the world showed high percentages of encounters with antibiotics prescribed ranging from 17.8% to 61.8% [12] [13] [14] [15] [16]. Most countries have reported high percentages of antibiotics thus indicating indiscriminate use of antibiotics. This poses a global threat as irrational antibiotic prescribing contributes to the development of antimicrobial resistance. According to studies done in the African region, there is a trend for increasing drug resistance [18]. The high percentage of antibiotics prescribed in Kenya is alarming and needs to be controlled. Irrational antibiotic prescribing also increases the overall drug costs. However, the high burden of communicable diseases implicates the extensive use of antimicrobials in Kenya and Africa [18]

Percentage of encounters with an injection prescribed

Only 9.71% of the encounters had an injection prescribed as shown in figure 3. This was in accordance with the recommended reference value of WHO which is less than 20%, and there were no over-prescription of injections. This was commendable as over prescriptions of injections increases the risk of infection and transmission of blood-borne diseases such as HIV/AIDS and hepatitis. Other than that, injections are relatively more expensive than other dosage forms and also require trained personnel for their administration.

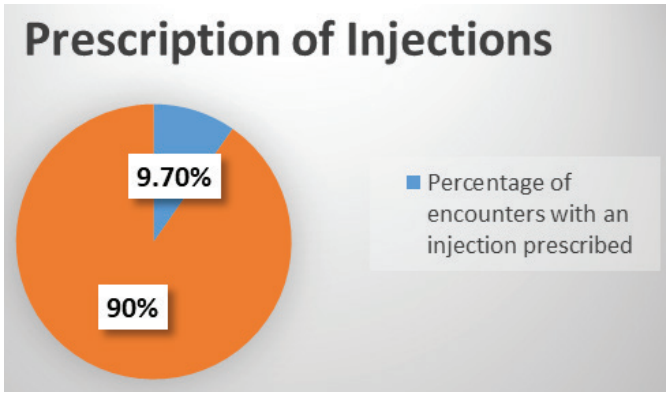


Figure 3. Percentage of encounters with an injection prescribed

In comparison to other findings in Kenya, the percentage of encounters with an injection prescribed ranged between 9.5% to 24.9% [8] [9] [10]. The findings from studies done in Kenya are mostly within the recommended range or slightly higher which is encouraging. Compared to other findings around the world, the percentage of encounters with an injection prescribed ranged between 5.43% to 24.3% [12] [13] [14] [15] [16]. Most of the countries had the percentage of encounters with an injection prescribed lower than the reference value set by WHO, thus suggesting there is no increased use of injections.

Percentage of drugs prescribed by generic name

About fifty-nine percent of the drugs prescribed were prescribed using the generic name as shown in figure 4 and table 1. The findings indicate that there are poor generic prescribing habits in the hospital as this was much lower than the standard set by WHO which is 100%.

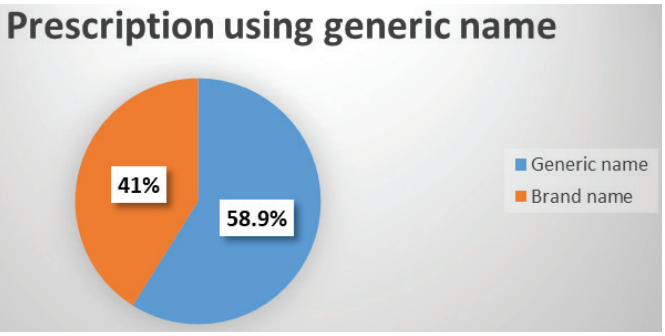


Figure 4. Percentage of drugs prescribed using the generic name.

The findings from various studies conducted in Kenya were comparable, where the percentage of drugs prescribed by generic name was between 25.6% and 52% [9] [10] [8] [11]. In comparison to the other health care facilities in Kenya, Thika level 5 had a high percentage of drugs prescribed using a generic name, however, it does not meet the standard set by WHO which is 100%.

In contrast to our findings, the percentage of drugs prescribed by the generic name in other developing countries ranged between 7.8% to 94.9% [12] [13] [14] [16]. Most countries had a high percentage of drugs prescribed using generic names but none comply completely with the reference value indicated by WHO. Prescribing using the generic name is not widely practiced in Thika Level 5 hospital and also other hospitals in Kenya. This high rate of prescribing using brand names instead of the generic name in most healthcare facilities in Kenya and around the world may indicate that the physician may be having a marketing influence and thus only prescribe drugs of a particular brand. The prescriber's perception of the effectiveness of the generic drug and the availability of cost-effective generic drugs may also contribute to the poor generic prescribing habits. The use of generic names when prescribing is encouraged as it promotes rational use of drugs and also provides the patient with more alternatives for drug purchase. The patient is also allowed to choose the most cost-effective drug. A comparison of prescribing inidcators at Thika level V hospital against WHO standards are summarized in table 1.

Table 1. Drug Prescribing Indicators at Thika Level V Hospital

Indicator	Findings obtained from this study	Standard Values by WHO
Average number of drugs per encounter	3.18	<2
Percentage of encounters with an antibiotic prescribed	52.4%	<30%
Percentage of encounters with an injection prescribed	9.71%	<20%
Percentage of drugs prescribed by a generic name	58.9%	100%

Assessment of prescribing practices in hospitals and healthcare facilities is essential as the findings provide prescribing patterns that indicate if rational prescribing and rational use of drugs and vital resources is practiced in the facility. Rational prescribing and use of drugs are necessary to achieve treatment outcomes, prevent adverse drug reactions and prevent the unnecessary cost of medical care. Based on the standards set by WHO for the prescribing indicators, the findings of this study indicate that there is a practice of irrational prescribing in Thika Level V hospital. From the four core prescribing indicators that were analyzed in this study, only one indicator conformed to the set standards.

Recommendations

Recommendations for improvement

The study revealed that there was practice of polypharmacy, irrational prescribing of antibiotics, and increase prescription of drugs using the brand name instead of the generic name in the hospital. The prescribing practices in the hospital can be improved by increasing awareness of rational prescribing practices by providing training to the health care practitioners. Various clinical guidelines such as the standard treatment guidelines and formularies should be prepared, made available in the facility and their compliance needs to be enforced to promote rational prescribing of drugs.

Recommendations for further studies

The three key areas that indicate rational use of drugs in a facility include prescribing practices, patient care, and facility-specific factors. This study only focused on the assessment of prescribing practices using the prescribing indicators. Further studies should be done using the patient care indicators and the facility indicators to further investigate drug use in health facilities. Under the patient care indicators, the average consultation time, the average dispensing time, the percentage of drugs dispensed, the percentage of drugs adequately labeled, and the patient's knowledge of correct dosage should be assessed. From these findings, the quality of healthcare provided to the patient by the prescriber can be assessed.

Under facility indicators, the availability of a copy of the essential drug list or national formulary in the facility and the availability of the essential drugs in the facility is assessed. This indicator is used to assess if the national formulary and the essential drug list are available and accessible to the prescribers in the facilities. Very few studies are done in Kenya in the past decade on the assessment of prescribing practices, thus the data available is inadequate to conclude that poor prescribing habits are practiced among prescribers in Kenya. Thus more studies need to be done in Kenya to assess the prescribing pattern and drug use practices in health care facilities.

Conclusion

This study showed that irrational prescribing practices were common in the hospital. Prescribers were not compliant with the WHO prescribing indicators. Out of the four indicators, only prescription of injections was within the reference value recommended by WHO.

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Herbal Medicine used for Treatment of Erectile Dysfunction available in Nairobi County, Kenya: Labelling and Regulatory Compliance

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Abstract

The use of herbal medicine, including those used for the treatment of erectile dysfunction, has been increasing rapidly in the recent past. This study surveyed herbal medicines used for the treatment of erectile dysfunction in Nairobi County, Kenya, and evaluated the labelling and regulatory compliance of these products. The study methodology involved randomly selecting the stockist of the target products. This was followed by identification of the products, collation of the unit prices and purchase of samples. The study site involved the following administrative units: Starehe, Westlands, Langata, Makadara, Dagoreti and Kamukunji. A total of 99 products were sampled. The study revealed that samples available were either locally manufactured or imported, mainly from China/Hong Kong, India, France, the United Kingdom and Tanzania.

The study showed that the range of products in various dosage forms (tablets, capsules, liquids, gels and powders) were available in Nairobi county. Most of the samples did not meet the minimum requirements with regard to labelling and regulatory compliance but were at varying levels of compliance with regard to labelling requirements.

The Pharmacy and Poisons Board had licensed some of the healthcare stores keeping the products like wholesale and retail pharmacies while others like herbal shops and street hawkers had not been licensed. There is a need to strengthen regulatory requirements for these products in the Kenyan market.

Key words: *Erectile dysfunction, Herbal medicine, labelling requirements, locally manufactured, Regulatory compliance.*

Introduction

There are numerous forms of Complementary Alternative Medicine (CAM) practiced worldwide. These include homeopathic, traditional Chinese medicine, Ayurveda, reflexology, aromatherapy, massage and herbal medicine. Herbal medicine is mainly obtained from plants, and is the most widespread. The plant kingdom is the largest source of complementary drugs, which are at different levels of development worldwide [1]. In 2003, WHO estimated that approximately 30% of modern medicines are derived from plants. Most of these are derived from the tropical rain forests of Africa, Asia, South and North America. The use of plants and herbs for medicinal purposes traces their origin in China and India, dating back thousands of years. Traditional medicine (TM) has emerged as an alternative to modern medicine. It is the combination of skills, knowledge, and practices established on beliefs, theories, and experiences indigenous to various cultures that are used to maintain health, and to diagnose, prevent, improve and treat mental and physical illnesses.

Today many of the drugs in clinical use have been derived from plants arising from their traditional uses. Examples include Artemisinin used in Artemisinin Combination Therapy (ACT) regimens for malaria treatment derived from *Artemisia annua* the Chinese worm herb and Madagascar periwinkle (*Catharanthus roseus*), which is the source of anticancer drugs; *Prunus africanus,* the source of medicine for treating benign prostate hyperplasia [2,3], and *Wabugia ugandensis* which contains compounds with activity on influenza [4].

The World Health Organization (WHO) has developed strategies to develop and promote the use of Traditional Medicine in healthcare systems in countries worldwide [1, 5]. According to WHO, USD 65 billion is used globally for purposes of treatment with herbal medicine and the expenditure is growing rapidly [5]. The growth in supply and sale of these products is because they are directly marketed in drug stores and pharmacies. In addition, hypermarket advertising on both electronic and print media has increased the sale of herbal products [6]. In developing countries, the use of herbal medicine has increased mainly because it is affordable and accessible to most of the population. In developed countries, the use of herbal medicine has increased due to concerns on adverse effects of conventional medicine and greater public access to information.

According to a study by Mwangi [7], for many patients, herbal medicine offers gentler means of managing chronic debilitating conditions such as heart disease, rheumatoid arthritis, cancer and mental disorders compared to conventional medicine. In developing countries, the ratio of patients to conventional medical practitioners is so high (1: 600) that many patients prefer to visit traditional medicine practitioners for their primary healthcare needs [8]. This is because the different specialists treat patients in a nonintegrated manner hence eroding patient confidence in the care received [7].

All pharmaceutical products for human use, including herbal medicine, must be appropriately labelled. According to the National Agency for Food and Drug Administration Control (NAFDAC) [9] of the US, the information on labels, which must be clear and adequate, should include the following: declaration of the content of drug and dose, and the trademark - which shall not give the wrong impression of nature, quality or substance of drug product. The label should show indications and dosage for use, precautions and warnings, and there must be a package insert/patient information leaflet [9]. According to the Medicines and Healthcare Regulatory Agency (MHRA) guidance document, the use of medicine requires clear labelling for the user to take the right dose, take necessary safety precautions and for safe use. Legible labelling is critical for easy understanding by those involved in handling the medicine. Legibility in labelling helps in avoiding the potential for confusion between similarities in the drug names. All these are important in preventing medication errors [10,11]. In Sri Lanka, the National Medicines Regulatory Authority has instituted a guideline for labelling medicinal products for human use [12]. In Australia, the Government department of health regulation; "Therapeutic Goods Administration (TGA)" has established guidelines for labelling medicines [13]. In Canada, Health Canada has put in place a guidance document addressing plain language labelling requirements for non-prescription drug products [14]. The objective of the guidance is labelling that supports the safe and effective use of drug products by healthcare providers and consumers [15].

In the US, herbal products are regulated as food supplements. The efficacy, safety and quality are the responsibility of the manufacturer. Label claims are unregulated and there is no standardization. There lacks guarantee that the manufacturing process of the product is proper and it does not have harmful contaminant levels. In Asia, the Association of Southeast Asian Nations (ASEAN) have developed harmonized guidelines to help avoid fraudulent labelling of traditional medicines [16]. In Kenya, traditional medicine regulation is limited to listing in the retention register though the guidelines developed by the Pharmacy and Poisons Board (PPB) in 2010 [17]

Objective

The main objective of the survey was to review the range of herbal medicinal products used for the treatment of erectile dysfunction available in Nairobi County, Kenya. The specific objectives were to evaluate samples for regulatory compliance and labelling requirements as directed by the Pharmacy and Poisons Board (PPB).

Methodology

The study methodology involved randomly selecting the stockist of the target products. This was followed by identification of the products, collation of the unit prices and purchase of samples. Samples were purchased from healthcare stores (pharmacies, supermarkets, health stores, herbal shops and open-air hawkers). Identification was made by asking the attendants and checking the indications on the labels. The budget for the purchase of the samples was then prepared and used to purchase samples from the identified stores. The market survey was done between July 2017 and August 2018. The sample information was entered into a Microsoft Excel spreadsheet [18] for data analysis. The results were presented in descriptive percentages and tables.

Study site and sampling

The study was carried out in Nairobi County, Kenya. The products were purchased from all distribution outlets (supermarkets, wholesale and retail pharmacies, health stores, herbal shops and street hawkers). Nairobi County has the highest concentration of Pharmacies, so it was deemed representative of the whole Country [19]. The study was done as per administrative regions of Nairobi County, namely: Starehe, Westlands, Langata, Makadara, Dagoreti and Kamukunji. The sampling was done between July 2017 and August 2018. Randomly selected outlets stocking herbal products for the treatment of erectile dysfunction were visited [18,19]. All the 99 products surveyed in the outlets were sampled (100% sampling), which was deemed representative. The samples collected were all-inclusive with respect to dosage forms: tablets, hard gelatin capsules, liquids, gels and powders. The samples only included intact packs; broken/loose packs were not sampled as such products were deemed to have been exposed to the environment hence the quality may have been compromised.

Results

A total of 99 products were sampled and analyzed for labelling requirements and regulatory compliance. A wide range of Herbal Medicinal Products (HMPs) used for the treatment of erectile dysfunction were readily available in Nairobi County. The samples were both locally manufactured and imported. The imported ones originated from countries including Tanzania, China/Hong Kong, India, France, and the United Kingdom. The samples were analyzed for country of origin, manufacturer details, ingredients in the sample, dosage form, indications/dosage, date of manufacture/ expiry, batch number, Patient Information Leaflet (PIL) and registration status. Some of the samples had not been listed on the PPB register to be in the Kenyan market. Most of the sellers were not licensed by PPB. The samples were at

different levels of compliance with labelling requirements. Table 1 is the list of the samples analyzed with regard to labelling requirements. Table 2 is a summary of the labelling requirements.

Table 1. Sample Labelling Analysis

S/No.	Product Code	Manufa-cturer's Details	Ingredients in the sample	Dosage/ Indications	Date of Manufacture/ expiry	PIL	Label Score
1	MWS	✓	x	✓	✓	✓	4/5
2	NKE	✓	x	✓	✓	✓	4/5
3	SE5	✓	x	✓	✓	✓	4/5
4	NJT	✓	x	✓	✓	✓	4/5
5	MLP	✓	x	✓	✓	✓	3/5
6	JMS	x	x	x	x	x	0/5
7	PLV	✓	x	✓	x	x	2/5
8	MXC	✓	✓	✓	x	x	3/5
9	MEG	x	✓	✓	✓	x	3/5
10	VHL	✓	✓	✓	✓	x	4/5
11	SGK	✓	x	✓	✓	✓	4/5
12	FT253	✓	✓	✓	✓	✓	5/5
13	HVP	✓	x	✓	x	✓	3/5
14	VTL	✓	✓	✓	✓	x	4/5
15	TKA	✓	✓	✓	✓	x	4/5
16	VOI	✓	✓	✓	✓	x	4/5
17	TN S	✓	✓	✓	x	x	3/5
18	MP	✓	x	✓	✓	x	3/5
19	SC	✓	✓	✓	✓	✓	5/5
20	VG	✓	✓	✓	✓	✓	5/5
21	THB	✓	x	✓	✓	x	3/5
22	SAT	✓	x	✓	x	x	2/5
23	SMB	✓	✓	✓	✓	✓	5/5
24	SPN	✓	x	✓	✓	x	3/5
25	MS F	✓	x	✓	x	x	2/5
26	MF	✓	x	✓	x	x	2/5
27	AMN	✓	✓	✓	✓	x	4/5
28	MRG	✓	x	✓	✓	x	3/5
29	VXB	✓	✓	✓	x	✓	5/5
30	BMC	✓	x	x	✓	x	2/5
31	VG800	✓	✓	✓	x	x	3/5
32	MRC	✓	✓	✓	✓	✓	5/5
33	VXM	✓	✓	✓	✓	✓	5/5
34	HVMS	✓	x	✓	x	✓	3/5
35	MPR	✓	✓	✓	✓	x	5/5
36	OMP	✓	✓	✓	✓	x	4/5
37	MPS	✓	✓	✓	✓	x	4/5
38	MPP	✓	✓	✓	✓	x	4/5
39	AVM	✓	✓	✓	✓	✓	5/5
40	FGT	✓	✓	x	✓	x	3/5
41	MkR1	✓	x	✓	✓	✓	4/5
42	EKU	✓	✓	✓	✓	x	4/5
43	LMR	✓	✓	✓	✓	x	4/5
44	VEM	✓	✓	✓	✓	✓	5/5
45	MEP	✓	✓	✓	✓	✓	5/5
46	TGB	✓	✓	✓	✓	✓	5/5
47	L7PF	✓	✓	✓	✓	x	4/5
48	MXC	✓	✓	✓	✓	✓	5/5
49	ZMP	✓	✓	✓	✓	x	4/5
50	VGM	✓	✓	✓	✓	✓	5/5
51	SWP	✓	✓	✓	✓	✓	5/5
52	LBP	✓	x	x	✓	x	2/5
53	STL	✓	✓	✓	✓	x	4/5
54	MPP	✓	✓	✓	✓	x	4/5
55	MXC	✓	✓	✓	✓	x	4/5
56	TMT	✓	✓	✓	✓	✓	5/5
57	MP	✓	✓	✓	x	x	3/5
58	LMM	✓	✓	✓	✓	x	4/5
59	AMX	✓	✓	✓	✓	x	4/5
60	YHP	✓	✓	✓	x	x	3/5
61	NMVP	✓	x	x	x	x	1/5
62	MSW	x	x	x	x	x	0/5
63	NHGW	✓	x	x	x	x	1/5
64	NGR	✓	x	x	x	x	1/5
65	MBF	x	x	x	x	x	0/5
66	RHC	x	x	x	x	x	1/5
67	VCPG	x	x	x	x	x	0/5
68	GS	✓	x	✓	x	x	2/5
69	AEF	✓	x	✓	x	x	2/5
70	BS	✓	x	x	x	x	1/5

71	BH	✓	✓	x	x	x	2/5
72	BS	✓	x	x	x	x	1/5
73	BF	✓	✓	x	x	x	2/5
74	SMNP	x	x	x	x	x	0/5
75	MT	x	x	x	x	x	0/5
76	MNP	✓	x	✓	x	x	2/5
77	NSP	✓	x	✓	x	x	2/5
78	MDMP	x	x	x	x	x	0/5
79	OBMV	✓	✓	✓	x	x	3/5
80	HV	x	x	✓	x	x	1/5
81	VB	x	x	✓	x	x	1/5
82	GH	x	x	✓	x	x	1/5
83	SPS	x	x	x	x	x	0/5
84	PA	x	x	x	x	x	0/5
85	TDH	✓	✓	✓	✓	x	4/5
86	AH	✓	✓	✓	x	x	3/5
87	GM	x	x	x	x	x	0/5
88	GA	✓	x	✓	x	x	1/5
89	SB	x	x	✓	✓	x	2/5
90	GHS	x	x	✓	x	x	1/5
91	VM VE	✓	✓	✓	✓	x	5/5
92	MVIP	x	x	✓	x	x	1/5
93	SSMG	x	x	✓	x	x	1/5
94	VPC	x	x	✓	x	x	1/5
95	VV	x	x	✓	x	x	1/5
96	MG	x	x	✓	x	x	1/5
97	BB	x	x	✓	x	x	1/5
98	DEA	✓	✓	✓	x	x	3/5
99	EM	✓	✓	✓	✓	✓	5/5

Source: Health Canada Guidance Document; Labelling of Pharmaceutical Drugs for human use.

Table 2. Sample Labelling Analysis Summary

SIGN for requirements	Manufacturer's Details	Ingredients in the sample	Dosage/ Indications	Date of Manufacture/ expiry	PIL
✓	76	48	79	51	25
x	23	51	20	48	74
TOTAL	99	99	99	99	99

✓ Meets the requirement x Does not meet the requirement
Source: Created for this research

From table 1, out of ninety-nine products, seventy-six (76.77%) met the requirements for manufacturer's details while twenty-three products (23.23%) did not meet this. Forty-eight products (48.48%) met the requirements for ingredients in the sample, while fifty-one (51.51%) did not. Seventy-nine products (79.80%) met the requirement for dosage/indications, while twenty products (20.20%) failed to meet this requirement. Fifty-one products (51.51%) met the requirements for the date of manufacture/expiry, while forty-eight products (48.48%) did not. Finally, only twenty-five products (25.25%) met the requirements for patient information leaflet (PIL), leaving seventy-four products (74.75%) not meeting the PIL requirement. Therefore, the requirements that are met by most products are manufacturer's details, dosage and indications. Seventeen samples (17.17%) met all five requirements, while ten samples (10.10%) did not meet any of the requirements. This leaves seventy-two samples (72.73%) that met at least one of the requirements but not all the five requirements. There is need to formulate a policy to ensure herbal medicine for human use adhere to the labelling requirements for the safety of the patients and proper use of the products.

Discussion

Regulatory compliance and labelling requirements of Medicinal Products (MPs) has been reviewed elsewhere [18,20,21]. MPs should comply with regulatory standards and labelling requirements, but there are no unified guidelines. The guidelines vary from country to country globally [19,20]. The National regulator in Kenya, PPB has established a guideline for labelling of pharmaceutical products for human use and a guidance document for grant of market authorization (MA).

This study revealed that the products surveyed were at varying levels of regulatory compliance and labelling requirements. With regard to regulatory compliance, all the surveyed products had not been granted market authorization by PPB. This poses health risks to the consumers. Therefore, it is recommended that PPB puts in place mechanisms to protect consumers against using non-compliant herbal products.

Seventy-six (76.77%) products met the requirement for manufacturer's details while twenty-three (23.23%) did not meet this requirement. PPB should put in place mechanisms to ensure that all the herbal products in the Kenyan market meet this requirement as stipulated in the PPB labelling guideline. Forty-eight (48.48%) products met the requirement for indication of ingredients on the label while fifty-one (51.51%) products did not; therefore, the bigger percentage did not meet this requirement. PPB should take regulatory action to ensure that this requirement is met for

all the herbal products in the Kenyan market to protect consumers against fraudulent products. Twenty (20.20%) products failed to meet the requirement for indication/ dosage on the label. This poses a health risk to consumers since the products may be taken for the wrong indication and dosage. PPB should take appropriate actions to enforce the requirement. Fifty-one (51.51%) products met the requirement for date of manufacture/date of expiry while forty-eight products (48.48%) did not. This poses a risk of consumers taking expired drugs which may be poisonous and ineffective. PPB should enforce this requirement to protect consumers against using expired herbal products used for the treatment of erectile dysfunction. Finally, twenty-five (25.25%) products met the requirement for PIL leaving seventy-four (74.75%) products non-compliant. PIL is useful in guiding consumers on the indications and dosage of the products, the absence of which may expose consumers to risks such as overdose and wrong indication. PPB should enforce this requirement to protect consumers.

Only seventeen (17.17%) products met all five requirement leaving eighty-three (83.83%) products non-compliant. Ten (10.10%) products did not meet any of the requirements evaluated. This calls for the regulator's action to enforce compliance with all the requirements.

Study limitations

There were some limitations in this study that could be addressed in future research. Foremost, the study focused on labelling and regulatory compliance of herbal medicine used for the treatment of erectile dysfunction in Nairobi County. Nairobi being the capital city of Kenya, the erectile dysfunction products surveyed may not entirely be the same as those available in rural-urban areas. Most of the rural-urban areas are likely to stock more of the locally manufactured products, while Nairobi has both locally manufactured and imported products. The labelling and regulatory compliance are likely to be different; hence information obtained might not be applicable for generalization to the entire Kenyan market. In future studies, the study site should include rural-urban areas all over Kenya to give more inclusive information. Secondly, Nairobi County was chosen for the study due to the high concentration of healthcare outlets. Several outlets could not be surveyed as they operated 'clandestinely' by hiding the products underground while they had space in the front street where they met the clients. Such products are more likely to be non-compliant with labelling and regulatory requirements. A method should be devised to include such products in future studies. Lastly, sample identification was solely done by the researcher; consequently, there is always the fear of sample bias which the researcher acknowledges could have been caused by the need to balance samples from different sampling blocks within Nairobi County. Nonetheless, all sample collection procedures were done consistently among all sampling blocks.

Conclusion

The study revealed that the herbal products used for the treatment of erectile dysfunction in Nairobi County did not meet regulatory requirements since all of them were not granted marketing authorization by PPB. A very small percentage (17.17%) of the products met all the five labelling requirements evaluated leaving, 83.83% of the products non-compliant. Furthermore, 10 (10.10%) products did not meet any of the five labelling requirements evaluated. There is need for PPB to enforce the regulatory and labelling requirements to protect the consumers from health risks associated with these herbal products. Consumer education is also necessary to further curb the circulation of non-compliant herbal products in the Kenyan market.

Abbreviations

PPB - Pharmacy and Poisons Board; WHO - World Health Organization; HMPs - Herbal Medicinal Products; TM - Traditional Medicines; CBD - Central Business District; CAM - Complementary Alternative Medicine; ACT - Artemisinin Combination Therapy; PIL - Patient Information Leaflet; OTC - Over the Counter; ED - Erectile Dysfunction, TGA - Therapeutic Goods Authority.

Declarations

Availability of data and materials.

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The ethical approval was obtained from the Institutional Review Committee of Kenyatta National Hospital.

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Authors' contributions

*Shabaan W. A Sifuma conceptualized the study, did data collection, performed analysis, interpreted data and drafted the manuscript. The rest of the authors reviewed the research work. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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