Phytochemical Composition and Antioxidant Activities of Phosphate Buffered Saline and Aqueous Extracts of *Aloe barbadensis* Miller Leaf Latex and Gel from Three Counties of Kenya

ABSTRACT

Aims: *Aloe barbadensis* Miller (*A. barbadensis*) is one of the most treasured species from the *Aloe* genus used in management of various ailments. However, there are few reports on the secondary metabolites, total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activities of its leaf latex and gel. This study aimed at comparing the phytochemicals and antioxidant activity of phosphate buffered saline (PBS) and distilled water extracts of latex and gel of *A. barbadensis* leaves from Kisumu, Elgeyo Marakwet and Baringo Counties of Kenya.
Study design: The study employed quantitative and qualitative research designs.
Place and Duration of Study: This study was conducted at the Department of Chemistry and Biochemistry, School of Sciences and Aerospace Studies, Moi University, Eldoret, Kenya between May 2021 and June 2021.
Methodology: Leaf samples were extracted by maceration using distilled water and PBS. Phytochemical screening was performed following standard screening procures while TPC, TFC and antioxidant activity were determined using Folin-Ciocalteau method, Aluminium Chloride assay and DPPH radical scavenging assay, respectively. One-way analysis of variance (ANOVA) was performed with Tukey post hoc test at $p < 0.05$. Correlations among TPC, TFC and antioxidant activity of the extracts were assessed using Pearson's bivariate correlation. The analyses were performed using GraphPad Prism for windows (v9.0, GraphPad Software, California, USA).
Results: Phytochemical screening results indicated the presence of alkaloids, phenols, saponins, flavonoids, cardiac glycosides, tannins, steroids, terpenes and quinones as the main secondary metabolites in the extracts. TPC and TFC were found to be highest for the aqueous extracts, with the highest contents (102.393 ± 0.121 mg GAE/ g DW and 47.228 ± 0.248 mg QE / g DW) being for dry latex of leaves from Baringo. The highest antioxidant activity (IC_{50} = 21.900 ± 0.0594 mg/mL) was for aqueous extract of fresh latex of leaves from Baringo, followed by those from Elgeyo Marakwet and then Kisumu.
Conclusion: This study established that dry and fresh latex and gel extracts of *A. barbadensis* leaves possesses therapeutic phytochemicals with antioxidant activities, which support their use in traditional phytomedicine in Kenya.

Keywords: Total polyphenols, Aloe vera, DPPH assay, secondary metabolites.

1. INTRODUCTION

Plants have remained a veritable source of medicine used in both traditional and modern medicine [1]. This is particularly evident in developing countries where there is limited access to health services, chronic poverty and the general belief that herbal medicines are not only efficacious and safe but also affordable, available and culturally acceptable [2]. Further, natural products have been the source of most known commercialized drugs. At least 25% of the known drugs used globally are derived from plants. Over 11% of the 250

basic and essential drugs used globally were reported to be from plants. Some important drugs derived from plants include antitumor drugs vincristine and vinblastine from *Catharanthus roseus*, morphine and codeine from *Papaver somniferum*, digoxin from *Digitalis* species, quinine and quinidine from *Cinchona* species and atropine from *Atropa belladonna* [3, 4]. The complete trust in natural products of plant origin is because they possess unique features vis-à-vis synthetic molecules which confers both advantages and challenges for the drug discovery process [5]. Thus, ethnobotanical surveys and scientific validation studies have been conducted on medicinal flora worldwide in the past decades, with the hope that novel therapeutic molecules will be developed from natural biodiversity in close association with leads furnished by traditional knowledge and experiences [6].

The genus *Aloe* L. (Asphodelaceae) encompasses at least 548 known species [7, 8]. It houses some of the most cultivated medicinal plants for both traditional and industrial (commercial) uses [9, 10]. The botanical name *Aloe vera* derives from "*Allaeh*" (Arabic for shining bitter substances) and *vera* (the Latin word for true) [11, 12]. *Aloe barbadensis* Miller (synonym: *Aloe vera*), *A. arborescens* and *A. chinensis* are the most common *Aloe* species worldwide [13].

Aloe barbadensis is a cactus-like perennial herb of family Liliaceae (sub-family of Asphodelaceae). It is native to the Arabian peninsula and North Africa but has been extensively distributed in hot and dry areas of the world where it is considered an invasive species [11, 14, 15]. Its turgid green leaves joined at the stem in a rosette pattern is the most used part [16]. *Aloe barbadensis* is considered to be the most bioactive *Aloe* [15] as evidenced by the latex and mucilaginous gel from its leaves being widely used in traditional medicine as well as pharmaceutical, cosmetic and food industries [7, 11].

Kenya belongs to the East African botanical plate and is known for her rich biodiversity of *Aloes* [8, 17-20]. Historically, *A. barbadensis* leaves have been used in Kenya for phytotherapeutical management of malaria [21, 22]. Though *Aloe* species have been shown to elicit therapeutic effects including antimalarial, anticancer, antioxidant, antidiabetic and antihyperlipidemic properties [10], there is paucity of information on the phytochemicals and the pharmacological profile of *A. barbadensis* used in Kenyan phytomedicine [23]. This study therefore aimed at determining the phytochemicals and antioxidant activity of latex and gel extracts of *A. barbadensis* leaves from Kisumu, Elgeyo Marakwet and Baringo Counties of Kenya.

2. MATERIAL AND METHODS

2.1 Chemicals and reagents

The chemicals and reagents used in this study were of high analytical purity, supplied by Merck and Sigma Aldrich.

2.2 Collection, authentication and preparation of samples

Mature *A. barbadensis* leaves were collected from plants in their natural habitats in Kisumu, Elgeyo Marakwet and Baringo Counties, representing the hot, moderately hot and cold regions of Kenya, respectively (**Table 1**). The samples were collected between May 2021 and June 2021. They were identified and authenticated by Tepeny Taabu Too, a taxonomist at the Department of Biological Sciences (School of Sciences and Aerospace Studies), Moi University, Kenya where voucher samples (CKN 010521, CKN 020521, CKN 030521) were deposited. Voucher samples were also deposited at the Herbarium of University of Eldoret, Eldoret, Kenya for future reference.

Table 1: Sampling location of *A. barbadensis* plants where leaves were sampled in Kenya

County	Sampling site	Geographical position system location
Kisumu	Katho (Upper Bwanda)	0^{o}11”44.5” S 34^{o}51”46.8” E
Elgeyo-Marakwet	Birweto	0^{o} 32”34.32N 35’26.17”E
Baringo	Timboroa	0^{o}18’59.3N 35^{o} 20’05.6”E

Extraction was done according to the method used by Sánchez-Machado, López-Cervantes [24]. Fresh latex, fresh gel, dry latex and dry gel were extracted and stored separately. The leaf latex of *A. barbadensis* was obtained by cutting the leaves transversally near the base using a knife and arranging them concentrically. They were drained for 30 minutes into a clean beaker. Then the collected latex was left in open air for 3 days to allow complete evaporation of water [24, 25].

The resulting leaves were washed under running tap water and then distilled water. They were surface sterilized with 10% sodium hypochlorite to prevent microbial contamination. Fresh gel from the leaf skin were removed and the inner and middle layers were scraped [26] and homogenized in a blender. For dry samples, fresh gel was dried at 40 °C for 24 hours in an oven while the fresh latex was dried at room temperature (25 ℃) for 24 hours. The dried samples from both gel and latex were separately extracted. The crude extracts thus obtained were kept at 4 °C in a refrigerator until commencement of analysis [24].

Measured 5 mg of dry latex were dissolved in PBS at pH 3 and topped up to the 5 mL mark. A total of 50 mg of dry gel was prepared by dissolution in PBS to make a 10 mL solution. All samples were sonicated on a digital Ultrasonic Cleaner (LMUC Series, Model–USBT-6-Liters) for 10 minutes and filtered through 0.45 µm pore size nylon membrane filters [24, 27]. The extraction process was replicated twice. All the extracts obtained were freeze dried using a FD5-serries freeze dryer (MRC Scientific Instruments). The yields of the extracts were computed using **Equation 1.**

$$\text{Percentage yield} = \frac{A}{A_o} \times 100 \qquad (2)$$

From which *A* denotes the mass of crude extract obtained after drying and A_o denotes the mass of the leaves used for extraction.

2.3 Classical phytochemical screening of *A. barbadensis* leaf extracts

The different solvent extracts were dissolved in their respective solvents of extraction (1:10, w/v) and subjected to phytochemical screening following the standard screening procedures of Trease and Evans [28]. The secondary metabolites screened for included phenols, saponins, flavonoids, anthraquinones, cardiac glycosides, alkaloids, quinones, glycosides, steroids, sterols, tannins and terpenes and coumarins. The screening results were reported in terms of the relative abundance of the secondary metabolites identified based on colour or foaming intensities [29-31].

2.4 Determination of total phenolic and flavonoid contents and *in vitro* antioxidant activity of the extracts

2.4.1 Total phenolic content

The total phenolic content (TPC) of *A. barbadensis* extracts were determined using the Folin-Ciocalteau method [32] with some modifications [31]. Briefly, dry extracts (0.01 g) were dissolved in 10 mL of distilled water and used to prepared 0.01 mg/mL solutions. Measured 0.5 mL of these extract solutions were mixed with 2.5 mL of Folin-Ciocalteau reagent in test tubes. After 7 minutes, exactly 2.5 mL of 6% sodium carbonate solution was added to the solutions and then incubated in the dark at room temperature (25.0 ± 2.0 ℃) for 30 minutes. The absorbances of the solutions were measured at 725 nm on a general-purpose UV-Visible spectrophotometer (Beckham Coulter DU 720, Beckham Coulter Inc., USA).

A calibration curve was constructed using various concentrations of gallic acid ranging from 20 to 100 mg/mL for quantification of TPC in the extracts. The gallic acid solutions received the same treatments as the extracts. The TPC of the extracts were estimated in mg gallic acid equivalents per gram of dry weight (mg GAE/g DW) from the gallic standard calibration curve [31].

2.4.2 Total flavonoid content

Determination of total flavonoid content (TFC) of *A. barbadensis* leaf extracts was achieved using aluminium chloride colorimetric assay as used by Pękal and Pyrzynska [33] with some modifications [31]. Briefly, the extracts (0.01 g) were dissolved in 5 mL of methanol. Different concentrations of methanolic standard quercetin solutions (5, 10, 25, 50, 75 and 100 ppm) were prepared. Measured 0.6 mL of the quercetin solutions and the extracts were separately mixed with 0.6 mL of 2% methanolic aluminium chloride solution in test tubes. The resultant solutions were incubated for 60 minutes at room temperature. Their absorbances were measured against methanol blank at 420 nm on a UV-Vis spectrometer. A calibration curve for TFC quantification was constructed using quercetin absorbances. The TFC of the extracts were determined in mg quercetin equivalents per gram dry weight (mg QE / g DW) of the extracts.

2.4.3 Total *in vitro* antioxidant activity

The antioxidant potential of *A. barbadensis* leaf extracts were assessed using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical scavenging assay as described by Khiya et al. [34] with some specific modifications [31]. The extracts were dissolved in methanol to produce concentrations of 10, 15, 25, 50 and 60 µg/mL. Measured 75 µL of freshly prepared methanolic DPPH solution (1.3 mg/mL) was then added to 200 µL of the extracts and incubated in the dark for 30 minutes. Ascorbic acid was used as the standard but was dissolved in distilled water to make solutions with the same concentrations as that of the extracts. The absorbances of the assay mixtures were read at 517 nm on a UV-Vis spectrophotometer. The percentage DPPH radical inhibition was computed using **Equation 2**. The half-inhibitory concentration (IC_{50}) was determined from the inhibition percentage curve as a function of the extract concentrations [34].

$$\text{Percentage inhibition} = \frac{A_0 - A_S}{A_0} \times 100 \qquad (2)$$

From which A_o = absorbance of the solution containing only DPPH radical solution as a negative control and A_S = absorbance of the sample solution in the presence of DPPH.

2.5 Statistical analysis of results

Quantitative data obtained from triplicate analyses were expressed as means ± standard deviations of replicates. To identify any significant differences between means, one-way analysis of variance (ANOVA) was performed with Tukey post hoc test at $p < 0.05$. Correlations among TPC, TFC and antioxidant activity of the extracts were assessed using Pearson's bivariate correlation. The analyses were performed using GraphPad Prism for windows (v9.0, GraphPad Software, California, USA).

3. RESULTS

3.1 Percentage yield of *A. barbadensis* leaves

The percentage yields (**Fig. 1**) were expressed as percentages of the initial mass of *A. barbadensis* leaf latex and gel extracted. Extraction using PBS recorded higher yields in comparison to distilled water with PBS extract of dry latex of *A. barbadensis* leaves from Kisumu having the highest average percentage yield of 78.4%. One-Way ANOVA indicated that the choice of the extraction solvent had no significant effect on the percentage yields ($P = .5176$).

3.2 Classical phytochemical screening results

Phytochemical screening of ethnomedicinal plants forms the basis for their further pharmacological evaluation and phytochemical investigation [35]. The results of screening extracts of *A. barbadensis* leaf latexes and gels revealed the presence of various secondary metabolites including alkaloids, phenols, saponins, flavonoids, cardiac glycosides, tannins, steroids, terpenes and quinones as shown in **Table 2**. Extracts from Elgeyo Marakwet had all the secondary metabolites screened. However, alkaloids were absent in aqueous extract of fresh gel from Baringo and PBS extracts of dry gel and dry latex from Kisumu. Anthraquinones were also not detected in aqueous and PBS extracts of fresh gel and PBS extract of dry gel from Kisumu. Other secondary metabolites that were absent in extracts from Kisumu included phenols, quinones and cardiac glycosides (in PBS extract of fresh and dried gels), terpenoids, steroids, sterols and glycosides (in PBS and aqueous extracts of fresh and dried gels) and tannins in PBS extract of fresh gel.

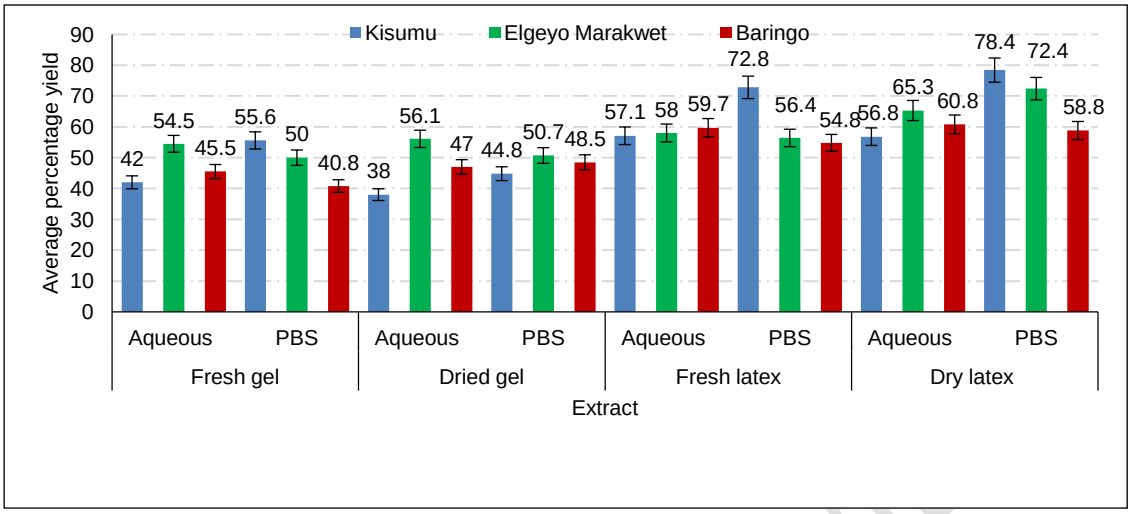

Fig. 1. Average percentage yield of different solvent extracts of A. barbadensis leaf latex and gel from the selected regions of Kenya.

Table 2: Secondary metabolites identified in *A. barbadensis* leaves from Kenya.

Phytochemicals	Part used	Extract	Kisumu	Elgeyo Marakwet	Baringo
Alkaloids	Fresh gel	Aqueous	++	++	-
		PBS	+++	+++	+++
	Dried gel	Aqueous	+++	+++	+++
		PBS	-	++	+++
	Fresh latex	Aqueous	+++	+++	+++
		PBS	++	++	++
	Dry latex	Aqueous	+++	+++	+++
		PBS	-	+	++
Anthraquinones	Fresh gel	Aqueous	-	+	++
		PBS	-	+	++
	Dried gel	Aqueous	++	+++	+++
		PBS	-	+	++
	Fresh latex	Aqueous	+++	+++	+++
		PBS	+++	+++	+++
	Dry latex	Aqueous	+++	+++	+++
		PBS	+	++	++
Flavonoids	Fresh gel	Aqueous	+	++	++
		PBS	+	++	++
	Dried gel	Aqueous	+++	+++	+++
		PBS	+	++	++
	Fresh latex	Aqueous	++	+++	+++
		PBS	++	+++	++
	Dry latex	Aqueous	+++	+++	+++
		PBS	+++	+++	+++
Phenols	Fresh gel	Aqueous	+	++	++
		PBS	-	++	++
	Dried gel	Aqueous	+++	+++	+++

Phytochemicals	Part used	Extract	Kisumu	Elgeyo Marakwet	Baringo
		PBS	++	++	+++
	Fresh latex	Aqueous	+++	+++	+++
		PBS	+++	+++	+++
	Dry latex	Aqueous	+++	+++	+++
		PBS	+++	+++	+++
Quinones	Fresh gel	Aqueous	+	++	++
		PBS	-	+	+
	Dried gel	Aqueous	+	++	+++
		PBS	-	+	++
	Fresh latex	Aqueous	+++	+++	+++
		PBS	+++	++	++
	Dry latex	Aqueous	+++	++	+++
		PBS	++	+	++
Saponins	Fresh gel	Aqueous	+++	+++	+++
		PBS	++	+++	++
	Dried gel	Aqueous	+	+++	+++
		PBS	+	++	++
	Fresh latex	Aqueous	+++	++	+++
		PBS	+++	+++	++
	Dry latex	Aqueous	+++	+++	+++
		PBS	+++	+++	+++
Steroids	Fresh gel	Aqueous	-	+	+
		PBS	-	++	++
	Dried gel	Aqueous	++	+++	++
		PBS	-	++	+
	Fresh latex	Aqueous	+++	+++	++
		PBS	++	++	++
	Dry latex	Aqueous	+++	++	+++
		PBS	+	+	++
Tannins	Fresh gel	Aqueous	+	++	++
		PBS	-	++	++
	Dried gel	Aqueous	+++	+++	+++
		PBS	++	++	+++
	Fresh latex	Aqueous	+++	+++	+++
		PBS	+++	+++	+++
	Dry latex	Aqueous	+++	+++	+++
		PBS	+++	+++	+++
Terpenoids	Fresh gel	Aqueous	-	++	++
		PBS	-	++	++
	Dried gel	Aqueous	++	++	+++
		PBS	+	+++	++
	Fresh latex	Aqueous	+++	+++	++
		PBS	+++	+++	++
	Dry latex	Aqueous	+++	+++	+++
		PBS	+	++	+++
Cardiac glycosides	Fresh gel	Aqueous	++	-	++
		PBS	-	+	++
	Dried gel	Aqueous	+++	+	+++

Phytochemicals	Part used	Extract	Kisumu	Elgeyo Marakwet	Baringo
		PBS	-	++	++
	Fresh latex	Aqueous	+++	+++	+++
		PBS	+++	+	++
	Dry latex	Aqueous	+++	+++	+++
		PBS	+	++	++
Sterols	Fresh gel	Aqueous	-	+	++
		PBS	-	-	+
	Dried gel	Aqueous	+++	++	++
		PBS	+	++	+
	Fresh latex	Aqueous	+++	++	+++
		PBS	+++	++	+++
	Dry latex	Aqueous	+++	++	++
		PBS	+	++	++
Coumarins	Fresh gel	Aqueous	+	++	+
		PBS	+	+	+
	Dried gel	Aqueous	+++	+++	++
		PBS	+++	+++	++
	Fresh latex	Aqueous	++	+	++
		PBS	+++	++	++
	Dry latex	Aqueous	+++	+++	+++
		PBS	+++	+++	+++
Glycosides	Fresh gel	Aqueous	-	++	++
		PBS	-	++	++
	Dried gel	Aqueous	++	++	+++
		PBS	+	++	++
	Fresh latex	Aqueous	++	+++	++
		PBS	++	++	++
	Dry latex	Aqueous	+++	+++	+++
		PBS	++	++	++

Where: +++ represents abundant, ++ represents moderate, represents traces, and – represents absent. The quantifications are based on colour/foam intensities observed [29-31].

3.3 Total phenolic content, total flavonoid content and *in vitro* antioxidant activity of the *A. barbadensis* extracts

3.3.1 Total phenolic content

The TPC of *A. barbadensis* extracts were determined using the Folin-Ciocalteau method. A calibration curve was prepared for quantitative analysis and the linearity for gallic acid standard was established from 10 ppm to 80 ppm which was fitted on the line $y = 0.0117x + 0.054$ with $R^2 = 0.9941$. As depicted in **Table 3**, TPC was highest for aqueous extracts, with extracts of dry latex from Baringo recording the highest TPC of 101.72 ± 0.22 mg GAE / g DW. Extracts of *A. barbadensis* leaf latex and gel from Baringo had the highest TPC followed by Elgeyo Marakwet and lowest in leaves from Kisumu. One-Way ANOVA results showed that there were no significant differences (P = .1672) between the mean TPC of the different solvent extracts of *A. barbadensis* leaves latexes and gels from the different Counties of Kenya.

Table 3: TPC of the *A. barbadensis* leaf latex and gel from the selected Counties of Kenya

County	Part used	Extract	Total phenolic content (mg GAE / g DW)
Kisumu	Fresh gel	Aqueous	1.026 ± 0.252
		PBS	9.373 ± 0.176
	Dried gel	Aqueous	8.575 ± 0.201
		PBS	2.593 ± 0.827
	Fresh latex	Aqueous	9.573 ± 0.185
		PBS	12.108 ± 0.224
	Dry latex	Aqueous	39.031 ± 0.315
		PBS	19.801 ± 0.176
Elgeyo Marakwet	Fresh gel	Aqueous	4.957 ± 0.604
		PBS	2.137 ± 0.230
	Dried gel	Aqueous	18.775 ± 0.213
		PBS	6.581 ± 0.140
	Fresh latex	Aqueous	28.148 ± 0.0403
		PBS	20.712 ± 0.384
	Dry latex	Aqueous	90.598 ± 0.849
		PBS	45.185 ± 0.542
Baringo	Fresh gel	Aqueous	5.328 ± 0.040
		PBS	2.992 ± 0.252
	Dried gel	Aqueous	10.114 ± 0.464
		PBS	6.724 ± 0.107
	Fresh latex	Aqueous	55.385 ± 0.304
		PBS	71.937 ± 0.029
	Dry latex	Aqueous	102.393 ± 0.121
		PBS	80.969 ± 0.040

Values are means ± standard deviations of triplicates.

3.3.2 Total flavonoid content

Aluminium chloride colorimetric assay was used for determining the TFC of the extracts. The assay is based on the formation of aluminium-flavonoid complexes [33]. A calibration curve prepared using quercetin standard was used in TFC quantification. Linearity of the standard was established from 5 ppm to 100 ppm which was fitted on a straight line that gave the equation $y = 0.0109x + 0.0851$ with $R^2 = 0.9987$. The mean TFC of the extracts was highest for aqueous extracts, with the highest content of 47.228 ± 0.248 mg QE / g DW being for dry latex of leaves from Baringo County (**Table 4**). One-Way ANOVA results indicated that there were no significant differences ($p = 0.223$) between the average TFC of the different extracts. Further, the TFC obtained for the extracts were lower than their corresponding mean TPC values.

Table 4: Total flavonoid content of *A. barbadensis* leaves from selected region of Kenya

County	Part used	Extract	Total flavonoid content (mg QE / g DW)
Kisumu	Fresh gel	Aqueous	4.684 ± 0.263
		PBS	1.702 ±0.150
	Dried gel	Aqueous	4.246 ±0.152
		PBS	2.404 ± 0.328
	Fresh latex	Aqueous	22.491± 0.402
		PBS	20.912 ± 0.248
	Dry latex	Aqueous	10.211 ± 0.263
		PBS	15.474 ± 0.212
Elgeyo Marakwet	Fresh gel	Aqueous	6.877 ± 0.124
		PBS	9.333 ± 0.445
	Dried gel	Aqueous	10.035 ± 0.447
		PBS	6.450 ± 0.329
	Fresh latex	Aqueous	35.386 ± 0.248
		PBS	37.561 ± 1.124
	Dry latex	Aqueous	24.965 ± 0.328
		PBS	22.246 ± 0.328
Baringo	Fresh gel	Aqueous	7.755 ± 0.328
		PBS	9.684 ± 0.213
	Dried gel	Aqueous	6.439 ± 0.328

County	Part used	Extract	Total flavonoid content (mg QE / g DW)
		PBS	9.684 ± 0.214
	Fresh latex	Aqueous	47.228 ± 0.248
		PBS	29.333 ± 0.124
	Dry latex	Aqueous	33.194 ± 0.155
		PBS	19.947 ± 0.215

Results are presented as means ± standard deviations of triplicates.

3.3.3 Free radical scavenging activity

The *in vitro* antioxidant potential of the *A. barbadensis* leaves latex and gels was established using the DPPH assay, which measured the hydrogen atom or electron donating capacity of the extracts to the stable radical DPPH formed in solution [36]. The lowest minimum inhibitory concentration, IC_{50} (21.900 ± 0.0594 mg/mL) was recorded for aqueous extract of fresh latex of leaves of *A. barbadensis* from Baringo (**Table 5**). This IC_{50} values was lower than that of ascorbic acid (22.113 ± 0.230 mg/mL), implying that it is a better antioxidant than the standard used.

Table 5: Minimum inhibitory concentration of *A. barbadensis* latex and gels from Kenya

County	Part used	Extract	IC_{50} (mg/mL)
Kisumu	Fresh gel	Aqueous	420.875 ± 0.0184
		PBS	651.644 ± 0.019
	Dried gel	Aqueous	324.675 ± 0.037
		PBS	46.500 ± 0.017
	Fresh latex	Aqueous	26.392 ± 0.01
		PBS	30.166 ± 0.141
	Dry latex	Aqueous	32.045 ± 0.112
		PBS	38.140 ± 0.127
Elgeyo Marakwet	Fresh gel	Aqueous	168.600 ± 0.024
		PBS	221.043 ± 0.1340
	Dried gel	Aqueous	110.156 ± 0.033
		PBS	152.341 ± 0.032
	Fresh latex	Aqueous	23.551 ± 0.084
		PBS	28.301 ± 0.0547
	Dry latex	Aqueous	25.018 ± 0.066
		PBS	31.568 ± 0.041
Baringo	Fresh gel	Aqueous	143.242 ± 3.100
		PBS	324.486 ± 0.039
	Dried gel	Aqueous	174.685 ± 0.048
		PBS	208.481 ± 3.951
	Fresh latex	Aqueous	21.900 ± 0.0594
		PBS	30.546 ± 0.0247
	Dry latex	Aqueous	23.638 ± 0.059
		PBS	28.954 ± 0.055

There were insignificant differences (P = .432) between the IC_{50} values of aqueous and PBS extracts of *A. barbadensis* leaf latexes and gels from the three Counties of Kenya.

3.3.4 Correlation between total phenolic content, total flavonoid content and *in vitro* antioxidant activity (IC_{50}) of *A. barbadensis* extracts

Pearson's bivariate correlation indicated that there is a positive correlation (R = 0.6302, P = .001) between the TPC and TFC of *A. barbadensis* extracts (**Table 6**). Pearson's bivariate correlation coefficient between TPC and antioxidant activity of the extracts revealed that TPC exhibited a negative correlation with the IC_{50} values obtained in the DPPH assay (R = − 0.4905, P = .0149). Similarly, TFC negatively and highly correlated (R = − 0.6197, P = .0012) with IC_{50} values obtained in DPPH assay.

Table 6. Pearson's correlation coefficients for TPC, TFC and antioxidant activity (IC_{50}) of the *A. barbadensis* extracts

Parameter	Total flavonoid content	Antioxidant activity
Total phenolic content	0.6302 (P = .001)**	-0.4905 (P = .0149)*
Total flavonoid content	1.000	− 0.6197 (P = .0012)*

*Correlation is significant at P < 0.05, **Correlation is also significant at P < 0.01.

4. DISCUSSION

4.1 Extraction yield

Considering the yields obtained, it can be inferred that PBS is a good solvent for extraction of phytochemicals in *A. barbadensis* latexes and gels, corroborating a previous observation by Muñoz et al. [37] in Chile where a high percentage yield of 64.03% was recorded in gel filet of *A. barbadensis* leaves. Earlier, Mpala et al. [38] had reported a yield of 7.82% for *A. barbadensis* leaves extracted using methanol. Another study reported a yield of 66.67% for aqueous *A. barbadensis* leaf skin extracts from Kairouan, Tunisia [16]. Differences in solvent polarity influences the solubility of phytochemicals from plant matrices [39, 40]. In addition, disparities in the functional groups of phytochemicals, time, temperature and solvent concentration also influences the solubility of phytochemicals in organic or polar solvents, or solvents of different polarities [41]. These could on the one hand, explain the differences in the yields of the latexes and gels of *A. barbadensis* from the different regions of Kenya.

Among the three Counties, the yield for PBS extract of *A. barbadensis* leaf latexes from Kisumu were nearly 1.5 times higher than that of aqueous extracts of both the gel and latex despite using the same method of extraction. For Elgeyo Marakwet and Baringo Counties, the PBS extract of the dry latex and aqueous extract of the dry latex had the highest average percentage yields of 72.4% and 60.8%, respectively. These intraspecific variation in the percentage yields of the extracts of *A. barbadensis* leaf latexes and gels could have been

due to extrinsic factors. For example, variations in soils, topography and climate which have been reported to cause variations in the yield of plant organs [31, 42-44].

Kisumu for instance has a tropical rainforest climate by virtue of its location along the equator and in the province of Nyanza on Lake Victoria [45]. Kisumu has two distinct rainy seasons: from March through June, and then November through December [46, 47]. It however receives a significant quantity of rainfall all year round, even in the driest months. The temperature in Kisumu averages at 23.1 °C, with rainfall of about 1966 mm per year [48]. Morning humidity levels in Kisumu ranges between 80 to 90% and drops in the evening to between 40% to 50%. The soils in this area is eutric cambisol [49, 50] i.e. dark, reddish brown friable sandy-clay-loam underlain by gravely red loam to light clay. The soil is well drained, with good physical properties but are slightly acid [51].

Elgeyo Marakwet on the other hand has three distinct topographical (agroecological) zones: the highlands, the escarpment and the valley (semi-arid) [52]. The temperatures range from 12 °C (extremely cold with mists and even frost to some extent) to 35 °C [53]. Rainfall ranges annually from 800 mm in the valley to 2300 mm in the escarpment [54]. The samples used in this study were taken from the valley, which is dry with sandy soils that supports growth of only drought-resistant crops [55].

Baringo County is divided into three major ecological zones: The Highlands, the mid and the Lowlands [56]. The Highlands are in the modified tropical zones with soils that are generally well drained and fertile [57]. The Lowland is a semi-arid area with complex soils. Thus, the variations in altitudes result into annual rainfall disparities (between 1000–1500 mm in the Highlands to 600 mm in the Lowlands). Temperature in the region varies from 10°C to 35°C, resulting into humid climate in the Highlands to arid climate in the Lowlands [56-59]. The samples used in this study were taken from the cold highlands.

Thus, the highest yields obtained for leaf latexes and gels from Kisumu could possibly be explained by the fertile soils in the County than in the other two counties. The County is also the hottest of the three studied regions of Kenya. Previous authors have reported that plant materials sampled from plants growing in hot areas (at lower altitudes) usually have higher extraction yields than those from cold regions [42, 60].

4.2 Phytochemical screening results

The identified secondary metabolites such as alkaloids, saponins, flavonoids, glycosides and tannins have been previously identified in *A. barbadensis* leaf gel and concentrate [13, 61-63], leaf extracts [64-67] and leaf skin extracts [16]. Moreover, the identified metabolites have been also detected in aqueous leaf extracts of other *Aloe* species such as *Aloe ferox* Mill. [68] and *A. turkanensis* [69]. The variations in secondary metabolites in *A. barbadensis* leaves could be attributed to the variations in soil fertility, rainfall, topography and climate that are reported to influence plant-environment interactions, and subsequently, the composition of secondary metabolites in plant organs [31, 70, 71] as discussed for the percentage yields obtained in this study.

4.3 Total polyphenolic content of the extracts

The TPC was highest for aqueous extracts, with extracts of dry latex from Baringo recording the highest TPC of 101.72 ± 0.22 mg GAE / g DW (**Table 3**). In comparison to extracts from Kisumu and Marakwet, all extracts of *A. barbadensis* leaf latex and gel from Baringo had the highest TPC followed by Elgeyo Marakwet and lowest in leaves from Kisumu. This could be

because of the relatively fertile soils in the County and the very cold temperatures experienced in the Highlands where the samples were drawn. Earlier reports [31, 72, 73] has emphasized that cold climates increase the production of phenolic compounds as a plant defense mechanism against environmental stress.

One-Way ANOVA results showed that there were no significant differences (P = .1672) between the mean TPC of the different solvent extracts of *A. barbadensis* leaves latexes and gels from the different Counties of Kenya. The relatively higher TPC of aqueous extracts could be because water is a polar protic solvent. As such, it is capable of extracting more polyphenols which are inherently polar through hydrogen bond formation [74, 75].

Previous studies indicated that *A. barbadensis* is rich in phenolic compounds [76-78] with a high content of 1,8-dihydroxyanthraquinone derivatives (aloe emodin) and glycosides (aloins) which are used as cathartic [79]. Kammoun et al. [16] reported a TPC of 2.072 ± 0.002 mg GAE/g of extract for *A. barbadensis* leaf skin extracts from Tunisia. A recent report [64] indicated TPC of 30.53 ± 0.30 mg GAE/g DW and 14.29 ± 0.44 mg GAE/g DW for methanolic and ethanolic extracts of *A. barbadensis* leaf extracts, which are comparable to some of the TPC reported in extracts of latex and gel of *A. barbadensis* leaves in this study.

The mean TFC of the extracts was highest for aqueous extracts (**Table 4**) though the differences were not significant. In addition, TFC obtained for the extracts were lower than their corresponding mean TPC values. High TPC of plant extracts than TFC is usually because most flavonoids are also phenolics [80]. The low levels of total flavonoids in the extracts also agreed with some of the phytochemical screening results (**Table 2**) which indicated that there were only traces of flavonoids in PBS extracts of fresh and dry gels from Kisumu. These results also indicate that most of flavonoids in *A. barbadensis* leaves latex and gels are polar as they were able to get extracted more by distilled water than PBS. A recent report by Bista, Ghimire [64] indicated comparatively higher TFC of 73.26 ± 2.46 mg RE/g DW and 54.95 ± 4.15 mg RE/g DW for methanolic and ethanolic extracts of *A. barbadensis* leaf extracts than obtained in this study. This could be due to the differences in the solvents used. For example, water is reported to extract even non-bioactive compounds (such as proteins and sugars) in plant leaves which do not usually contribute to the TFC and some biological activities of plant extracts [31, 39]. As explained for the TPC of the extracts, the relatively higher TFC of the extracts of leaves from Baringo could be explained by the cold climate experienced in the County and the variations in seasons in which the sampling was done [81].

4.4 Antioxidant activity of the extracts

There were insignificant differences (P = .432) The insignificant differences between the IC_{50} values of aqueous and PBS extracts of *A. barbadensis* leaf latexes and gels from the three Counties of Kenya could be possibly due to geographical, soil, climate and genetic variations experienced in the different regions chosen for the study [31, 42, 70, 71]. The high antioxidant activity of aqueous extracts could be because most phenolic compounds which accounts for antioxidant activity of plant extracts possess polar functional groups and are readily dissolved by polar solvents like water through hydrogen bond formation [82]. A preceding study by Kammoun, Miladi [16] reported an IC_{50} value of 2.072 ± 0.002 >1 mg/mL for aqueous *A. barbadensis* leaf skin extracts, which was lower than the radical scavenging activity of chloroform/ethanol (1/1) extract (IC_{50} = 0.274 mg/mL), ethyl acetate extract (IC_{50} = 0.326 mg/mL) and hexane extract (IC_{50} = 0.366 mg/mL). A previous study [63] reported

DPPH inhibition of 83.00 ± 0.05% and 81.00 ± 0.08% for *A. barbadensis* leaf extract and gel, respectively at a concentration of 100 µg/mL. Another report [37] recorded 46.67 ± 0.35% and 36.69 ± 1.60% DPPH inhibition by fresh and lyophilized extracts of *A. barbadensis* leaf gel at 680 µg/mL and 678 µg/mL, respectively. A recent investigation [64] reported 81.91 ± 0.04% DPPH inhibition by 99.5% methanolic extracts of *A. barbadensis* leaves. The current antioxidant activity report suggests that the aqueous extracts of this species have potential role in reducing oxidative stress, and the incidences of oxidative-stress mediated complications.

4.5 Correlation between total polyphenolic content and antioxidant activity of the *A. barbadensis* extracts

Positive correlation between the TPC and TFC of *A. barbadensis* extracts (**Table 6**) is because total phenolic compounds comprises of both flavonoids and non-flavonoid polyphenols [30]. Hence, a positive correlation depicts that TFC of the extracts contributes significantly to the TPC of the extracts [31, 83]. On the other hand, the negative relationships between IC_{50} values of DPPH radical quenching activity, TPC and TFC are explained by the fact that the radical content of DPPH bears inverse relationship with TPC and TFC i.e. the DPPH radical content decreases as the activity of the extract increases. This is thus indicative that the total phenolic or total flavonoid compounds may play a significant role in increasing DPPH radical scavenging activity of the extracts [30, 31, 83, 84].

5. CONCLUSION

This study revealed that phosphate buffered saline is a better extraction solvent for *A. barbadensis* than distilled water. The extracts of latexes and gels of *A. barbadensis* leaves exhibited intraspecific variation of secondary metabolites, total phenolic and total flavonoid contents, and antioxidant activity for the same solvent extracts of samples taken from different regions of Kenya. These variations could be attributed to the differences in the soils, climate (rainfall) and topography of the different regions which might have affected the interaction between the plants and the environment, and thus the quantities of the phytochemicals detected and quantified in the *A. barbadensis* leaves. The total phenolic and total flavonoid contents, and the antioxidant potential of the latexes and gels of *A. barbadensis* leaves are plausibly due to the secondary metabolites identified in the different solvent extracts. *In vitro* studies as well as toxicity testing of the extracts warrant further research, so as to establish their efficacy and safety when used in traditional herbal medicine. An extension of this study is quantifying the aloin content and investigating the antimalarial activity of the extracts.

The study highlights the efficacy of " HERBAL " which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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