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African Journal of **Plant Science**

November 2019
ISSN 1996-0824
DOI: 10.5897/AJPS
www.academicjournals.org



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Full Length Research Paper

Morphological characterization of blackberry (*Rubus* subgenus *Rubus* Watson) genetic resources in Kenya

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Received 17 August, 2018; Accepted 8 October, 2018

The variation of morphological and physiological traits of blackberry (*Rubus* subgenus *Rubus* Watson) is vital for successful breeding of the fruit crop. The objective of this study was to characterize blackberry accessions *in-situ* using morphological descriptors in Kenya. Each blackberry accession was nested within its county of collection. A phylogenetic tree was then constructed using the Gower's coefficient which clustered the accessions into two classes; I and II consisting of 1 and 89 accessions, respectively. The clustering of accessions did not show an association between the origin of collection and the accessions. Principal Component Analysis (PCA) revealed ten axes of which seven had a cumulative variation of 96.30% with the first two axes having a discriminatory variance of 52.71%. This suggests that variables identified in this study could be used to differentiate blackberry accessions morphologically. This study demonstrated that the number of internodes per average growing shoots, thorniness of the plant and length of internode were associated with the first axis with Eigenvalue of 27.79%. Plant thorniness was also associated with the second axis with Eigenvalue of 24.92%. These results suggest that there exists qualitative and quantitative variation among blackberry accessions in Kenya that can be utilized in breeding.

Key words: Morphological diversity, *Rubus* subgenus *Rubus* Watson, accessions, cluster analysis.

INTRODUCTION

The assessment and monitoring of diversity of plant genetic resources *in-situ* and *ex-situ* is essential for germplasm management and for establishing core breeding stocks (Oyoo et al., 2015; Orobiji et al., 2017). Knowledge of morphological variability of germplasm collections improves understanding of the relationship between the structural morphology of plants and their

corresponding functional botany (Lauri and Normand, 2017). Characters that show diversity within each species are commonly used in the characterization process. Attributes of the edible part of the plant such as leaf shape, length, persistence and total foliage cover are taken in by many crops (Chweya and Edmonds, 1997). For those crops whose fruit is the edible part, fruit size,

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texture, colour, length and weight are used. In addition to the nutritive aspects of each species, phenology and attributes related to storability of the harvested part for consumption are often considered (Human and Rheeder, 2004). Breeding in blackberry focus on specific characters which include adaptation, pest and disease resistance, plant habit, primocane fruiting, thornlessness, fruit size and shape, fruit quality and yield (Clark et al., 2007).

Blackberry (*Rubus* subgenus *Rubus* Watson) is a cross-pollinated, fruiting plant species formerly of subgenus *Eubatus*. The fruits aggregate around a receptacle and consist of fleshy drupelets, each with a single seed (Finn, 2008). Blackberry is a perennial plant with biennial canes and is of three types in reference to cane architecture; erect, semi-erect and trailing (Clark et al., 2007). There are 84 wild species of blackberry in Kenya (Chittaranjan, 2011) and only two plant introductions; one hybrid berry (definitive genetic origin unknown but is believed to be an interspecific cross between European Raspberry, *Rubus idaeus* and another European blackberry, *Rubus fruticosus*) (Wood et al., 1999) and the other, a European berry, *Rubus fruticosus* cultivated mainly for export market. Blackberries have a complex reproductive (sexual, facultatively apomictic to obligately apomictic), ploidy (autoploidy and alloploidy) and inheritance strategies (disomic and tetrasomic) (Clark et al., 2007). Thus, there is difficulty in identifying superior berry as well as designation to definite groups which are sometimes misclassified. Blackberry fruits have varied health benefits and are rich in natural phytochemicals (Rao and Snyder, 2010), vitamin C and E (Hirsch et al., 2013), and contains phenolic compounds that are secondary plant metabolites integral in human and animal diets (Siriwoharn et al., 2004; Lee et al., 2011) due to their antioxidant properties (Hirsch et al., 2013). They are also used to prevent lifestyle diseases like diabetes, cancer, cardiovascular diseases and other pathogens (Bravo, 1998; Hollman et al., 1996). Blackberry fruits are consumed fresh or processed as individually quick frozen (IQF), canned, pureed, juiced or freeze-dried (Finn, 2008). The crop is gaining prominence in Kenya and Africa at large due to its possible health benefits and the influx of a more informed, aggressive middle-class population.

Modern breeding objectives emphasize on the evaluation of the characteristics of importance to production and productivity within genetic resources and concentration of the same in one cultivar (Bozovic et al., 2016). Analysis of genetic diversity can be achieved through molecular and morphological markers. Some of the molecular markers that have been used in the assessment of genetic diversity of blackberry are random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLP), restriction fragment length polymorphisms (RFLP), ISSR-EST, and simple sequence

repeats (SSRs) (Clark et al., 2007). *In-situ* hybridization techniques (ISH) such as genomic *in-situ* hybridization (GISH) and fluorescence *in-situ* hybridization (FISH) have also been used to infer blackberry phylogeny genus (Yan et al., 2015). Morphological markers are still useful in phenotypic descriptions of plant populations. Some morphological traits have been associated with influencing some other trait that has great economic importance but difficult to measure such as disease susceptibility (Karimi et al., 2009). Phenotypic descriptors are widely used to classify cultivars, genotypes and landraces based on discriminant variables for the plant genetic resources (PGR) studied (Orobiyi et al., 2017). Consequently, a comparative analysis is done of the composition of PGR with those of the classes obtained from principal component analysis and correlation analysis. This can better reveal the constitution of each group with respect to the landraces, cultivars or genotypes studied.

In Kenya, blackberry is still a minority crop and grown mainly for the export market or the suburban population. Smallholder farmers are still few as the crop gains prominence in the region. Statistics for yield in Africa are only available for South Africa (220 tonnes) and still, this is very low in comparison to other regions of the world (Strik et al., 2007; Finn, 2008). Being a minority crop, challenges abound; inadequate breeding programs and funding targeting blackberry in Kenya, little understanding of population structures within repositories and the available breeding program, inaccurate identification of species and misclassification in gene banks, difficulty in identification of duplicate accessions in germplasm repositories as well as unavailability of improved local cultivars. In addition, the available varieties experience pest and disease problems coupled with abiotic stresses that are not well documented. Phenotypic expression such as objective descriptions of tree and fruit characteristics discriminating against undesirable traits in the process is unreliable and may not provide an accurate indication of genetic diversity (Menkir et al., 1997). This preference for specific traits based on phenotypic descriptions has also previously led to the discarding of potentially important and advantageous germplasm. In addition, expression of morphological data are greatly influenced by environment, phenological stages of development and can be subjective in nature resulting in errors (Marinoni et al., 2003). This assessment of the morphometric diversity of the fruit tree species in core germplasm collections, wild and introduced, whether *in-situ* or *ex-situ* is however, necessary. It offers prerequisite remedies to the challenges mentioned above and is vital for the thorough understanding of these genetic resources, breeding options and subsequent conservation efforts. The objective of this study was to characterize wild blackberry types in selected counties in Kenya and Plant Introductions (PIs) using morphological markers.

MATERIALS AND METHODS

Experimental site

This study was conducted *in-situ* in selected counties in Kenya. These counties included Kericho (0.3689° S, 35.2863° E), Nakuru (0.3031° S, 36.0800° E), Uasin Gishu (0.5143° N, 35.2697° E), Nandi (0.1036° N, 35.1777° E), Laikipia (0.3970° N, 37.1588° E), and Baringo (0.4897° N, 35.7412° E). In each county, five random districts or sub-counties were selected out of which five random locations and villages were chosen for germplasm sampling.

Germplasm sampling

Sampling of blackberry for morphological trait analysis was carried out in the areas mentioned above. Fruit trees were coded to reflect the county, district, division, subdivision, village and the collection number (Oyoo et al., 2015). If the collection was from Baringo county, Tinet district, Torongo division, Lembus Mosop location, Makutano village and it was the first blackberry sampled, the code given was; BRG/TIN/TOR/LM/MAK/01 (Supplementary material 1). Sampling was done to reflect different agro-ecological zones in the counties where blackberry is reportedly growing. The selected agro-ecological zones were different and are designated pyrethrum (*Chrysanthemum cinerelifolium*)-wheat (*Triticum aestivum*) zone (UH2), tea-dairy zone (LH1), wheat-maize-pyrethrum zone (LH2) wheat-barley zone (LH3), cattle-sheep-barley (*Hordeum vulgare*) zone (LH4), coffee (*Coffea arabica*) zone (UM2) and sunflower (*Helianthus annuus*)-maize zone (UM4). The altitude varied from 1650 m to 2743 m above sea level. Samples were taken in areas where the fruit trees are morphologically different and there are marked changes in altitude, cropping systems, a formidable barrier such as a mountain, river, valley or local people are ethnically different (dialect) from previous collection sites. Here, quantitative and qualitative attributes of the plants were taken along edaphic, topographic and climatic gradients. Data stations in a location were within 200 m intervals. This was done to minimize redundancies. For each fruit tree sampled, Global Positioning System (GPS) data were taken and the plant photographed. This was vital for mapping of these areas using ArcGIS software (Figure 1). Plants with similar features growing in ecologically distinct locations were assumed to be of different eco-strain and hence, were sampled and characterized. Blackberry accessions were evaluated for population structure, architecture and fruit tree characteristics.

Evaluation of traits

Seven qualitative and three quantitative important traits to blackberry breeding were characterized (Table 1) according to Yin, (2017). These included tree, stem, leaf, reproductive characteristics and stress severity assessment. The descriptors of blackberry were as further discussed.

Pre-harvest

Vegetative observations

The cane architecture, showing the degree of creeping for each plant (denoted for each individual as erect "E," semi-erect "S," or trailing "T"); stem type, whether malformed, symmetrical or asymmetrical; thorniness, indicating whether the plant is thorny or not (denoted for each individual as thorny "T" or thornless "N"); overall plant health, showing the degree of infestation, apparent nutrient deficiencies and general abiotic stress susceptibility (subjectively assessed from 1 to 10, where 10 = excellent health);

overall plant vigor, examining leafiness, length of current season's growth, and relative number of actively growing shoots (subjectively assessed from 1 to 10, where 10 = extremely vigorous).

Reproductive observations

Number of internodes/actively growing shoot; internode length (mm), the average length of the fourth internode of four plants; pubescent colour (white, varied or purple); flower colour (white, varied or purple).

Data analyses

Multivariate analysis

Multivariate analysis was carried out using GENSTAT 15th Edition programme on morphological data to identify discriminant variables amongst the 90 accessions. Means of quantitative traits were first obtained using PROC GLM in Statistical Analysis System (SAS) version 9.1 (SAS Institute Inc., Cary, 2001) to determine the significant differences among the accessions. The following statistical model was used

$$Y_{ijk} = \mu + \alpha_i + \beta_{(i)j} + \epsilon_{ijk} \quad (1)$$

Where, μ = Overall mean, α_i : effect of the i^{th} county, β_{ij} = a random effect due to the j^{th} blackberry accession nested within the i^{th} county, ϵ_{ijk} : random error component associated with each observation.

Factorial Component Analysis (FCA) based on discriminant variables obtained was performed using DARwin 6.0 (Perrier et al., 2003; Perrier and Jacquemoud-Collet, 2006) to illustrate the different grouping of the germplasm available. R program for statistical computing version 3.4.1 (R Development Core Team, 2015) was used for the construction of a hierarchical dendrogram to show the overall similarity between the morphological data by plotting the results in homogenous groups. Since the data under consideration were of mixed types (qualitative and quantitative characters), the UPGMA dendrogram was constructed using Gower's dissimilarity coefficient (Gower, 1971) as shown below

$$S_{\text{Gower}} = \frac{\sum_{i=1}^m S_i W_i}{\sum_{i=1}^m W_i} \quad (2)$$

Where; $S_i = 1$ if $x_i = y_i$ (binary or qualitative data); $S_i = 0$ if $x_i \neq y_i$ (binary or qualitative data); $S_i = 1 - |x_i - y_i|/R_i$ (quantitative data): where; $W_i = 1$ if x_i can be compared to y_i and $W_i = 0$ if x_i cannot be compared to y_i . Therefore

$$D_{\text{Gower}}(x, y) = 1 - S_{\text{Gower}}(x, y) \quad (3)$$

The silhouette width was determined using R for statistical computing ver. 3.4.1 as follows;

$$S_i = \frac{b_i - a_i}{\max\{a_i, b_i\}} \quad (4)$$

Where; S_i = the silhouette width, a_i = the average dissimilarity ($d_{i,k}$) between the i^{th} accession and all others in its cluster; b_i = the average dissimilarity ($d_{i,k}$) between the i^{th} accession and its neighbour cluster. Identification of the discriminant variates by the PCA (Principal Component Analysis) and correlation was done using GENSTAT 15th Edition programme.

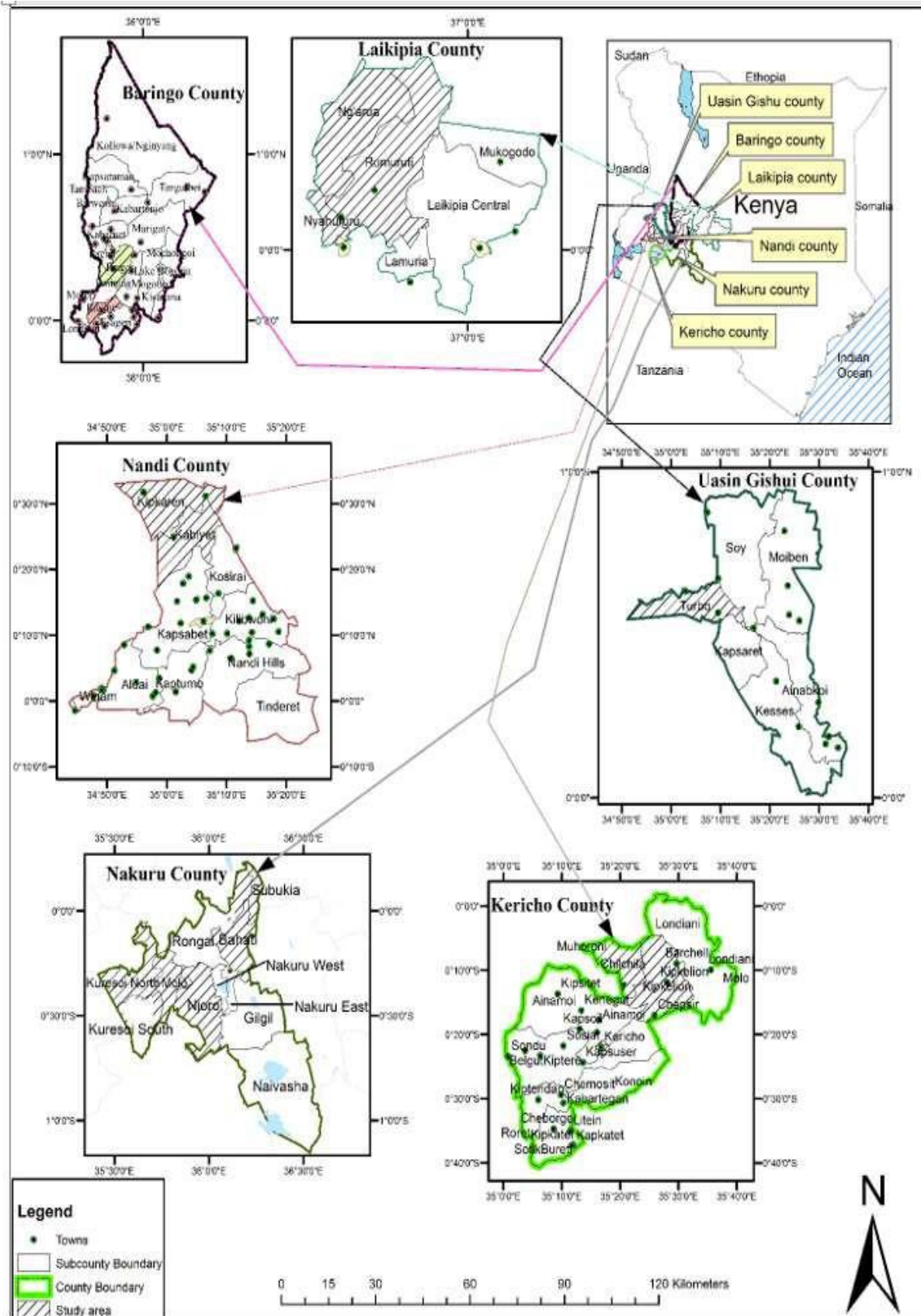


Figure 1. Map showing blackberry germplasm occurrence in six counties in Kenya generated using ArcGIS.

Table 1. Measured variables and observation criteria used to characterize blackberry accessions in Kenya.

Code	Qualitative traits	Phenotypic scale
PT	Plant Thorniness	1 = thornless, 2 = thorns very small and sparse, only detectable upon touch, 3 = small, sparse thorns visible, 4 = small to medium thorns, sporadically spaced, 5 = medium thorns, spaced evenly, 6 = medium thorns closely spaced, 7 = medium to long thorns sporadic, 8 = medium to long thorns evenly spaced, 9 = long thorns, closely spaced
OPH	Overall Plant Health	1 = dead, 2 = extreme biotic/abiotic stress (B/AS), with < 50% leaves green, 3 = obvious symptoms of B/AS with 50% of leaves green, 4 = obvious symptoms of B/AS with > 50% of leaves green, 5 = apparent majority of leaves green but with obvious symptoms of B/AS, 6 = leaves mostly green with minor symptoms of B/AS, 7 = sporadically spaced symptoms of B/AS, 8 = B/AS only observable upon close inspection, 9 = no symptoms of B/AS
OPV	Overall Plant Vigour	1 = no PC or new leaf growth, 2 = leaf growth with no primocane (PC) development, 3 = 1-2 PC or PC < 15 cm in length, 4 = PC > 15 cm, shorter than florican (FC), 5 = PC growth approximately same length as FC, 6 = 3-5 PC, length similar to FC, 7 = 3-5 PC slightly longer than FC, 8 = PC significantly longer than FC, vigorous growth, 9 = 5 or more PC significantly longer than FC with overly vigorous growth
SS	Stem Symmetry	1 = extremely malformed and asymmetrical, 2 = very asymmetrical, 3 = significantly asymmetrical, 4 = asymmetrical, 5 = somewhat asymmetrical, 6 = slightly asymmetrical, 7 = very slightly asymmetrical, 8 = symmetrical, 9 = completely symmetrical
PFC	Plant Flower colour	1 = white, 2 = purple, 3 = pink
PPC	Plant Pubescent colour	1 = white Light Green, 2 = brown, 3 = green

RESULTS

Qualitative phenotypic variability

The accessions exhibited a wide range of differences in qualitative morphological features in plant architecture which refers to the growth habit of canes. The semi-erect type was the most dominant with 97% of the fruit trees sampled, with trailing and erect types being 1 and 2%, respectively (Figure 2). For the fruit trees sampled, 36% had small to medium thorns, sporadically spaced; 22% had medium to long thorns, evenly spaced; 13% had small, sparse visible thorns; 12% had medium to long thorns, sporadically spaced; 9% had medium thorns,

evenly spaced; 6% had medium thorns, closely spaced while only 2% had long thorns that are closely spaced. White was the most frequent flower colour representing 62% of all sampled fruit trees, followed by purple (33%). Pink flower colour accounted only for 4%. The plant (leaf) pubescence colours were categorized into 3; white was the most dominant (54%), with brown and green types being 37 and 9%, respectively.

For all the germplasm sampled, 31% had their leaves mostly green with minor symptoms of biotic and abiotic stress (B/AS), 23% had apparent majority of leaves green but with obvious symptoms of B/AS, 16% had obvious symptoms of B/AS with more than 50% of leaves green, 13% had obvious symptoms of B/AS with 50% of leaves

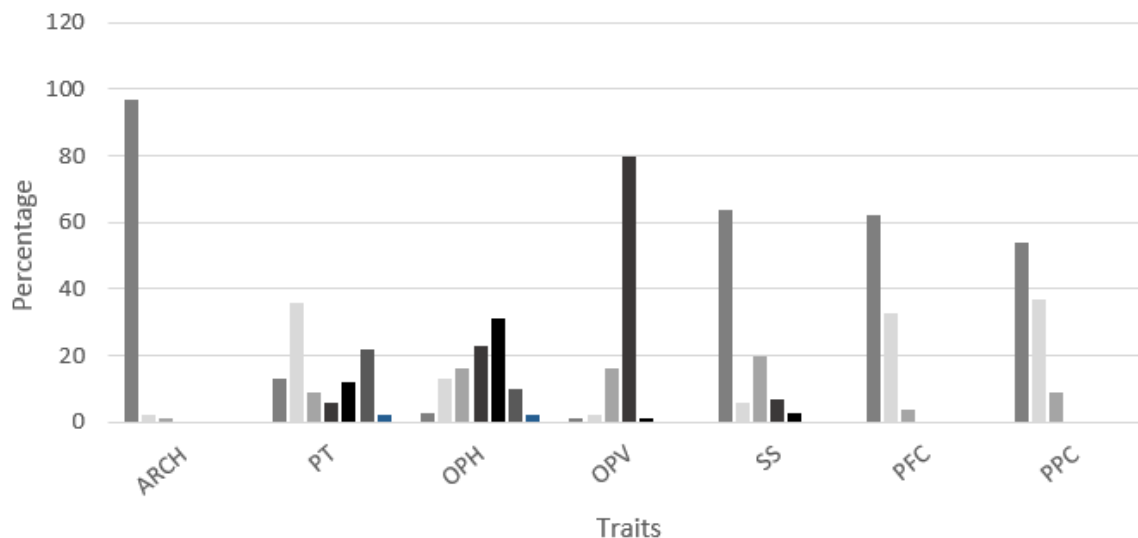


Figure 2. Distribution of some qualitative morphological features among the blackberry accessions collected from different regions in Kenya. ARCH, architecture; PT, plant thorniness; OPV, Overall plant vigour; SS, stem symmetry; PFC, plant flower colour; PPC, plant pubescence colour.

green, 10% had B/AS only observable upon close inspection; 3% had extreme B/AS with less than 50% leaves green and only 2% had no symptoms of B/AS (Figure 2). In addition, 80% of the plant genetic resources studied had vigorous growth with long primocanes. In terms of stem type, 64% were asymmetrical; 20% slightly asymmetrical; 7% very slightly asymmetrical; 6% somewhat asymmetrical while 3% were symmetrical. No malformed stems were observed.

Principal component analysis (PCA)

Principal component analysis allowed the association of axes to the variables and out of the ten axes generated, seven had a cumulative variation of 96.30% with the first two axes having a discriminatory variance of 52.71% (Figure 3). Three variables were associated with the first axis with Eigenvalue of 27.79% (Table 2). These were number of internodes per average growing shoots, plant thorniness and internode length. Plant thorniness was also associated with the second axis with Eigenvalue of 24.92%. One variable was associated with the third (Eigenvalue = 14.29%), fifth (Eigenvalue = 8.38%), sixth (Eigenvalue = 6.78%) and seventh (Eigenvalue = 2.37%) axes. These were overall plant health, number of leaflets, symmetry of stem and the colour of plant during pubescence (Table 2). The number of internodes per average growing shoots and length of internode were associated with the fourth axis with Eigenvalue of 11.77%. Discriminant analysis of the variables taken for all the accessions for the first two principal scores

clustered the wild blackberry types together in comparison to the cultivated types (Figure 4).

Factorial component analysis carried out using dissimilarity coefficients obtained from the usual Euclidean distance was conservative and splits the accessions into the 4 planes (Figures 5 and 6). Most of the accessions overlapped, demonstrating redundancies in the morphology of the characterized germplasm. From the PCoA plot generated (Figures 5 and 6), principal axes 1 and 2 showed that NKR/NJR/EGER/EGER/F7/01 (90), NKR/NJR/NES/NES/KIM/01 (9) from Nakuru county; BRG/TIN/TOR/LM/MAK/06 (42), BRG/TIN/TOR/LM/MAK/02 (39), BRG/ERN/IGE/MM/KIN/04 (53), BRG/ERN/TIM/MBE/KMA/O2 (56) from Baringo county; NDI/NN/KUR/CKO/SUR/03 (60) from Nandi County, LC/LKN/GMA/GMA/KBI/RK/01 (77) from Laikipia county, UG/KKB/ABK/KPG/CHES/03 (80) and UG/KKB/ABK/KBG/CHES/01 (78) from Uasin Gishu and an introduced germplasm CV/RBN/01 (85) (from South Africa) were the most distinct from the other accessions.

Cluster analysis

Cluster analysis split the accessions into two clusters, I and II (Figure 7). The Plant Introductions (CV/RBN/01) had its own cluster, I, while the other (CV/BYN/01) clustered with the rest of the wild accessions collected from different regions of Kenya. The accessions were clustered together according to the traits (quantitative and qualitative) measured. The accessions did not cluster

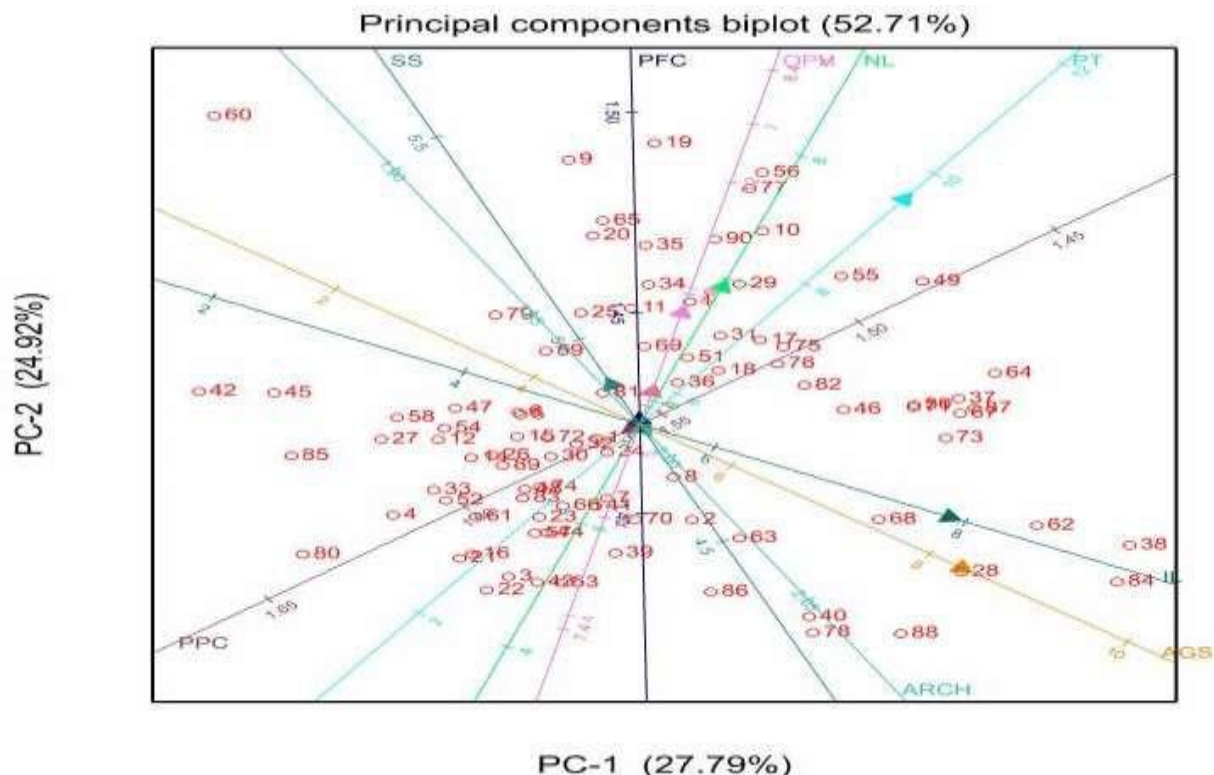


Figure 3. Principal components biplot for the first two principal components of a morphological principal components analysis of blackberry accessions. SS, Stem Symmetry; PFC, Plant Flower Colour; PPC, Plant Pubescence Colour; ARCH, Architecture; OPV, Overall Plant Vigour; OPH, Overall Plant Health; AGS, Number of internodes per average growing shoot.

Table 2. Principal Component loadings of ten traits for 90 blackberry accessions collected from different regions in Kenya.

Variables	Principal component loadings						
	Axis 1	Axis 2	Axis 3	Axis 4	Axis 5	Axis 6	Axis 7
Architecture	0.009	-0.015	0.012	0.039	0	0.032	0.006
No. of internodes per shoot	0.609	-0.425	0.015	0.558	0.339	0.129	0.012
No. of leaflets	0.156	0.407	-0.319	0.419	0.595	0.412	-0.079
Overall Plant Health	0.079	0.331	0.86	0.272	-0.129	-0.173	-0.059
Overall Plant Vigour	0.023	0.097	0.083	0.004	-0.024	-0.071	-0.242
Plant Flower Colour	0	0.014	0.057	-0.02	0.125	0.113	-0.108
Plant Pubescent colour	-0.015	-0.011	0.055	0.078	-0.147	-0.051	0.947
Plant Thorniness	0.5	0.667	-0.216	-0.219	0.389	-0.21	0.107
Stem Symmetry	-0.053	0.118	0.27	-0.258	0.302	0.846	0.104
Internode length	0.586	-0.274	0.16	-0.563	-0.484	0.037	-0.032
Eigen value	4.843	4.344	2.491	2.052	1.46	1.181	0.414
Variation (%)	27.79	24.92	14.29	11.77	8.38	6.78	2.37
Cumulative variation (%)	27.790	52.710	67.000	78.770	87.150	93.930	96.300

according to counties of origin. However, most of the accessions from all the six geographical regions clustered in Group II. Cluster II had the highest number of

genotypes of 89 based on the morphological descriptors used. Cluster II also had sub-clusters. Grouping of these accessions into these sub-clusters indicated a substantial

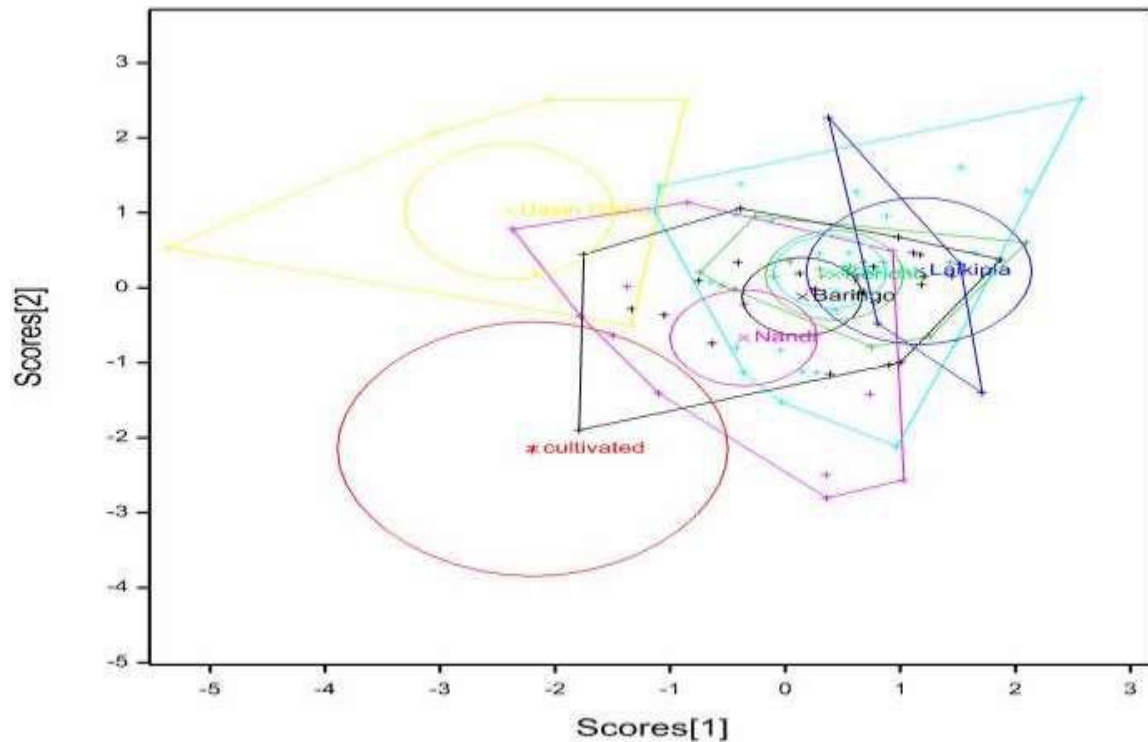


Figure 4. Variables factor biplot for the first two principal scores of a discriminant analysis for the Blackberry Introductions (which is cultivated) and other accessions from 6 different regions in Kenya.

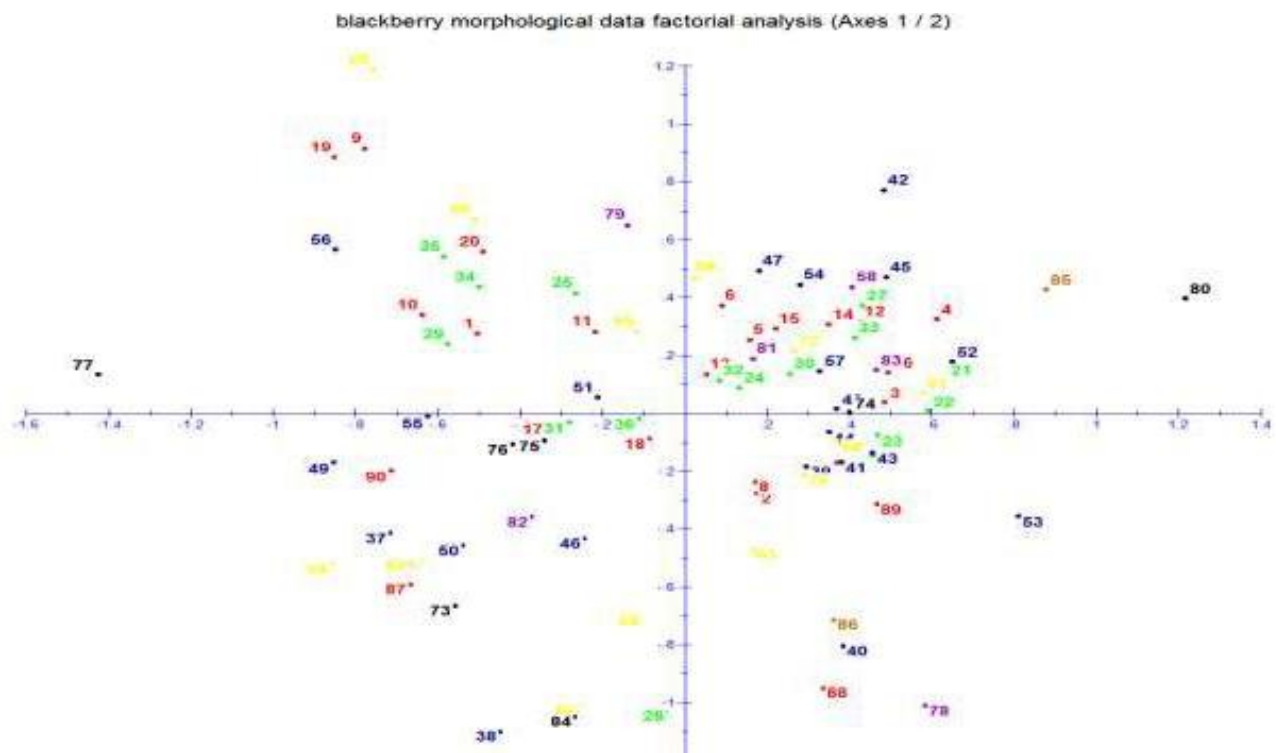


Figure 5. PCoA of axes 1 and 2 based on the dissimilarity of 90 blackberry accessions. The accessions are depicted using the following colour codes: Red = Nakuru; Green= Kericho; Blue= Baringo; Yellow = Nandi; Black = Laikipia; Purple = Uasin Gishu and Orange = introductions.

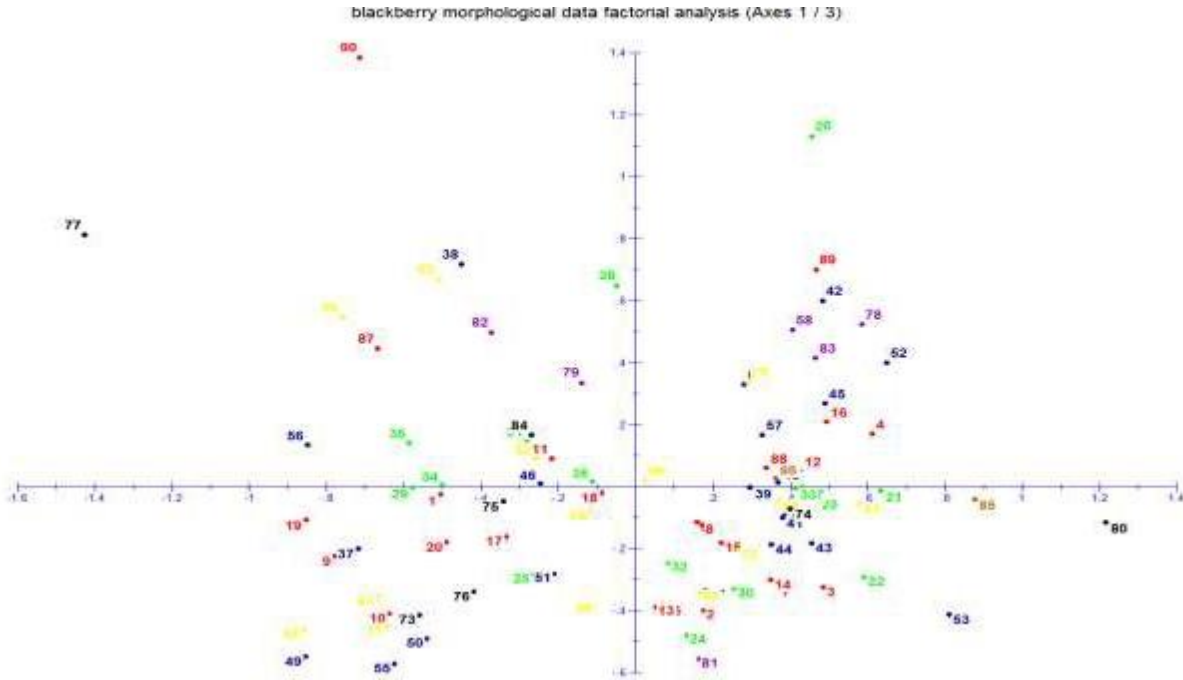


Figure 6. PCoA of axes 1 and 3 based on the dissimilarity of 90 blackberry accessions. The accessions are depicted using the following colour codes: Red = Nakuru; Green= Kericho; Blue= Baringo; Yellow = Nandi; Black = Laikipia; Purple = Uasin Gishu and Orange = introductions.

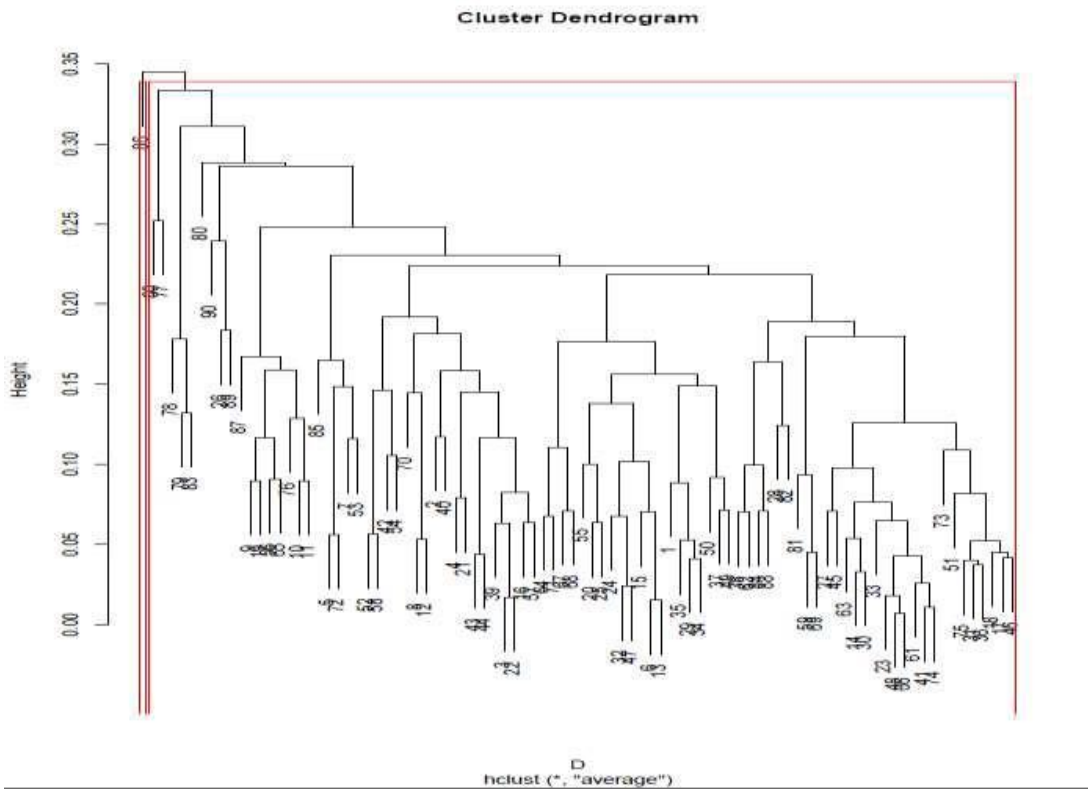


Figure 7. Hierarchical dendrogram based on Gower's dissimilarity matrix calculated from the dataset of 90 blackberry accessions clustered in two groups as revealed by Gowers based clustering model. The two groups are demarcated using the red border line.

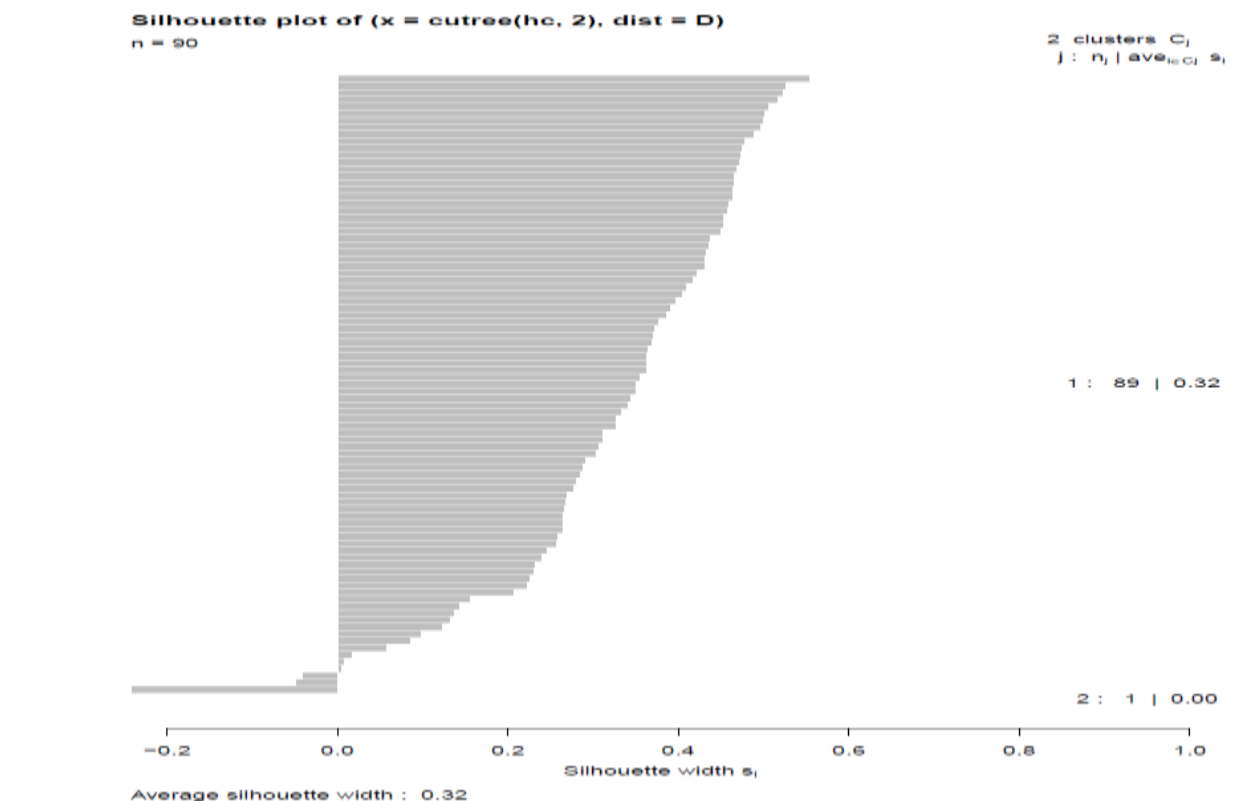


Figure 8. A silhouette plot showing a graphical display of the structure of the blackberry germplasm.

level of intra-polymorphism within the wild blackberry population in the country. Cluster 1 only had one genotype indicating inter-polymorphism with the rest of the accessions collected.

Structure analysis was illustrated using a silhouette plot and was used to compare the minimum average dissimilarity of each accession to other clusters with the average dissimilarity to accessions in its own cluster. There were two main groups within this set of germplasm, hence objective determination in the number of stable clusters. Observations close to 1 (large s_i) indicate that the individual (s_i) is very well clustered. Clusters with observations close to 0 (small s_i) indicate that the germplasm lie between two clusters. Based on the silhouette plot generated, the natural number of clusters in this particular germplasm, given by the traits analysed, is $k = 2$. The average silhouette width (ASW) from this structure analysis is 0.32 (Figure 8). This shows that the structure of the population of the accessions under study was weak and could therefore be artificial.

DISCUSSION

The assessment of variation in morphological germplasm is the first step in the determination of genetic diversity. It

is a prerequisite for conservation and utilization of plant genetic resources (Mason et al., 2017). Morphological characterization can also be useful in selection of parents for breeding (Orobiyi et al., 2017; Kagimbo et al., 2017). Therefore, there is a need to assess the diversity of any crop prior to selection and crossing to better utilize the resources in any breeding program. In this work, blackberries were studied at different agro-ecological areas and therefore, had differences in morphological expressions (Figure 2). According to tree characteristics, most of the wild blackberry accessions were semi-erect (87%) and 80% of genotypes studied had vigorous growth. This study anticipated higher morphological diversity due to the inclusion of introduced germplasm from South Africa. However, a narrow diversity was observed (ASW=0.32) as per the silhouette plot (Figure 8). This shows that the structure of the population of the accessions under study was weak and could be artificial. The introduced germplasm from South Africa had their origins from Europe and North America. Neither Kenya nor South Africa is a centre of origin for blackberry.

The observed low genetic diversity might be due to a number of reasons including nature of propagation of the crop, method of dispersal of the crop, effect of environment, nature of breeding and farmer to farmer exchanges of germplasm. Blackberry reproductive nature

is complex. This varies from sexual to facultatively apomictic to obligately apomictic. Blackberry (*Rubus* subgenus *Rubus* Watson) are often hermaphrodites (Nybom, 1986) and outcrossing has also been observed (Antonius and Nybom, 1995). Additionally, self-fertilization is frequent (Nybom 1988). Infertility or partial fertility may also occur in some plants, and this is attributed to genetic factors such as poor pollen production, unattractive nature to pollinators, lack of pollinators and environmental effects. Open pollinators are likely to have higher diversities compared to inbreds or vegetatively propagated berries (Stafne and Clark, 2004). Most wild blackberries are clonally propagated by way of root sprouts, underground stems (rhizomes) and branches that has its root at the tips (stolons). Therefore, the number of breeding parents may be few, thus, low diversity. Low morphological diversity of blackberry could also be due to the fact that the crop is an invasive species, and propagate vegetatively very vigorously, enabling the clonal spread of single individuals in a patch of habitat. This, however, does not mean that there is a narrow genetic base or higher homozygosity of blackberries in Kenya. In retrospect, the genetic base of fruit tree crop is varied and can be attributed to the different species available.

The progress of breeding blackberry genotypes is directly affected by the plant thorniness. Thornlessness is the most bred qualitative trait in blackberry. Four genes have been detected to be responsible for thornlessness, and they can vary from recessive to dominant for the trait. Breeding progress is thus, hampered by the source of the thornless genotypes and the ploidy of the blackberry type in question.

Plant flower colour varied from white, pink to purple in which white was the most dominant color (62%). Differences in plant flower colour and plant pubescence colours are commonly noticed in natural and introduced blackberry germplasms. This was evident in the data taken and is important as it influences pollination and diversity of the accessions. Some accessions, for instance CV/RBN/01, were more divergent than others and this may be attributed to their outcrossing nature (Figures 4 and 7).

Cluster analysis on the characteristics split the accessions into two groups, I and II. These groups were in a random manner irrespective of their geographical origin. The two distinct groupings were due to the availability of introduced germplasm (CV/RBN/01) that singly constituted Group I. The rest of the landraces grouped together, albeit with subgroups. Although there was no clear association between the subgroups and counties of origin, most germplasm from Nakuru county tended to cluster together. This may be explained in terms of gene pool concept where the wild types formed the primary gene pool, which consists of the crop species itself and other species that can be easily crossed with it. The cultivated type may have grouped alone (CV/RBN/01) as variability in cultivated plant species depends on how

evolutionary forces impact on natural populations. The sole grouping of CV/RBN/01 over the rest of the accessions in this study may also be due to the selective advantage it has over the rest of the accessions. This can be by way of mutation, genetic drift that is as a result of random changes in allele frequencies for generations due to the finite size of populations, gene exchanges or gene flow among populations and selections (both natural and artificial selection). The high similarity of the wild types in morphometry and agro-morphological traits across the different agro-ecological zones may be attributed to the invasive nature of the accessions characterized.

Principal Component Analysis (PCA) also did not associate the accessions with their regions of origin. Of the ten traits subjected to PCA, eight were able to differentiate the collected accessions and were considered as variables that are capable of discriminating accessions on the basis of morphology. It was evident that PCA also categorized assessed phenotypic traits in the population into several related groups (Figure 3). This can also be explained by the reproductive and often invasive nature of the fruit tree species over wide ethno-geographical regions as well as the folk nomenclature that exists in these areas. Apomixis also occurs in some blackberry species (wild and introduced). Therefore, clones dispersed by man or birds spatially across habitats can be a cause. This often results in misclassification of genotypes and existence of duplicates (Mason et al., 2015; Agre et al., 2017).

Conclusion

This work is important as a treatise to breeding of blackberry and can be used with DNA genotyping information to understand the morphological variations that are present in blackberries. Although the study covered only six counties in Kenya, this work is of great significance in management of these genetic resources. It highlights the contribution of key qualitative and quantitative traits to morphological differences of blackberry and their variation across environments. The agro-morphological characterization is however, ambiguous. Moreover, the phenotypic characters are influenced by the environments in which each plant was collected. A more detailed high throughput phenotyping study should be carried out including fruit characteristics such as yield, fruit quality and quantity variables to determine the health benefits of blackberry with possibility of improvement. It is also important to exploit molecular techniques to generate non-ambiguous information to know more about the genetic variation that really exists in these materials.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

This work was funded by RUFORUM Grant award (RUFORUM-RU/CGS/CRG/30/03/14) in collaboration with Egerton University, Kenya. The authors would like to thank Prof. John R. Clark (University of Arkansas, Fayetteville), Dr. Chad Finn (USDA-ARS) and Dr. P. Okwiri (Egerton University) for reviewing this manuscript; KALRO-Njoro (Food Crops Institute) staff for their insights and assistance during field work; along with colleagues and staff from the Department of Crops, Horticulture and Soils, Egerton University for their support during the research.

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Full Length Research Paper

An ethnobotanical study of medicinal plants in chiro district, West Hararghe, Ethiopia

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Received 1 October, 2019; Accepted 27 November, 2019

Ethnobotanical study of medicinal plants is inadequate in Ethiopia in general, and in Chiro District in particular. Therefore, this study documents medicinal plant utilization, management and the threats encountered on them. The study was conducted from April 2017 to June 2018. Forty eight informants were purposively selected. Socio-economic and botanical data were gathered using group discussions, semi-structured interviews, and field observations and analysed using informant consensus factor, preference ranking and paired comparison methods. The result revealed that 60 plant species from 42 families and 58 genera were used as traditional medicine to treat human and animal diseases. The Euphorbiaceae were represented by 7 species, followed by Asteraceae (5 species), Myrtaceae and Solanaceae (3 species each), Lamiaceae, Brassicaceae and Polygonaceae (two species each). Of the 60 species, 22 (36.67%) were herbs, followed by shrubs (n=19, 31.67%), trees (n=16, 26.66%) and climbers (n=3, 5.0%). In the study area the most significant threat to medicinal plants is agricultural expansion. Even though the study revealed that the area is enriched with medicinal plant diversity, awareness should be done to enhance the conservation of medicinal plants.

Key words: Ethnomedicine, Chiro district, medicinal plants.

INTRODUCTION

World Health Organization (WHO, 2002) defines traditional medicine as 'the the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether justifiable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and social discrepancy, and relying exclusively on practical experience and observation transferred from generation to generation, whether verbally or in writing'. Since traditional medicine is the most affordable, simple to use

and easily accessible source of treatment, especially in developing countries (Haile and Delenasaw, 2007), it became an integral part of many cultures (Pankhurst, 1965). Studies indicate that deforestation, urbanization, agricultural expansion and lack of awareness among the community are the critical threats to medicinal plants (Hunde et al., 2015; Dida, 2017).

Ethiopia is endowed with diverse biological resources (About 6,500 are higher plants) of which approximately 10% are medicinal plants (Abera, 2014; Tadesse et al., 2018). In addition, Ethiopians have used traditional

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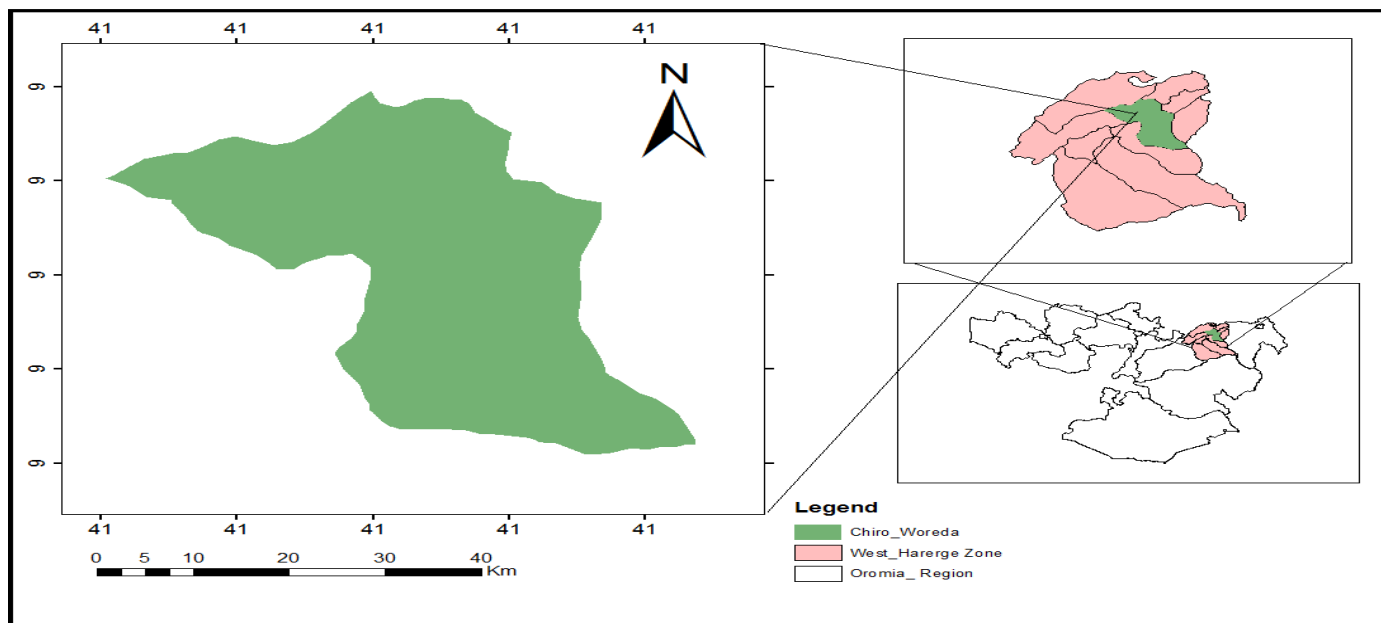


Figure 1. Location of Chiro district in the West Hararghe Zone, Ethiopia.

medicines for many centuries. The use of which has become an integral part of the different cultures in the country. Traditional people around the world have developed their own specific knowledge of plant resource uses, management, and conservation on which they depend on for food, medicine and general utilities (Zewudu, 2013).

According to Zewudu Birhanu (2013), traditional remedies are the source of therapeutics for nearly 70% of Ethiopian population and 90% of livestock in the country. There is, however, a need for sustainable use of medicinal plant materials and the associated indigenous knowledge as wild plants are under extreme pressure of increased demands (Haile and Dilnesaw, 2007). Moreover, in Ethiopia the use of wild or uncultivated plants is a common custom and this has been accelerating the deterioration of useful plant population in addition to agricultural expansion accompanied by wide cutting original forest species and environmental degradation (Abera, 2014). Hence, the current study aimed to document the indigenous knowledge of the local people on the use, threat and conservation of medicinal plants in Chiro district. This study has value in that it could be used as basis for further studies on medicinal plants in Chiro District and for future phytochemical and pharmacological studies.

MATERIALS AND METHODS

Description of the study area

Chiro District (Figure 1) is one of the 18 Districts of West Hararghe

Zone in the Oromia region of Ethiopia. Part of the West Hararghe Zone, Chiro district is located between 9°05' longitude and 40°52'E latitude. From the study district, 6 (Medicho No.3, Nejebas, Wachu Geleyi, Gara Nigus No. 1, Medicho No. 9 and Chiro Kela) kebeles were used as sampling sites for data collection.

The district is founded at an altitude ranging from 1826 to 1950 m above sea level. The district has undulating topography and mountainous characteristics with low vegetation cover and sparsely vegetated landscapes which is highly vulnerable to erosion problems. Drought, shortage of water, soil erosion, flooding, animal forage scarcity, and lack of income diversity are the main threats to food security and sustainability. The 2007 national census reported a total population for this district of 169,912, of whom 87,003 were men and 82,909 were women; none of its population was urban dwellers (CSA, 2007). The agricultural activities are mainly mixed type with cattle rearing and crop production undertaken sideways. Major annual crops include sorghum, maize, bean, barley, teff, wheat, and pea and from cash crops Khat and Coffee are widely produced. It has a maximum and minimum temperature of 23 and 12°C, respectively and maximum and minimum rainfall of 1800 and 900 mm, respectively. Rainfall type is bimodal and erratic in nature. Main rainy season of the study area is from June to September while short rainy season is from March to May. *Croton macrostachyus* Del., *Juniperus procera*, *Podocarpus falcatus* (Thmb.) R.B. ex. Mirb., *Vernonia amygdalina* Del., and *Hagenia abyssinica* (from the natural forests), and *Juniperus procera*, *Cupressus lusitanica*, and *Eucalyptus Camaldulensis* Dehnh., J.F Gmel. (from plantations) are some of the common vegetation types of the study area

Informant selection

Forty eight informants (36 males and 12 females) aged between 18 to 81 were selected by using judgment and volunteer sampling techniques, according to the method of Abera (2014). Out of these, 12 (7 males and 5 females) key informants, were selected based on recommendations from elders and local authorities (Kebele

administration leaders and religion leaders). These key informants were singled out due to their superior knowledge of medicinal plants over the other 36 interviewees. The informants are ethnically Oromo since they are inhabiting dominantly in the study area.

Study design

Data was collected between April 2017 and June 2018 using one-on-one semi-structured interviews, field observations, and group discussions. The semi-structured questionnaire sought to gain information on the following themes: Medicinal plants resource of the study area, preparation and administration methods of decoctions, medicinal plants species used to treat human diseases, major human diseases and plant species used for the remedies, medicinal plants species used to treat livestock diseases, major livestock disease and number of plant species used, acquisition and transfer of indigenous knowledge on medicinal plants and threats to medicinal plants.

Dried specimens of the plants collected from the Chiro District were taken to the Oda Bultum University Plant Herbarium for taxonomical identification. Voucher specimens were also deposited at this herbarium.

Data analysis

A descriptive statistical method such as frequency and percentage were employed to analyze and summarize the ethnobotanical data obtained from the interviews and group discussions on reported medicinal plants and associated knowledge.

Data obtained from the questionnaire was analysed by means of quantitative statistics. Different index methods were used to analyse data on Informant Consensus Factor, as well as Preference ranking and paired comparison.

The Informant Consensus Factor (ICF) value was calculated using the formula:

$ICF = \frac{(Nur - Nt)}{(Nur - 1)}$, where Nur is the number of use report of informants for each ailment, and Nt is the number of taxa used for a specific ailment (Trotter and Logan, 1986).

Preference ranking was calculated using the formula:

Preference ranking was conducted by using ten randomly selected key informants to rank four medicinal plants against Tufa being given the highest (4 = most effective) and the least (1 = less effective values to each medicinal plants species following Martin (1995).

Paired comparison was calculated using the formula:

Paired comparison was used to evaluate the degree of preference or levels of importance of 5 reported medicinal plants for treating Chancroid following Martin (1995). A list of the pairs of selected items with all possible combinations was made and sequence of the pairs and the order within each pair was randomized before every pair is presented to the 6 selected informants and their responses recorded and total value was summarized.

Ethical considerations

Ethical clearance was sought from Oda Bultum University Ethics and Code of Conduct Committee (ECCC). Informants gave their informed consent for the publication of all results and any accompanying images before commencing with the interview

schedules, as required by the Oda Bultum University Ethics and Code of Conduct Committee (ECCC).

RESULTS AND DISCUSSION

Medicinal plants resource of the study area

Overall, 60 medicinal plant species distributed under 42 families and 58 genera were identified in the Chiro District (Table 1). The dominant families included the Euphorbiaceae, which were represented by 7 species, followed by the Asteraceae with 5 species; Myrtaceae and Solanaceae with 3 species each, and Lamiaceae, Brassicaceae and Polygonaceae with two species each. The remaining 34 families were represented by one species each. This could be an indication that the study area consists of considerable diversity of plant species similar to other districts of the country (Kalayu et al., 2013; Zewudu, 2013; Abera, 2014).

From the total of 60 medicinal plant species documented in the study area, 39 (65%) species were wild vegetation, 11 (18.33%) species were cultivated and the remaining 10 (16.67%) were both cultivated and wild vegetation. This indicates that the practitioners mostly depend on wild vegetation than home garden vegetation for preparation of medicine which is also supported by other studies in the country (Kalayu et al., 2013; Abera, 2014; Mulugeta, 2017).

Habit

Out of 60 species, 22 (36.67%) species were herbs followed by shrubs 19 (31.67%) species, tree 16 (26.66%) species and climber 3 (5.0%) species (Figure 2). The current findings show that most widely used medicinal plants in the study area are shrubs and herb in habit. High number of shrubs and herbs for medicinal plants were explained in Ethiopia in the previous studies (Bayafers, 2000; Debela, 2001; Ermias, 2005).

Preparation of medicine

The practitioners employed 10 cultural medicine preparation methods (Table 2) to prepare 87 types of cultural medicines. From the cultural medicine preparation methods used in the study area, pounding and grinding were the most popular methods of preparation contributing to 43 (49.43%) and 15 (17.24%) cultural medicine preparations, respectively. Both pounding (Solomon et al., 2015) and grinding (Kalayu et al., 2013) methods were also reported as among the dominant cultural medicine mode of preparations. Moreover, these pounding and grinding are also importantly used by the local community to preserve the medicinal plants in the form of powder. The next ranks were taken by boiling

Table 1. List of medicinal plants encountered in the study area (T= Tree; Sh= Shrub; H= Herb; L= Liana; Cl=Climber; WC= Wild and Cultivated; W= Wild; Ct= Cultivated).

No.	Scientific name	Oromipha name	Family	Habit	Degree of management	Parts used	Ailment type	Mode of preparation	Use	Route of administration
1	<i>Acacia abyssinica</i> Hochstex. Benth.	Dadecca/Lafto	Euphorbiaceae	T	WC	Leaf	Physical injury with ulcer	Pounding the leaves of <i>A. abyssinica</i> plant and creaming on the affected body	H	dermal
						Bark	Inflammation	Grinding of the root bark and mix it with water and drink it		
2	<i>Acalypha fruticosa</i> Forssk.	Baaltokkee	Euphorbiaceae	Sh	W	Root	Chest pain (stabbing pain)	Pounding fresh root and mixing it with coffee and drunk orally	H	Oral
3	<i>Acanthospermum hispidum</i> DC.	Qummuxxo	Asteraceae	H	W	Leaf	Tetanus	Pounding the leaf and put on affected area with ulcer	H	Dermal
4	<i>Achyranthes aspera</i>	Darguu	Amaranthaceae	H	W	Root	Chankroid	Grinding the root part boil it with water and drink it	H	Oral
						Leaf	Inflammation	Grinding dried leaf pound it and put the powder on affected skin part	H	Dermal
5	<i>Allium sativum</i> L.	Qullubi addii	Alliaceae	H	Ct	Bulb	Cough	Pound the bulbs of <i>Allium sativum</i> and Eucalyptus globulus and mix with butter fire it and fumigate it	H	Nasal
6	<i>Aloe macrocarpa</i> Tod.	Argiisa	Asphodelaceae	H	W	Leaf	Diabetes	Pounding the leaf and drinking	H	Oral
							Hypertension	Pounding the leaf and drinking	H	Oral
7	<i>Aloysia triphylla</i> (L 'Herit.) Britton	Xuxxoo	Verbenaceae	T	Ct	Leaf	Tonsillitis	Pounding the leaves of <i>A. triphylla</i> and mix it with ash and water drinking drop by drop	H	Oral
8	<i>Artemisia absinthium</i> L.	Harritta/Harii yuyoo	Asteraceae	H	WC	Leaf and root	Dengetegna	Drying and pounding the leaf and roots of <i>A. absinthium</i> and mixed with water	H	Oral
9	<i>Arundinaria alpine</i>	Qacc'ee	Gramineae	H	W	Young leaf	Intestinal ulcer	Pounding the young leaves and mix with water and drink it	H	Oral
10	<i>Asparagus africanus</i> Lam	Saritii	Asparagaceae	Sh	W	Root	Chankroid	Grinding the root part boil it with water and drink it	H	Oral
11	<i>Azadirachta indica</i> A. Juss.	Kinninii	Meliaceae	T	WC	Leaf	Dingetegna	Leaves of <i>A. indica</i> pounded, mixed with water and be taken orally	H	Oral
						Root	Amoebiasis	Pounding the root and drink it with water		
12	<i>Calpurnia aurea</i> (Ait.)	Ceekaa	Fabaceae	T	W	Seed	Amoebiasis	Roasting the seed and pound it and boil the powder with water and drink it	H	Oral
						Leaf	Pneumonia	Pounding the leaf and drinking it Or Boiling the leaf and fumigate the steam		
13	<i>Carica papaya</i> L.	Papaya	Caricaceae	T	Ct	Seed	Cough	Grinding and mixing the <i>C. papaya</i> seeds with the leaves of <i>Cynodon dactylon</i> and drinking with water	H	Oral
						Root	Diarrhea	By grinding the roots and mixed with water and drink it		
						Leaf	Pneumonia	Pounding the leaf and drinking it Or Boiling the leaf and fumigate the steam		
14	<i>Carisa Spinarum</i> L.	Agamsa	Apocynaceae	Sh	W	Root	Evil Spirit Un milking cow	Pounding the dry roots of <i>C. Spinarum</i> , <i>Solanum incanum</i> and <i>Dovyalis abyssinica</i> and mix it with water and administer it into ear and nose parts of the cow	A	Nasal and dermal

Table 1. Contd.

							Dermatitis	Pounding the roots of <i>C. Spinarum</i> and mix it with water and drink		Oral	
15	<i>Cassipourea malosana</i> Aubl.	Xilloo	Rhizophonaceae	T	W	Leaf	Tinea Versicolor	Pounding the leaves of <i>C. malosana</i> and <i>Bidens pilosa</i> L. plants together and rub over the affected skin part	H	Dermal	
16	<i>Celtic Africana</i>	Mataqomaa	Cannabaceae	T	W	Leaf	Body swelling	Pounding the leaf and drink it	H	Oral	
17	<i>Cissampelos mucronata</i> A.Rich.	Bal tokkee	Menispermaceae	L	W	Leaf	Tufa	Pounding the <i>C. mucronata</i> and <i>Biclens pilota</i> leaves and creaming on affected skin and drinking it	H	Dermal and Oral	
							Rabies	Pounding the leaves of <i>C. mucronata</i> , the roots of <i>Impatiens rothii</i> together and mix it with water and drinking it or eat it with injera	A	Oral	
							Root	Abdominal pain	By grinding the root mix it with water and drink it	H	Oral
18	<i>Coffee arabica</i> L.	Buna	Rubiaceae	T	Ct	Seed	Abdominal pain	Pounding the roasted seed and mixed with honey and administer it orally			
19	<i>Commelina africana</i> L.	Mandar buqas	Commelinaceae	H	W	Flower	Hemorrhoid	Pounding the flower part and rub put on affected body	H	Dermal	
20	<i>Crabbea velutina</i> S.Moore.	Laafaa	Acanthaceae	Sh	Ct	Leaf	Urinary problems	Drying and pounding the leaf	H	Oral	
21	<i>Crambe hispanica</i> L.	Fujul	Brassicaceae	H	W	Fruit	Ear Eche	Squeezing the <i>Crambe hispanica</i> fruit and mix it with salt and water and screen the juice and put on affected ear area	H	Dermal	
							Leaf	Ear ache	Pounding the leaves of <i>C. macrostachus</i> and <i>Thymus schmperi</i> and squeeze the liquid filter it and add in the affected ear part	H	Dermal
							Shoot	Wart	Rubbing the young shoots of <i>C. macrostachyus</i> on affected skin and let it dry over it every morning for seven consecutive days	H	Dermal
22	<i>Croton macrostachyus</i> Del.	Bakkannisa	Euphorbiaceae	T	WC	Bark	Inflammation	Grinding the dried barks of <i>C. macrostachus</i> and put on affected area	H	Dermal	
							Pneumonia	Grinding the barks of <i>C. macrostachus</i> and boil it with water and drink it	H	Oral	
23	<i>Cucumis ficifolius</i> A. Rich.	Haregoge	Solanaceae	Cl	W	Roots	Rabies	Pounding Roots of <i>C. ficifolius</i> , <i>Eragrostis teff</i> (red), and roots and leaves of <i>Verbascum sinaiticum</i> together and deliver orally with injera.	A	Oral	
							Abdominal pain	Pounding fresh roots of <i>Cucumis ficifolius</i> , mix with water and drink it	H	Oral	
24	<i>Cuminum cyminum</i> L.	Kemmuuna	Apiaceae	H	WC	Leaf	Chancroid	Pounding leaves of <i>Cuminum cyminum</i> and <i>Tragia cinerea</i> together and mix with water and drink it	H	Oral	
							Urinary problems	Pounding leaves of <i>Cuminum cyminum</i> and mix with water and drink it	H	Oral	

Table 1. Contd.

						Fruit	Kidney problems	Boiling the fruit with water and drink it	H	
						Leaf	Mastitis	Pounding and creaming on the affected animals udder	A	Dermal
25	<i>Datura stramonium</i> L.v	Banji	Solanaceae	H	W	Seed	Tooth ache	Grinding the seeds mix with water and put on affected tooth part	H	Oral
26	<i>Dodonaea angustifolia</i> L. f.	Ittaacha	Sapindaceae	Sh	W	Shoot	Eye disease	Pounding shoot and young leaves together with water and squeeze and drop on affected eye	H	Ocular
27	<i>Echinops longisetus</i> A.Rich.	Qoree Adi	Asteraceae	Sh	W	Shoot	Ulcer	Cutting young shoot and collect the yellowish juice by squeezing it and put on affected skin part	H	Dermal
28	<i>Ehretia cymosa</i> Thonn.	Ulagaa	Boraginaceae	T	W	Leaf	Febrile illness (Mich)	The leaves of <i>Ehretia cymosa</i> , and <i>Cucumis ficifolius</i> pounded and squeezed and mixed with water	H	Oral and dermal
29	<i>Eleusine floccifolia</i> Forssk.	Coqorsa/Akrima	Poaceae	H	W	Root	Vomiting	By chewing the root and swallow it	H	Oral
30	<i>Eucalyptus globulus</i> Labill.	Bar gamoo	Myrtaceae	T	WC	Leaf	Megagna , common cold (Mich)	Boiling the lef in water and fumigate it	H	Nasal
31	<i>Euphorbia tirucalli</i> L.	Qinchibe	Euphorbiaceae	Sh	W C	Stem	Hemorrhoid	Cutting the stem and collect the juice and put on the affected body part	H	Oral
32	<i>Euclea racemosa</i> subsp. Schimper	Me'essaa	Ebenaceae	Sh	W	Root	Chancroid	Boiling the root of <i>Euclea racemosa</i> with water and drink it	H	Oral
33	<i>Ficus sycomorus</i> L.	Oda	Moraceae	T	W	Leaf	Befta (Dermal disease)	Roasting the leaven with fire and pounding it and put the powder on the affected part	H	dermal
34	<i>Fuerstia africana</i> T.C.E.Fr.	Guran qaayee	Lamiaceae	Sh	W	Root and leaf	Mastitis	Pounding the roots and leaves of <i>F. africana</i> and drink it	A	Oral
35	<i>Gomphocarpus purpurascens</i> A. Rich.	Ari-Yuyo	Asclepiadaceae	Sh	W	Root	Pneumonia	Fumigate the root and /or boiling and drink it	H	Oral, Nasal
36	<i>Helichrysum elephantinum</i> Cufod.	Arado	Asteraceae	Sh	W	Leaf	Head ache	Squeezing the leaves of <i>H. elephantinum</i> and drop the liquid through nose	H	Nasal
37	<i>Impatiens rothii</i> Hook. F	Buri	Balsaminaceae	H	W	Young stem	Anthrax	Burying the young stem of <i>I. rothii</i> in the necks of affected animals	A	Dermal
						Leaf	Mastitis	Pounding and creaming on the affected animals udder	A	Dermal

Table 1. Contd.

38	<i>Jasmiium floribundum</i> L.sub sp. Floribundum (R.Br. ex. Freesen.) P.S. Green	Biluu	Oleaceae	Sh	W	Leaf, roots	Tufa	Pounding Leaves of <i>J. floribundum</i> , <i>Premna schimperi</i> , <i>R. glutinosa</i> , <i>Ehretia cymosa</i> , <i>Grewia bicolor</i> and roots of <i>C. spinarum</i> and wrap over affected body parts	H	Dermal
39	<i>Justicia schimperiana</i> (Nees) T. Anderson	Loomii	Euphorbiaceae	T	Ct	Fruit	Tonsillitis	Smash and Mix the <i>C. aurantifolia</i> juice with ash and administer it orally	H	Oral
40	<i>Lepidium sativum</i> L.	Fexxoo	Brassicaceae	H	Ct	Leaf	Febrile illness (Mich)	Squeezing the leaves of <i>Ocimum gratissimum</i> and <i>Lepidium sativum</i> and applying on the skin and /or drinking orally	H	Dermal /oral
41	<i>Myrtus communis</i> L.	Adasii	Myrtaceae	Sh	WC	Leaf	Pneumonia	Boiling the leaf and fumigating the steam	H	Nasal
42	<i>Nigella sativa</i> L.	Asmudi gurati	Ranunculaceae	H	WC	seed	Dermal Ulcer	Grinding the seeds of <i>Nigella sativa</i> and put it on affected body	H	Dermal
							Milk production	Grinding the seeds of <i>Nigella sativa</i> and mix it with water and administer orally and rub over the udder	A	Oral and dermal
							Kidney problems	Grind the seeds of <i>Nigella sativa</i> mix with honey eat every morning before meal	H	Oral
43	<i>Ocimum gratissimum</i> L.	Daamakasee	Flacourtiaceae	Sh	WC	Leaf	Tooth ache	Chewing and holding the leaves of <i>Ocimum gratissimum</i> with affected tooth part	H	Oral
							febrile illness (Mich)	Squeezing the leaf and cream over the skin	H	dermal
44	<i>Ocimum lamiifolium</i> Hochst. ex Benth	Damakesse	Lamiaceae	H	WC	Leaf	Febrile illness (Mich)	Squeezing the leaves of <i>O. lamiifolium</i> applying on the skin and /or drinking orally	H	Oral
45	<i>Pavonia patens</i> (L.F). Redoute	Hiccini	Malvaceae	Sh	W	Leaf	Intestinal Parasites (Diarrhea)	Pounding the <i>P. patenis</i> and <i>Calpurnia auria</i> leaves together and drink it	A	Oral
46	<i>Phytolacca dodocandra</i> L. Herit.	Haandoodee	Phytolacaceae	L	W	Leaf	Dermatitis	Pounding the leaf mix with oil and cream on affected body	H	Dermal
47	<i>Podocarpus falcatus</i> (Thunb.) Mirb.	Birbisa	Podocarpaceae	T	W	Leaf	Snake bite	Pounding together leaves of <i>Podocarpus falcatus</i> , <i>Cynoglossum coeruleum</i> and <i>Impatiens rothii</i> together and mix with water and drink it and put on affected body	H	Oral, Dermal
48	<i>Polygala sphenoptera</i> Fresen.	Harmel	Polygonaceae	H	W	Root	Chancroid	Boiling the root with water and drinking	H	Oral
							Mastitis	Pounding the roots of <i>Polygala sphenoptera</i> , <i>Tragia cinerea</i> , <i>Carisa Spinarum</i> , and <i>Allium sativum</i> together and mix with water and administer it in left nose	A	Nasal

Table 1. Contd.

							Anthrax	Stem of <i>I. rothii</i> , roots of <i>Senna petersiana</i> , and roots of <i>Solanum incanum</i> mixed and pounded together and administered orally	A	Oral
49	<i>Prunus persica</i> (L)Batsch	Kookii	Rosaceae	T	Ct	Shoot	Infertility	Pound the shoots of <i>Prunus persica</i> , <i>Cuminum cyminum</i> , <i>Premna schimperi</i> , <i>Croton macrostachyus</i> and <i>Rumex nervosus</i> drink with one cup of water	H	Oral
50	<i>Psidium guajava</i> L.	Zeyituna	Myrtaceae	T	Ct	Root	Diarrhea	Grinding the roots of <i>Psidium guajava</i> and <i>Impatiens rothii</i> mix with water and drink it	H	Oral
51	<i>Rhus glutinosa</i> Hochst. Ex.A.Rich	Xaxeeyisa	Anacardiaceae	T	W	Leaf	Tufa	Pounding the leaves of <i>R. glutinosa</i> , <i>Jasmiun floribundum</i> , <i>Olea europea</i> , <i>Juniperus procera</i> , <i>Croton macrostachus</i> and wrap on the affected body for Seven days.	H	Dermal
52	<i>Rhus vulgaris</i> Meikle	Dabobechaa	Anacardiaceae	Sh	W	Leaf	Hepatitis	Pounding the young leaves of <i>R. vulgaris</i> and mix with water and drinking it for seven consecutive days	H	Oral
53	<i>Rumex nervosus</i> Vahl	Dhangaggo	Polygonaceae	Sh	W	leaf	Tufa	Smashing the leaves of <i>Rumex nervosus</i> and <i>P. schimperi</i> mix with honey and administer either orally or wrap on affected body	H	Oral and dermal
54	<i>Ruta chalpensis</i> L.	Xalatom/ciraad ama	Rutaceae	H	Ct	Leaf	Psychosis	Burn the dried leaves with fire and fumigate it	H	Nasal
55	<i>Solanum incanum</i> L.	Hiddi Bude	Solanaceae	H	W	Root	Milking Phobia	<i>S. incanum</i> root, <i>Urtica simensis</i> , <i>Grewia beguinotleaf</i> and root <i>C. macrostachus</i> root and aloe leaf smashed together mixed with water and drink	A	Oral
56	<i>Suregada procera</i> (Prain) Croizat	Xilloo	Euphorbiaceae	H	W	Stem	Hemorrhoid	Burn the stem and fire it on affected area	H	Dermal
57	<i>Tragia cinerea</i> (Pax) Gilbert & Radcl. Smith	Laalessaa	Euphorbiaceae	H	W	Leaf	Inflammation	Pounding the leaves of <i>Tragia cinerea</i> and <i>Impatiens rothii</i> together with water and drinking and creaming on affected skin part	A,H	Dermal
58	<i>Urtica simensis</i> Steudel.	Dobii	Urticaceae	H	W	Root	Erectile dysfunction	Chewing the roots of <i>Urtica simensis</i> and /or pounding the root with water and drink it.	H	Oral
59	<i>Vernonia amygdalina</i> Del.	Eebicha	Asteraceae	Sh	W	Leaf	Dingetegna	Pounding the leaves of <i>V. amygdalina</i> and mix it with water and drink it	H,A	Oral
							Hepatitis	Pounding the leaves of <i>V. amygdalina</i> and mix it with water and drink it	H	Oral
							Hypertension	Pounding the leaf of <i>V. amygdalina</i> mix with water and drink it	H	Oral
60	<i>Zingiber officinale</i> Roscoe	Jinjibila	Zingiberaceae	H	Ct	Root	Alzheimer	Chewing the root part thoroughly	H	Oral
						Stem	Cough	Grinding the stem mix it with sugar and water boil it and drink it	H	Oral

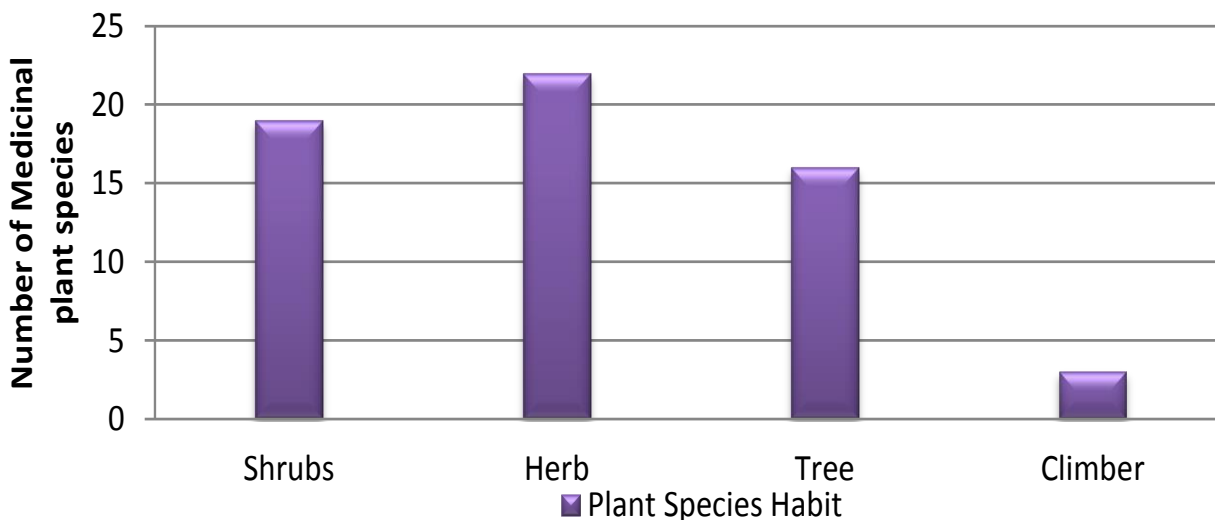


Figure 2. The growth forms of the medicinal plant species.

Table 2. Methods of cultural medicine preparation by people of the study area.

No.	Methods of preparation	Medicinal treatments	Percentage
1	Pounding	43	49.43
2	Squeezing	6	6.90
3	Roasting	2	2.30
4	Boiling	7	8.05
5	Grinding	15	17.24
6	Crushing	2	2.30
7	Smashing	4	4.59
8	Chewing	5	5.74
9	Burning	2	2.30
10	Burying	1	1.15
Total		87	100

7 (8.05%), squeezing 6 (6.90%), chewing 5 (5.74%), smashing 4 (4.59%) etc. Others collectively constitute 7 (8.0%) preparation methods.

The current finding showed that in the study area most remedies 66 (75.86%) were prepared from single plant species while, 21 (24.14%) remedies were prepared from combined plants. This finding agrees with finding of Dawit Abebe (1986) and Debela Hunde (2001).

Administration methods

In the study area, various routes of administration methods (Figure 3) were employed of which, oral administrations method leads the rank [60 (63.20%)].

Next to this dermal [26 (26.32%)] and nasal [8 (9.47%)] took the 2nd and 3rd ranks, respectively. The remaining administration method was ocular 1 (1.05%). The higher

employments of oral and dermal administration methods were in line with the works of Dawit and Ahadu (1993), Haile and Delnesaw (2007) and Kalayu et al. (2013).

Medicinal plants used for treating human and livestock ailments

Medicinal plants species used to treat human diseases

Out of the total 60 medicinal plant species collected from the study area, 55 (91.67%) species were used to treat 39 types of human diseases. Of these 55 medicinal plant species, 48 (87.27%) species were used only for human ailments and the remaining 7 (12.73%) species were used for both human and livestock treatments. These 55 medicinal plant species comprised 39 families and 53

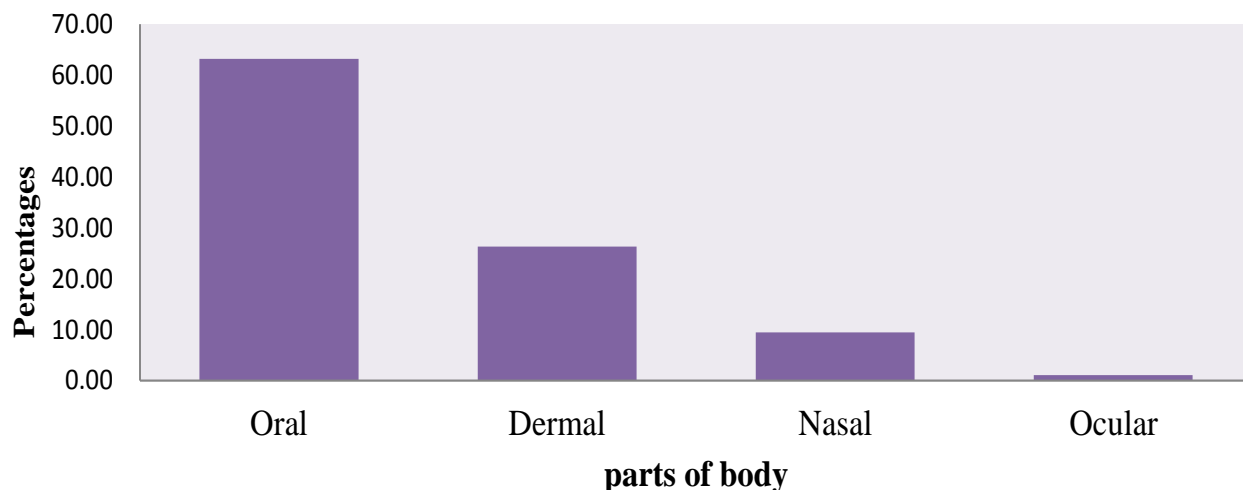


Figure 3. Cultural medicines routes of administration against human and livestock diseases.

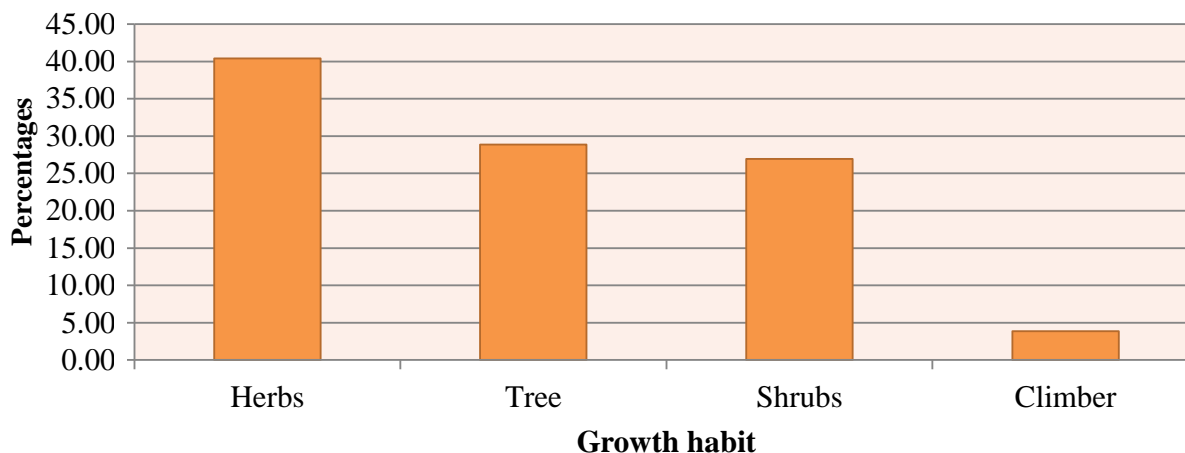


Figure 4. Proportion of medicinal plants used to treat human ailments in growth habit.

genera. The medicinal plants used for treatments of human ailments constitute herbs 20 (36.36%) species, tree 17 (30.91%) species, shrubs 15 (27.27%), species and Lianas 3 (5.45%) species (Figure 4). This indicates that most of the medicinal plants used for human ailments are herbs, trees and shrubs. This was in agreement with the works of Bayafers (2000) and Debela (2001).

Most (36 (65.45%) of the 55 medicinal plant species used for human ailments were collected from wild, followed by cultivated [11 (20 %) species], and wild and cultivated [8 (14.54)] species. This action has ecological meaning by the reduction of wild plant species. This finding also was explained by Mirutse (1999) and Bayafers (2000).

With regard to plant parts used, traditional practitioners mostly [31(46.96%)] harvested leaves and roots

[16(24.24%)] for treating human ailments (Figure 5). The other parts include seed 5(7.58%), shoots 4 (6.06%), fruits 3(4.55%), stem 3 (4.55%) and others 4(6.06%). Studies showed that the use of leaves for medicinal purpose has little effect for rare plants in the area

However, the use of root (that is, the second mostly harvested plant part) for medicinal purpose leads to the destruction of mother plants that could affect the survival and ecological aspect of the plant (Odera, 1997; Kalayu et al., 2013).

Major human diseases and plant species used for the remedies

This study showed that a total of 39 human diseases were recorded which are treated by the 55 plant species.

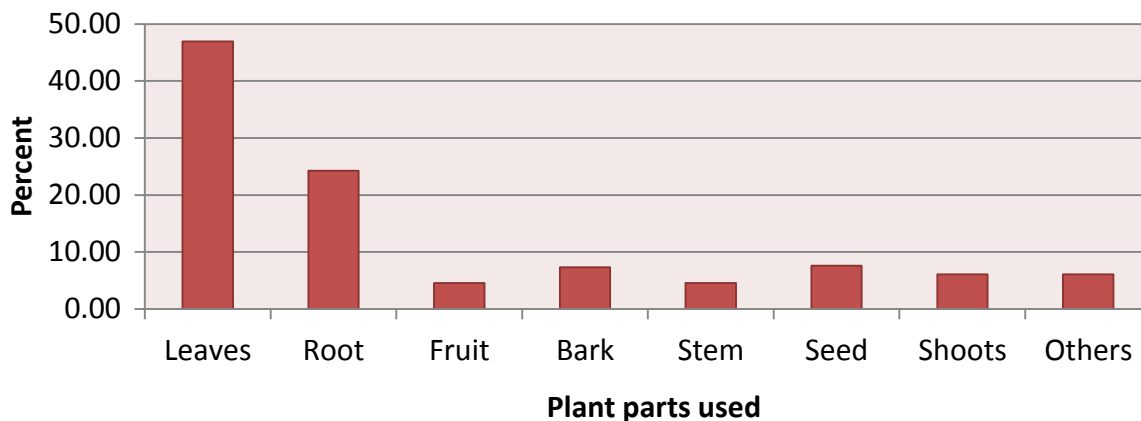


Figure 5. Medicinal plant parts used for human ailment treatments.

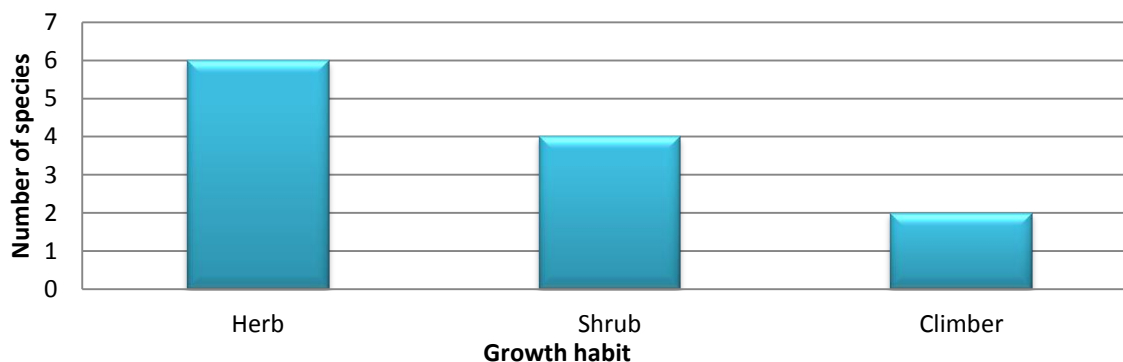


Figure 6. The growth form of medicinal plants used to treat livestock ailments.

The identified diseases may be treated by one or more species and vice versa. As a result, the total number of plants (71) used to treat human health in the study area were greater than the total number of medicinal plant (55) species which were documented for treatment of human disease. This clearly indicates that some plant species were used to treat more than one type of disease. For example; *Carissa spinarum* used to treat evil spirit, tonsillitis and snake bite. As shown in Table 4, the informants know more plant species to treat Chancroid, inflammation, fibril illness (Mich), Pneumonia, and Tufa (treated by medicines prepared from 4 and above plants each). This finding shows that about 50% of the diseases were treated with cultural medicines prepared from two or more different plant species which increases the conservation of medicinal plants.

Medicinal plants species used to treat livestock disease

Out of the total 60 medicinal plant species recorded in the study area, 12 (20%) species were used for treatment of

livestock ailment. Of which 7 (58.33%) species were used for treatment of livestock and human ailments. The remaining 5 (41.67%) species were used for treatment of only livestock ailments. These 12 species were distributed under 11 families and 12 generas. Family Solanaceae represent 2 species while the remaining 10 families were represented by single species each.

These medicinal plants which were used to treat livestock comprised herbs [6 (50%)] species, shrubs 4 (33.33%) species, and Lianas 2 (16.67%) species (Figure 6). This indicates that, most of the plant species used to treat livestock were herbs (50%) followed by shrubs [4 (33.33%)]. This finding was in agreement with the work of Etana (2007). The remaining was liana.

The rational practitioners were familiar with using varieties of plant parts to prepare different types of traditional medicines (Figure 4). Moreover, they show certain preferences over this plant parts for their medicine preparation. In doing so, the traditional practitioners mostly used leaves from 6 (46.15%) species, roots from 5 (38.46%) species, stems and seeds from 1 (7.69%) species each. This analysis clearly showed that leaves and roots were the most important plant parts used to

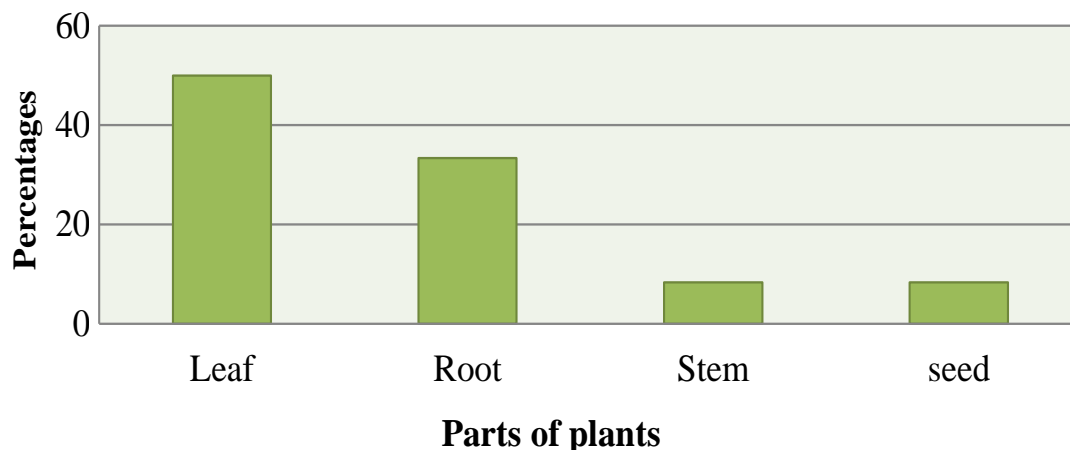


Figure 7. Medicinal plant parts used to treat livestock ailments.

treat different livestock diseases followed by seed and stem parts from single plant species each in the study area. The previous study in different part of Ethiopia also showed that leaves and roots are the most important plant parts used to treat various health problems (Dawit and Estefan's, 1991; Bayafers, 2000; Mirutse and Gobana, 2003).

Major livestock disease and number of plant species used

In the study area a total of 9 livestock diseases were recorded which are treated by the 12 plant species. The informants know more species to treat Mastitis and Anthrax.

Medicinal plant species used for both human and livestock

From the total 60 medicinal plant species recorded in the study area, 7 (3.33%) species were used for treatment of both human and livestock. These 7 species were distributed under 7 families and 7 generas. With regard to plant parts used, practitioners' harvested leaf parts from 4 plants, roots from 3 plants, fruits and seeds from single plants each to prepare the 19 cultural medicines used to treat the 14 human and livestock diseases.

Medicinal plants use report/Informant consensus

Some medicinal plants and their utilization were more popular than others. In the study area, plant species *Ocimum lamiifolium* (Damakesse) took the lead where it was cited by 42 (87.5%) informants. *Ruta chalpensis* L. (Xalatom/ciraadama) with 41 (68%) informants and *Allium sativum* L. (Qullubi addii) with 40 (66.67%)

informants took their consecutive ranks.

Informant consensus factor

The frequently observed diseases in the study area probably become the primary area of concern to treat them and therefore need to accommodate more indigenous knowledge than less frequently appeared disease categories. In the current study, most of the diseases mentioned in the table have higher ICF value. This indicates the diseases which mentioned in Table 7 were common for the study area and many people have great knowledge to cure the diseases. Among the 39 diseases encountered in the study area, only 17 of them were considered. In comparison, chancroid, cough, diarrhea, tonsillitis, diarrhea, ear ache, hemorrhoid, inflammation and tufa has the highest ICF values (0.99) each followed by Alzheimer, hepatitis, hypertension, and pneumonia with ICF value 0.98 each (Table 3).

Preference ranking

The study showed that diarrhea and tufa were among the common diseases in the study area. The highest rank was given for *Jasmiun floribundum* being as effective treatment against the disease called tufa (Table 4). *Cissampelos mucronata* and *Rhus glutinosa* took the 2nd and 3rd ranks (Table 4).

Paired comparison

Paired comparison was used to evaluate the degree of preference of 5 reported medicinal plants for treating chancroid following Martin (1995). Therefore the study identified that *C. cyminum* is the most preferred plant species used to treat chancroid in the study area.

Table 3. Informant consensus factor for certain disease

No.	Disease treated	No. of plant species used	No. of use citations	ICF value
1	Abdominal pain	2	40	0.97
2	Alzheimer	2	50	0.98
3	Chancroid	5	130	0.99
4	Cough	3	80	0.99
5	Diarrhea	2	135	0.99
6	Dingetegna	3	111	0.99
7	Ear ache	2	93	0.99
8	Hemorrhoids	3	75	0.99
9	Hepatitis	2	66	0.98
10	Hypertension	2	60	0.98
11	Inflammation	4	152	0.99
12	Fibrill Illness (Mich)	4	188	0.99
13	Pneumonia	4	45	0.98
14	Tonsilitis	2	70	0.99
15	Tooth ache	2	34	0.97
16	Tufa	4	198	0.99
17	Urinary problem	2	28	0.96

Table 4. Medicinal plant species preference ranking by informants to treat Tufa.

Plant species used to treat Tufa	Respondents										total	Rank
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10		
<i>Cissampelos mucronata</i>	8	8	9	7	8	9	8	9	6	8	80	2
<i>Jasmiun floribundum</i>	10	9	10	8	9	10	7	10	7	7	87	1
<i>Rhus glutinosa</i>	7	7	8	9	8	8	7	8	5	6	73	3
<i>Rumex nervosus</i> Vahl	6	7	8	7	8	7	6	7	5	7	68	4

Table 5. Paired comparison of medicinal plants used to treat Chancroid.

Plant species used to treat diarrhea	R1-R6					Total	Rank
<i>Cuminum cyminum</i>	-	Cc	Cc	Cc	Cc	4X	1
<i>Polygala sphenoptera</i>	-	-	Ps	Ps	Ps	3X	2
<i>Achyranthes aspera</i>	-	-	-	Aa	Aa	2X	3
<i>Asparagus africanus</i> Lam	-	-	-	-	Aal	1X	4
<i>Euclea racemosa</i> subsp. Schimperii	-	-	-	-	-	0X	5

Similarly, *Polygala sphenoptera* and *Achyranthes aspera* were cited as the second and third ranked plants respectively to treat Chancroid (Table 5).

Acquisition and transfer of indigenous knowledge on medicinal plants

In the study area, most 36 (75%) of the informants who have acquired the knowledge on medicinal plants were from their parents and close relatives. The other 12 (12%) got the knowledge by reading different written

material, by giving incentive for elders and by trial and error. Most of the informants 39 (81.25%) have already trained their family and close relatives. Some informants require incentive to give their knowledge for other person.

Threat to medicinal plants

In the study area, the survival of medicinal plants affected by both natural (dry time) and anthropogenic (fire wood, overgrazing, agricultural expansion, construction and medicine) activates. In the study area, the most threat for

Table 6. Priority ranking factors perceived as threats to medicinal plants based on the level of destructive effects (values 1-7 were given: 1 is the least destructive threat, and 7 is the most destructive threat).

Factor	Respondents							Total	Rank
	R1	R2	R3	R4	R5	R6	R7		
Dry season	5	6	3	4	6	5	6	35	3 rd
Land slide	2	3	1	1	1	1	1	10	7 th
Fire wood	6	7	7	6	5	4	6	41	2 nd
Over grazing	3	5	5	2	3	2	3	23	5 th
Agriculture	7	4	6	7	7	7	5	43	1 st
Construction	4	2	4	5	4	6	7	32	4 th
Medicinal value	1	1	2	3	2	3	2	14	6 th

distraction of medicinal plants is agricultural expansion. The 2nd and 3rd threats for disappearance of medicinal plants were fire wood and dry season (Table 6).

Conclusion

In this study, 60 plant species with medicinal value distributed under 42 families and 58 genera were identified and documented. This shows the area is rich in plant diversity. About 39 (65%) species were found from wild vegetation, 11 (18.33%) species were cultivated and the remaining 10 (16.67%) were both cultivated and wild vegetation. Herbs were the dominant growth forms used for the preparations of traditional remedies followed by shrubs.

In the study area, 48 ailments were reported (39 for human and 9 for livestock) to be treated by traditional medicinal plants of the area. As indicated by informants, high numbers of medicinal plant species were applied for treatment of the informants know more plant species to treat chancroid, inflammation, fibril illness (Mich), pneumonia, and tufa (treated by medicines prepared from 4 and above plants) for human and mastitis and anthrax for livestock.

Humans and natural factors are the major threats to plant species in general and to the medicinal plants in particular in the study area. As suggested by most informants, in the area, the human induced threats including agricultural expansion, fire wood, construction, over grazing, and natural factors such as extended dry times were cited to be major threats for reduction of medicinal plants.

Recommendations

1. The indigenous knowledge and skill of traditional medicine practitioners must be encouraged and protected. This could be the way through which such people could exercise their knowledge boldly.
2. Establishing conservation measures strategies to

ensure the sustainability of multipurpose and widely used medicinal plants as most medicinal plants are obtained from the wild.

3. Create awareness of the local people on the magnitude of loss of medicinal plants and associated knowledge to ensure sustainable harvesting of medicinal plants.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The author extends their thanks to Oda Bultum University for supporting with financial and logistic grants. They would like to express their deep sense of gratitude to the traditional healers for sharing their indigenous knowledge and invaluable time. Great thanks go to Mr. Getachew Bayable for the constructing map of the study area. The authors owe their sincere gratitude to Mr. Kaleab Terefe who gave constructive comments during the review session of the paper.

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