REVIEW ON VARIATION IN GENETIC AND CHEMICAL CONSTITUENTS OF *Strychnos henningsii* POPULATIONS IN KENYA

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Abstract

Strychnos henningsii is an indigenous medicinal plant species widely used in tropical Africa. Studies have revealed that this plant has been used as a remedy for various ailments including rheumatism, gastrointestinal complications, abdominal pains, syphilis, snakebites, diabetes malaria, and arthritis amongst others. Phytochemical and pharmacological studies have identified various compounds such as alkaloids, anthraquinones, cardiac glycosides, chalcones, flavonoids, phenolics, proanthocyanidins, saponins, steroids, tannins and triterpenes from the crude extracts of S. henningsii. These chemical constituents exhibited analgesic, antibacterial, antidiabetic, anti-inflammatory, antioxidant, antiplasmodial, antiprotozoal, antispasmodic as well as cytotoxicity activities. Secondary metabolites are known to aid plants in coping with various environmental stresses. Environmental stress triggers expression of genes for the enzymes involved in biosynthesis of secondary metabolites, many of which have higher medicinal value despite being useful in plant defense mechanisms. This paper is a review on the chemical constituents, pharmacological properties and genetic variation of S henningsii across its geographical range.

Key Words: *Strychnos henningsii, chemical constituents, genetic, medicinal, variation.*

INTRODUCTION

Botanical information of *S. henningsii.*

S. henningsii belongs to the family Strychnine but was earlier included in the family Loganiaceae. The species epithet honors Professor Paul Christopher Henning, a mycologist at the Royal Botanical Gardens, Berlin-Dahlem. The common names are Red bitter berry (English) (Gachathi, 2007), Henning's Strychnos (Maundu and Tengäs, 2005). The local names include Muteta (Kikuyu and Kamba), Maset (Kipsigis), Entuyesi (Maasai), Mutambi (Mbeere), Muchimbi (Meru), Kapkamkam (Pokot), Nchipilikwa (Samburu), Hadesa (Somali), Turukukwa (Tugen) and Yapoliss (Turkana) (Maundu and Tengäs, 2005).

It varies in size from a shrub or small erect tree, much-branched tree of about 2 to 15 m tall with green-reddish stem. The bark is pale grey and smooth in young trees but becomes dark brown and somewhat flaky in specimens. The twigs have pale ashy or straw-colored and waxy skin splitting lengthwise. Lenticles are few and inconspicuous. Leaves are opposite, sub-sessile or ovate, 2.5 to 6.5 cm long and 0.8 to 4.5 cm wide. They have an entire margin and acuminate leaf tips. The leaves are strongly; three to five nerved from base cuneate or rarely sub-cordate at base; a characteristic feature in Strychnos species (Van wyk et al., 1997). Floral cymes are borne on flat clusters in the leaf axils, 2 to 2.5 mm long and 4 mm wide when open, scented, yellowish-green in color turning orange with age. The ovary is globose with a short style. The fruit is up to 1.9 cm long and 6 to 11 cm wide, oblong or roundish with one to two seeds (coffee-like) red, brown or orange when ripe (Figure 1) (Beentje, 1994; Gachathi, 2007; Maundu and Tengäs 2005).

S. henningsii is a semi-deciduous plant commonly occuring in the dry and moist forests, wooded hillsides and thickets, on rocky hills, coastal forests and stream banks. It is native to Angola, Mozambique, South Africa, Swaziland, Tanzania and Uganda. In Kenya, it is widely distributed in Nairobi, Kakamega, and in the Central province. It is often associated with dry

Podocarpus and Olea forests, hillsides, thickets and *Combretum* bushland (Maundu and Tengäs, 2005**)**. It is raised from seedlings or wildings. The species also suckers well. The pulp is removed before sowing the seeds. The seeds exhibit orthodox storage behavior. It is managed through pruning and coppicing (Maundu and Tengäs, 2005).

In the African traditional medicine, it is used for treatment of various ailments including rheumatism, gastrointestinal complications, abdominal pains, syphilis, and possibly of value in dysmenorrhoea (Hutchings, 1989; Watt and Breyer, 1962; Pujol, 1993; Hutchings, 1996; Oyedemi *et al.,* 2009). Root's bark and green fruits of Strychnos species are used as a remedy for snakebites (Tits *et al*., 1991; Van Wyk *et al*., 1997) and hookworm infections in Tanzania (Oyedemi *et al.,* 2009). The bark decoction is employed as a remedy for rheumatism and arthritis (Palgrave, 1988; Beentje, 1994). A decoction of the plant has been used in traditional Kenyan medicine for the treatment of rheumatism, gynecological complaints, chest pain, internal injuries and malaria (Kareru *et al*., 2007). The ground bark is a mouth antiseptic and applied on the wounds in cattle and horses to hasten healing (Gachathi, 2007). In South Africa, the decoction or infusions from the stem bark is widely used for the management Diabetes mellitus (Oyedemi *et al.,* 2009). The aqueous bark extract is also used in South Africa for the treatment of stomach, colic, dizziness and as a purgative agent (Oyedemi *et al.,* 2013). Almost all parts of *S. henningsii* are used as a source of medicine, however studies conducted elsewhere (Alfred, 2021 and Kuria et al., 2012) revealed that the roots, stem and the bark are the most commonly used parts for medicinal purposes in different parts of Africa. This plant species is mainly used as an anthelmintic, appetizer, blood cleanser, purgative, and tonic as well as in ethnoveterinary medicine (Alfred, 2021). In traditional medicine, it is mainly used as a remedy for abdominal pain, bilharziasis, colic, diabetes mellitus, gastro-intestinal complications, headache, malaria, menstrual problems, respiratory diseases, rheumatism, snake bites and syphilis (Alfred, 2021 and Kuria *et al*., 2012).

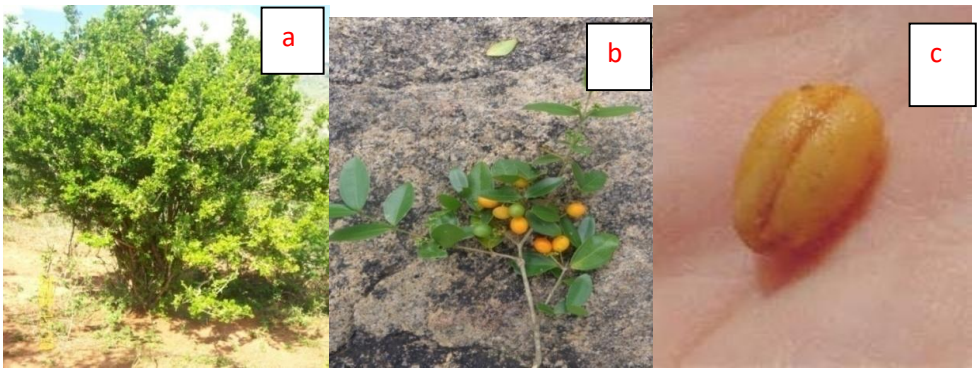

Figure 1 a, b and c: *S. henningsii* Shrub, fruits and seed

Variation in chemical constituents and pharmacological properties of *S. henningsii*

Various studies conducted by different authors have revealed various bioactive compounds isolated from different parts of *S. henningsii*. Such compounds include the indolinic alkaloids, strychnine, brucine, curanine, and bitter glycosides with significant values (Penelle *et al.*, 2000; Oyedemi *et al.*, 2010a). Other compounds including holstine, diaboline, strychnochromine and guianensine have been isolated from the stem and root bark of *S. henningsii* (Angenot and Tits, 1981). A research conducted by (Alfred, 2021) also revealed a wide range of biological compounds such as alkaloids, anthraquinones, cardiac glycosides, chalcones, flavones, flavonoids, flavonols, phenolics, proanthocyanidins, saponins, steroids, sterols, tannins and triterpenes produced by this plants species. These compounds have been isolated from the bark, leaves, roots, root bark, stem bark and twigs of *S. henningsii*. Some of these phytochemical compounds may be responsible for the various pharmacological properties exhibited by *S. henningsii.*

Pharmacological studies have shown that the different phytochemical compounds identified from extracts of *S. henningsii* have various biological activities. They included antibacterial (Tirop et al., 2019; Njire et al., 2010), antidiabetic (Ngugi *et al.*, 2011; Oyedemi *et al.*, 2012; 2013), anti-inflammatory (Tits *et al.*, 1991), antioxidant, (Oyedemi *et al.*, 2010a; 2013)

antiplasmodial (Phillipe *et al.,* 2005; Kirira *et al.,* 2006; Frederich *et al.,* 1999), antiprotozoal (Wright *et al*., 1994), antispasmodic (Tits *et al.,* 1991), cytotoxicity (Oyedemi *et al.,* 2012 and 2013) and toxicity (Ogeto *et al.,* 1984; Oyedemi *et al.,* 2010a; Tirop *et al.,* 2018). Although there are several reports about phytochemical constituents and pharmacological properties of *S. henningsii*, information about the variation of these chemical constituents of *S. henningsii* based on its geographical location is unavailable.

Plants of the same species growing in different geographical locations are subjected to a wide range of biotic and abiotic environmental factors (Hartmann *et al*., 2005; Rapinski *et al*., 2014 and 2015; Baille *et al*., 2016; Mahmoud *et al.,* 2016). These environmental factors trigger an adaptive response by stimulating gene expression for enzymes responsible for production of a wide array of secondary metabolites in plants which in turn may have medicinal value (Mahmoud *et al.,* 2016). The environmental stressors include temperature, low precipitation, solar radiation as well as edaphic factors. These environmental factors are subject to latitudinal, longitudinal and altitudinal gradients and hence the differences in the chemical constituents of plants (Dixon *et al*., 2006; Asensio *et al*., 2020).

There is a general perception among the traditional healers and elders that plants in the Northern latitude and coastal regions are more efficient sources of traditional medicine as well as higher altitude because they accumulate higher concentrations of secondary metabolites (Baille *et al*., 2016). For instance, higher contents of rutin were reported in the populations of *Casearia sylvestris* (SW) growing in Savannah (poor soils and higher solar radiation) as well as those in higher altitudes (Silva *et al*., 2006). Plants growing in higher altitudes were reported to contain high levels of flavonoids in *Calluna vulgaris* populations (Monschein *et al*., 2010), *Arnica montana* (Perry, *et al*., 2009) and *Quera robur* (Abdala-Roberts *et al.,* 2016). Higher contents of flavonoids and anthocyanins were reported in plants growing in higher latitude due to longer daylight periods and lower night temperature (Lätti *et al*., 2010). Higher levels of phenolic compounds were also reported in Bearberry plants growing

in the areas of higher radiation and temperature (Asensio *et al*., 2020). The island populations of *Prunus Africana* have been overexploited for medicinal purposes than the inland populations (Kadu *et al*., 2012).

S. henningsii is widely distributed in the tropical and subtropical areas in Africa. It occurs in wooded and open forests from sea level up to 2200 m altitude (Ruijter *et al*., 2008). This may explain the wide range of chemical variation from individuals of this plant species from various geographical locations. Additionally, ethnobotanical studies also revealed that different parts of this plant species are used as sources of medicine in different geographical areas (Kuria *et al*., 2012 and Alfred 2021). Plants exhibit differences in their chemical components not only according to their locality but their tissue types as well (Fraster *et al*., 2007). Differences in phenolic compounds and activities observed according to tissue type were supported by traditional healers who used decoctions from specific parts of the plant for different symptoms (Fraster *et al*., 2007). Inner bark is more preferred medicinally because it tends to show higher degree of antioxidant activity than leaves (McCune and Johns 2007; Fraster *et al*., 2007; Rapinski *et al*., 2014 and 2015). Ethnobotanical studies of *S. henningsii* revealed that the roots and the stem were the most widely used plant parts for medicinal purposes in the areas of study (Kuria *et al.,* 2012).

Further genetic variation in plants is partially reflected in the variation of concentrations and types of chemical constituents produced in a plant species growing in different geographical locations (Baille *et al*., 2016). This is because the genetic makeup of plants provides the ability or inability to synthesis certain compounds (Chaplain, 1975; Baille *et al*., 2016) and such potential depends on the differences in the environmental conditions prevailing in the geographical location of specific plant population (McCune and Johns 2007; Theis and Lerdau, 2003; Dixon and Paiva, 1995; Figueredo *et al.,* 2008; Fraster *et al*., 2007).

In literature, there is no available information explaining association between genetic variation and the phytochemical constituents of *S. henningsii*. Evaluating the link between

genetic diversity and chemical constituents of this plant will provide useful insight for designing strategies for sustainable utilization and conservation of this important plant species. Additionally, this information will also be useful in developing drugs from populations that show higher potential as a remedy for various ailments.

Genetic variation of *S. henningsii* **from different populations in Kenya**

Genetic variation accounts for the chemical diversity in plants (Moore *et al*., 2014). Genetic diversity promotes the adaptation of organisms to environmental conditions (Onda *et al.,* 2016). Environmental factors such as soil nutrients, temperature, water availability and light amongst others influence the genetic and chemical diversity of plant populations (Pacheco-Hernández *et al.,* 2021). These environmental conditions exert strong selective pressures that could influence the evolutionary course of plant populations (Pacheco-Hernández *et al.,* 2021). This natural phenomenon causes plant populations consisting of single species to show varied genetic patterns and chemical variations in different geographical locations (Chen *et al.,* 2015). Expression of genes for enzymes involved in production of secondary metabolites in plants varies and it's higher in plants subjected to areas characterized with stressful environmental conditions (Baille *et al*., 2016). Plants adapt to new environmental conditions due to their genetic variation that may be associated with specific chemical compounds produced (Via and Conner, 1995; Younsi *et al.,* 2018). Maintenance of genetic diversity of plant species is vital for selecting the best fit (adaptable) individuals and self-sustaining populations (Reed and Frankham, 2003).

The effects of genetic variation in the biosynthesis of secondary metabolites in medicinal plants have been reviewed (Iannicelli et al., 2020). Variation in genetic and chemical constituents has been reported in various plants (Silva *et al*., 2006; Khan *et al*., 2017; Asensio *et al*., 2020). High contents of flavonoids and anthocyanins were reported in plants growing in high latitude due to longer daylight durations and lower night temperatures (Lätti *et al*., 2010). High contents of phenolic compounds in bearberry plants were also reported in plant

species growing in areas of higher radiation and temperature (Asensio *et al*., 2020). Altitudinal variations also influence production of secondary metabolites, for instance higher levels of flavonoids were reported in *Calluns vulgaris* populations growing in higher altitudes, *Arnica Montana* (Perry *et al*., 2009), *Quera robur* (Abdala-Roberts *et al*., 2016) and in *Casearia sylvestris* (SW). Silvia *et al*., (2006) reported higher levels of rutin production in plants growing in high altitude and savannah regions.

A study on genetic diversity of *S. henningsii* was conducted by (Kuria *et al*., (2018) using ISSR markers (Figure 2). Nine populations were selected from areas identified from the following places: Taita-Taveta (Mwache forest), Kilifi (Arabuko Sokoke forest), Narok (Tipilikwani forest in Talek near Maasai Mara game reserve), Baringo (Tugen hills), Kitui (Ndumooni hills), Marsabit (Marsabit forest reserve), Nyeri (Kabiruini forest), Kiambu (Karura forest) and Kajiado (Ngong forest)**.** Each population comprised thirty individuals and therefore a total of two hundred and seventy individuals were randomly selected from the nine populations to conduct a study on genetic diversity of *S. henningsii* in Kenya. Nine markers that gave clear and reproducible bands were selected to help determine the genetic diversity among *S. henningsii* populations.

ISSR markers have been successfully used in other studies to determine the genetic diversity of medicinal plant species such as in *Croton heliotropiifolius* in (Rocha *et al*., 2016), *Varronia curassavica* (Jacq.) in (Brito *et al*., 2016), Rheum spp, in (Tabin *et al*., 2016), *Withania Somnifera* in (Khan and Shah 2016) and *Croton tetrandenius* in (Almeida-Pereira *et al*., 2017) amongst others.

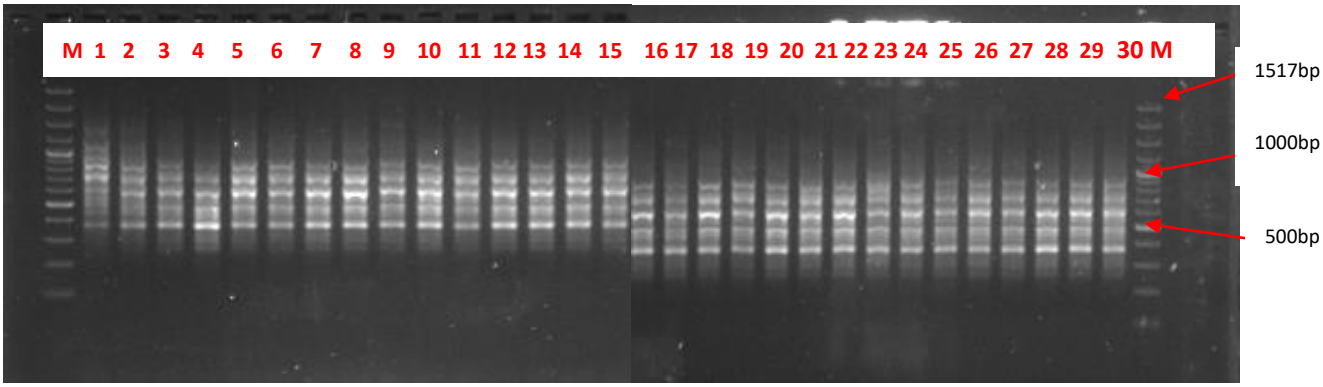

Figure 2: ISSR marker profile of amplified loci of samples from Baringo population using primer 862. Lane (1-30) are samples, M:-Marker DNA 100bp ladder

In this study, ISSR detected and amplified a total of 96 loci among *S. henningsii* genotypes, all of which were polymorphic. The mean percentage of polymorphism detected was 43.40%. The most polymorphic population was Ngong with 51 polymorphic loci (53.12 %) while Baringo was the least polymorphic population with 28 polymorphic loci (29.17%) (Table 1). Similar results were reported in other studies on genetic diversity using ISSR markers. For example, a percentage polymorphism of 42.47% was revealed in *Costus pictus* (Naik *et al*., 2017) and 59.13% percentage polymorphism in *Peganum harmals L.* (Zebarjadi *et al*., 2016). High polymorphism (94.8%) was reported in *Croton tetradenius* (Almeida-Pereira *et al*., 2017), 93.4% in *Ziziphus sphi-christi* l. (Alansi *et al*., 2016) and 76.1% in *Thuja sutchuenensis* (Liu *et al*., 2013). Other studies reported low polymorphism using the same markers such as 24.36% in *Bruguiera gymnorrhiza* and 12.73% in *Heritiera fomes* (Dasgupta *et al*., 2015).

Table 1 Genetic diversity analysis of nine populations of *S. henningsii* as revealed by ISSR markers in GenAlex software.

ISSR Markers								
Population	**%P**	**N**	**Na**	**Ne**	**I**	**He**	**UHe**	**PSL**
Kitui	43.75 %	30.00 0	0.917	1.307	0.251	0.172	0.175	5.000

	41.67 %	30.00 0	0.865	1.255	0.219	0.147	0.149	1.000
Marsabit								
	29.17 %	30.00 0	0.688	1.159	0.145	0.096	0.097	0.000
Baringo								
	39.58 %	30.00 0	0.802	1.282	0.232	0.159	0.162	0.000
Nyeri								
	42.71 %	30.00 0	0.885	1.271	0.230	0.156	0.158	2.000
Narok								
	51.04 %	30.00 0	1.063	1.376	0.299	0.207	0.211	2.000
Karura								
	53.13 %	30.00 0	1.115	1.315	0.273	0.183	0.186	2.000
Ngong								
	37.50 %	30.00 0	0.781	1.235	0.203	0.137	0.139	0.000
Jilore								
	52.08 %	30.00 0	1.052	1.298	0.267	0.177	0.180	2.000
Taveta								
	43.40 %	30.00 0	0.907	1.278	0.236	0.159	0.162	
Mean								

N= population size, PPL= population polymorphic loci, % P= percentage polymorphism, Na= Number of observed alleles, Ne= number of effective alleles, H= Nei's genetic diversity, I= Shannon information indices, He= expected Heterozygosity, UHe= unbiased expected Heterozygosity, PSL=population specific loci.

According to Nei (1978), percentage polymorphism is not a significant measure of genetic variation despite being the most commonly used indicator of genetic variation in many studies on natural population and that the parameter of genetic diversity (H) is more

appropriate. The values for genetic diversity (H) and Shannon index (I) ranged from (0.0955 – 0.1828 and 0.1448-0.2728 respectively according to a study conducted by Kuria *et al*., (2018) (Table 2). According to genetic diversity and Shannon index values this study showed that the Ngong population was the most diverse while Baringo was the least diverse. These values indicate a low genetic (allelic) diversity for *S. henningsii* populations. The results obtained could be attributed to the pollination, propagation and seed dispersal mechanisms.

Table 2 Genetic diversity analysis of nine populations of *S. henningsii* has revealed using ISSR markers in PopGene software

ISSR Markers							
Population	**N**	**PPL**	**%P**	**Na***	**Ne***	**H***	**I***
Kitui	30	42	43.75	1.4375	1.3067	0.1720	0.2514
Marsabit	30	40	41.67	1.4167	1.2548	0.1469	0.2189
Baringo	30	28	29.17	1.2917	1.1594	0.0955	0.1448
Nyeri	30	38	39.58	1.3958	1.2823	0.1590	0.2317
Narok	30	41	42.71	1.4271	1.2715	0.1558	0.2303
Karura	30	49	51.04	1.5104	1.3764	0.2071	0.2994
Ngong	30	51	53.12	1.5312	1.3148	0.1828	0.2728
Jilore	30	36	37.5	1.3750	1.2346	1.1366	0.2030
Taveta	30	50	52.08	1.5208	1.2977	0.1773	0.2673
Overall	270	96	100	2.0000	1.4683	0.2889	0.4473

Key words:

N= population size, PPL= population polymorphic loci, % P= percentage polymorphism, Na = Number of observed alleles, Ne = number of effective alleles, H= Nei's genetic diversity, I = Shannon information indices.

This plant species has cleistogamous reproduction (self-pollinating) (Bruce and Lewis, 1960). It bears small and brightly colored flowers which indicate a high possibility of entomophilous pollination. Insects transfer pollen for short distance mainly on flowers in a single tree resulting in the production of inbred seeds with poor germination (Bryndum and Hedgegart, 1969; Mathew *et al.,* 1987; Indira and Mohandas, 2002; Tangmitcharoen *et al.,* 2009). *S. henningsii* is also known to have restricted geographical zones within its natural environment. All these factors may have contributed to the low genetic diversity due to the narrow and common gene pools in the populations of this plant species.

Analysis of molecular variance (AMOVA) revealed a higher genetic variation $p < 0.001$ (58 %) among than within (42%) the *S. henningsii* provenances (Figure 3). This was possibly due to the self-pollinating nature of the species. Khan and Shah, (2016) reported a higher genetic variation among populations than within population in *Withania somnifera*, a self-pollinating plant species. Genetic drift may have also contributed to the higher genetic variation due to loss of some alleles through successive generations. The preserved alleles may be responsible for the adaptation of this plant species in its specific but wide geographical distribution range from the sea level up to about 2220 m above sea level. The geographical locations vary in the environmental conditions hence the individuals from the different geographical areas (populations) cope up differently by producing various chemical compounds that aid in the adaptation process (Baille *et al*., 2016). This may explain the wide range of chemical constituents produced by this plant species.

Habitat fragmentation and reduction in population size in the wild medicinal plants as a result of over exploitation is one of the main causes of increased genetic differentiation among populations and reduced gene flow between populations (Panda *et al*., 2015). In the study conducted by (Kuria *et al.,* 2018), Kitui, Taita–Taveta and Nyeri populations revealed

higher genetic variation due to the reduction in gene pool within these populations as a result of over exploitation for medicinal purposes.

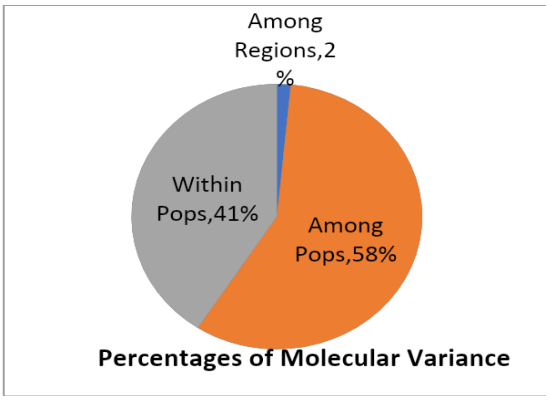

Figure 3 Percentage of Molecular variance of ISSR data

Cluster analysis of ISSR data based on the Nei's, (1978), unbiased genetic distance generated a dendrogram with three major groups. Cluster I consisted of three populations namely Kitui, Ngong and Jilore. Cluster II consisted of five populations (Marsabit, Taveta, Nyeri, Narok and Karura) and cluster III consisted of Baringo population (Kuria *et al.,* 2018) (Figure 4). The Principal Coordinate Analysis confirmed the results of the clustering analysis where there was dispersion in the genetically diverse populations (Figure 5). However, the UPGMA and PCA analyses did not reveal a clear pattern of clustering and the geographical trend among the populations (Figure 4 and 5). Therefore, genetic divergence did not match to the geographical places of collection. The lack of correlation between genetic distance and geographical locations indicate that genetic drift has played a significant role in shaping the genetic structure and variation among populations of *S. henningsii* (Fischer *et al.,* 2000).

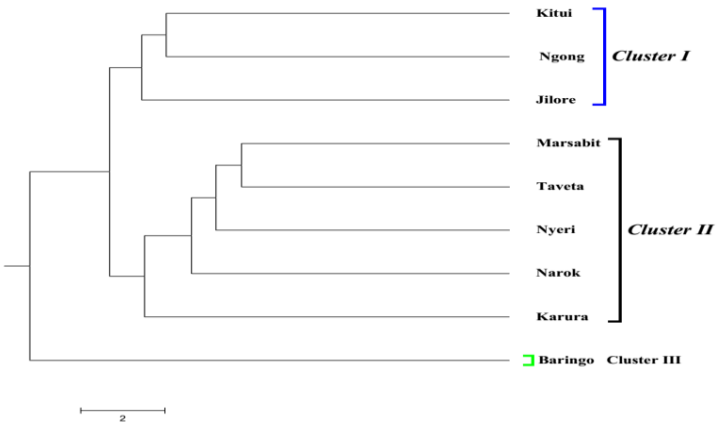

Figure 4 UPGMA clustering analysis of nine *S. henningsii* populations based on Nei's (1978) unbiased genetic distance

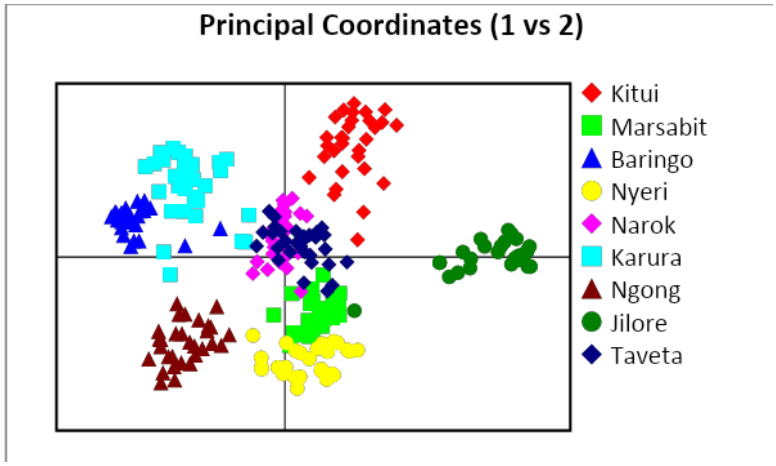

Figure 5 a three dimensional plot of the Principal Coordinate Analysis (PCA) of ISSR data showing the clustering of *S. henningsii* populations.

RECOMMENDATIONS AND CONCLUSIONS

Strychnos henningsii is a traditional medicinal plant species widely used in tropical Africa. Overview of this plant species has revealed that it has been used as a remedy for various ailments including rheumatism, gastrointestinal complications, abdominal pains, syphilis, among others in African traditional medicine. It is a source of several important chemical constituents which could possibly be responsible for its various pharmacological activities

exhibited by this plant species. Genetic diversity study revealed higher genetic variation among the populations than within the population. However, there are no reports describing association between the variation in genetic and chemical constituents of this plant species across its geographical range. There is therefore a need for further studies to provide more insights in the conservation strategies of genotypes that show superiority in their genetic and chemical constituent's variability. These genotypes can serve as sources of raw material for the development of new drugs that could be useful in treatment of chronic diseases in both medical and veterinary institutions.

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