International Journal of Sustainable Biome and Life Care
Volume No. 1; Issue No.4, July 2025, Pages 8-14
https://doi.org/10.31632/ijsblc.2025.v01i04.002

International Journal of Sustainable Biome and Life Care

Online ISSN: 2581-4877

Journal homepage https://ijsblc.org

Original Article

The Use of African Senna (*Senna didymobotrya)* Extract in the Treatment of H.I.V In Rodents

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Abstract

Senna *didymobotrya* belonging to the family Fabaceae (Leguminosae) is a widely used medicinal plant in East Africa, its potential as a medicinal plant is being exploited by traditional practitioners in different part of the world. The study is aimed at investigate the use of *Senna didymobotrya* extract in the treatment of HIV through the following objectives which are: to carry out phytochemicals screening of *Senna didymobotrya* leaves extract and to test the effectiveness of the plant leaves on HIV virus using rabbit as study animal. Method used was the leaves extract of *Senna didymobotrya* was successively extracted using n-hexane, ethyl acetate, methanol and distilled water. The phytochemical screening shows the presence of alkaloids, flavonoids, Terpenoids, anthraquinone, saponins, tannins, phenol, glycosides, and steroids. The leaves extract was tested for its Anti-HIV activity using experimental rabbit and RVS strip. The result of the experiment shows that the extract has a potential anti-HIV activity.

Keywords: *Extract; H.I.V and Rodents; Senna didymobotrya; Treatment*

Introduction

Senna didymobotrya belonging to the family Fabaceae *S* (Leguminosae) is a widely used medicinal plant in East Africa (Anywar *et al*., 2020). Worldwide, its potential as a medicinal plant is being exploited by traditional practitioners. In Kenya, Kipsigis community has traditionally been using these plants to control malaria as well as diarrhea (Anywar *et al*., 2020). In addition, it has been used to treat skin conditions of humans and livestock infections as well. In Congo, Rwanda, Burundi, Kenya, Uganda, and Tanzania, root decoction of these plants was used for the treatment of malaria, other fevers, jaundice and intestinal worm (Atieno, 2023) The roots or leaves are mixed with water or decoction of fresh parts is used to treat abscess of the skeletal muscle and venereal diseases. The plant is also useful for the treatment of fungal, bacterial infections, hypertension, hemorrhoids, sickle cell anemia, a range of women's diseases such as inflammation of fallopian tubes, fibroids and backache, to stimulate lactation and to induce uterine contraction and abortion (Husen, 2021). In the present study, *S. didymobotrya* root extract was selected for investigation because it is routinely used to cure diarrhea in the Kipsigis traditional medical practice. It is, therefore, of interest to scientifically evaluate its cytotoxicity and its effect on amoebiasis for potential ant amoebic activity *in vitro* and *in vivo*. The effectiveness of different solvents on the plant extract was also investigated (Jemutai Kotut, 2024).

Received on :21st May 2025; Revised version received on :15th June 2025; Accepted:207th June 2025

Senna didymobotrya is an evergreen rounded shrub or a small tree, growing up to 3m tall. The young stems are softly down. The leaves are alternate and compound with 8-18 pairs of opposite leaflets; these are oblong-elliptic with a rounded, asymmetric base, and a rounded apex with a short, sharp point. There are no glands on the leaf stalks. When crushed, the leaves smell somewhat like peanut butter (Nyamwamu, 2015). The rather large stipules are egg or heart shaped. Senna *Didymobotrya* flowers plentifully in showy upright clusters of flowers in the upper part of the branches. The petals are brown, and the petals are bright yellow. The fruit is a flattened blackish-brown pod with a short "beak" at the tip. It encloses 9-16 oblong seeds with a distinct elongated areole (sort of spot) on each side (Mongalo & Raletsena, 2023).

Table 1: Classification of Senna Didymobotrya

Kingdom	**Plantae**
Clade	Tracheophytes
Clade	Angiosperms
Clade	Eudicots
Clade	Rosids
Order	Fabales
Family	Fabaceae
Subfamily	Caesalpinioideae
Tribe	Cassieae
Genus	Senna mill

Senna didymobotrya is native to tropical Africa where it is found from Congo east to Ethiopia and south to Namibia, Zimbabwe and Mozambique. It has been introduced as an ornamental plant into many tropical countries including the Comoros, Madagascar, and Mauritius, South Africa and Nigeria. It was originally introduced into tropical Asia and America as a fodder, green manure & cover crop, but is now mainly cultivated as an ornamental. Senna *didymobotrya.* Presently is one of the 18 Senna species growing in Ethiopia (Husen, 2021).

The plant is known for its value in traditional medicine it is usually a several- stemmed shrub or small tree, 0.5- 5(-9) m tall. It is common in deciduous bush land, along lake shores, streams, rivers and other damp localities, in grassland and woodland, from sea-level up to 2500 m altitude. Sometimes it is found in old plantations and in hedges near buildings (Atieno, 2023).

In South Africa, it has become invasive in grassland, coastal scrub, woodland and on river banks. *Senna didymobotrya* is known for its anthraquinone glycosides and laxative action. The anthraquinone molecules have remarkable biological properties. Leaves and roots contain a number of anthraquinones, choline, and the trisaccharide raffinose. It is commonly used as a stupefacient poison for fishing (Jemutai Kotut, 2024). A decoction of the leaves is used against stomach complaints and treatment of ringworm infections, as a laxative and in the treatment of leprosy and syphilis. It is also used in the treatment of animal diseases such as removal of ticks (Maina *et al*., 2024).

The use of medicinal plants is as old as man (Maina *et al*., 2024). In the past few decades, medicinal plants have been tested extensively and found to have several pharmacological uses such as, antibacterial activity, antifungal activity, anti-diabetic activity, anticancer activity, antioxidant activity, hepatoprotective activity, haemolytic activity, anti-inflammatory activity, larvicidal (Jemutai Kotut, 2024).

Phytochemical screening of *Senna Didymobotrya*

The methanol crude extract of *Senna didymobotrya* was found to contain tannins, saponins, terpenoids, flavonoids, phenols, alkaloids, steroidal rings the phytochemical compounds detected such as saponins, tannins, flavonoids and alkaloids, have previously been reported to have antimicrobial and antioxidant activity.

The study therefore reveals that the phytochemicals extracted by methanol may be responsible for the wide antimicrobial activity. The chloroform: methanol extract was found to contain saponins, terpenoids, tannins, flavonoids, phenols, alkaloids, steroids and steroidal rings were present. These findings

suggest that antimicrobial activity of *S. didymobotrya* seed extract may primarily contain the presence of either tannins or alkaloids. The most important use of alkaloid originality from plant are in the treatment of malaria (Nagappan, 2012).

Materials and Methods

The collected sample of *Senna didymobotrya* leaves was washed in clean water to remove dirt. An air dried at room temperature for two weeks, pulverized with the aid of pestle and mortar into fine particles. it was stored in air tight container and kept away from moisture until required for the experiment.

Reagent/Chemical

The following chemicals chloroform, n-hexane, 2N HCl, Meyer's reagent, 10%ferric chloride, 5%ferric chloride, methanol, Alcl3, alcoholic a-naphtha, NoaH, concentrated Sulphuric acid, distilled water, mulish reagent, Muller Hinton agar, ethyl acetate, Dandruff's reagent, dilute hydrochloric acid, benzene. dilute ammonia was used for phytochemical screening test.

Apparatus/Equipment

The followings apparatus and equipment Petri dish, hot air ovum, test tube, filter paper, mortar and pestle, spatula, weighing balance, measuring cylinder, conical flasks, beaker, micropipette, funnel, round bottle, shaker, disposable glove, facemask, Bunsen Burner, refrigerator, autoclave, grinding machine, Bunsen burner, glass rod, rotary evaporator, dropper were accessed in the laboratory to carry out the experimental work.

Extraction of the Sample

The powdered dried leaves extract of *Senna* d*idymobotrya* was extracted using maceration method. Powdered leaves extract (200g) was each suspended in 400ml of extracting solvent (n-hexane, ethyl acetate, methanol, distilled water) for three days with occasional agitations. The extract was then decanted and filtered out using what man no.1 filter paper. The filtrates were each concentrated using rotary evaporation, weighed and store in the refrigerator at 4c.

Recovery of the extracted phytochemicals

The extracts were filtered through Whatman No. 1 filter paper into a clean dried 1000cm^3 round-bottomed flasks. This was then mounted to Rotary evaporator which is attached to a receiving flask and condenser and carefully inserted into water bath (attached to a rotary evaporator) which was then activated and solvent distillation carried out until the volume reduced to about 50cm^3 at the end. The concentrated extract was transferred into a crucible dish to dry in a fume cupboard for 24 hours and the flasks containing the dry samples were weighed. The drying and weighing exercise continued until a constant weight was obtained for each sample.

Phytochemical Screening Test

Phytochemicals are bioactive chemicals of plant origin. They are regarded as secondary metabolites because the plants that manufacture them have little or no need for them. They are naturally synthesized in all parts of the plants. Phytochemical screening is usually carried out to detect the presence of secondary metabolites. The following qualitative tests are carried out on the crude extract of *Senna didymobotrya.*

Test for alkaloids

a) The extract (0.5g) was dissolved in 5ml of 2N HCl and filtered. A few drops of Mayer's reagent (prepared by dissolving 1.358 g of mercury chloride in 60 mL of water and poured into a solution of 5 g of KI in 10 mL of water, made up to 100 mL with distilled water) were added. The presence of white (turbid) precipitate indicates the presence of alkaloids (Sukirtha & Growther, 2012).
b) The extract (0.5g) was dissolved in 5ml of 2N HCl and filtered. The filtrate was treated with Dandruff's reagent (Solution of potassium bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids (Njoroge & Bussmann, 2006).

Test for flavonoids

a) Dissolve small amount of the extract (about 0.5g) in ethanol. 5ml of the solution was added to concentrated Sulphuric acid (1ml) and 0.5g of Magnesium metal. A pink coloration indicates the presences of flavonoids (Sukirtha & Growther, 2012).

b) Treat the extract (0.5g dissolved in 2ml of methanol) with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids (Njoroge & Bussmann, 2006).

Test for saponins

a) Frothing test:
Dilute the extracts (0.5g) with distilled water to 20ml shake in a graduated cylinder for 15minutes indicates (over a vortex mixer or universal shaker). Formation of 1cm layer of foam indicates the presence of saponins (Njoroge & Bussmann, 2006).

b) Foams test:
Shake about 0.5g of the extract 2ml of water. Formation of foams which persist for over ten minutes indicates the presence of saponins (Sandhu & Singh 2007).

*Test for terpenoids (Salkowski's test***)**

1ml of chloroform was added to 2ml of the extract followed by a few drops of concentrated Sulphuric acid. A reddish-brown precipitate produced immediately indicates the presence of terpenoids (Reddy, Jose, Anjana, & Ruveena, 2010).

Test for tannins (Braymer's test)

2ml of the extract was treated with 10% alcoholic ferric chloride solution. Formation of blue/greenish coloration indicates tannins (Reddy *et al*., 2010).

Test for sterols (Libermann-Burchard test)

1ml of the extract was treated with few drops of chloroform, acetic anhydride and concentrated Sulphuric acid. The formation of dark pink or red color indicates the presence of sterols (Govindarajan *et al*., 2019).

Test for phenols

A fraction of the extract was treated with aqueous 5% ferric chloride solution. The formation of deep blue or black color indicates the presence of phenols (Govindarajan *et al*., 2019).

Modified anthraquinones test:

0.1 gm of extract was added to 5ml of 5% solution of ferric chloride and 5ml diluted hydrochloric acid and heat on boiling water-bath for 5 minutes, cool the solution and shake gently with an organic solvent like benzene. Separate the organic solvent layer and add an equal volume of dilute ammonia. A pinkish red color is formed in ammoniacal layer. This test is of C. glycoside.

Test for cardiac glycosides (Keller Kelliani's test)

Add 2ml of glacial ascetic to 5ml of each extract (0.5g dissolved in 5ml methanol) in a test tube, followed by a drop of ferric chloride solution. Carefully underlay the mixture with 1ml concentrated Sulphuric acid. A brown ring at the interface indicated the presence of deoxy sugar characteristic of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may form.

Anti- H.I.V Activity

The rabbit was injected with RVS virus, and it was observed for one month until the symptoms of virus appeared, then the animal's blood sample was taken for a test to be conducted to confirm it was affected by the virus. The result test of HIV showed that he has been affected by the virus. The sample leaves extract was then prepared according to the traditional preparation method as follows; The 80g of the

leaves extract of S*enna didymobotrya* was mixed with some ingredient and then boiled until the boiling water turns black. When the mixture was cooled to about 60^0C, 20 ml was taken and administered orally to the animal daily before breaking for four (4) days. At the end of the treatment, the animal's blood sample was taken again for RVS test.

Results and Discussion

The result of successive maceration extraction of *Senna didymobotrya* leaves extract with 400 % n-hexane, 400% ethyl acetate, 400% methanol, and 400% distilled water kept for 3 days via successive maceration method using n-hexane, ethyl acetate, methanol and distilled water. The table 1 show that the % yields of the n-hexane 10.6, ethyl acetate 11.7, methanol 12.7, and distilled water 11.6 respectively.

Table 2: Percentage Yield of Leaves Extract of Senna Didymobotrya

S/N	Weight of the used sample (g)	Solvents used	Percentage yield (%)
1.	50	n-hexane	10.6
2.	50	Ethyl acetate	11.7
3.	50	Methanol	13.7
4.	50	Distilled water	11.6

Source: Laboratory work, 2023

Table 3: Result of Phytochemical Screening of Leaves Extract of Senna didymobotrya

S/N	Phytochemicals	Reagents	n-Hexane	Ethyl acetate	Methanol	d. water
1.	Steroids	Liebermann Burchard	+	_	+	_
2.	Terpenoids	Salkowski`s	+	_	+	+
3.	Anthraquinone	Ferric chloride	+	_	_	_
4.	Tannins	Braymer's	+	+	+	+
5.	Saponins	Foaming	+	_	+	+
6.	Glycosides	Keller Kelliani's	+	+	+	_
7.	Flavonoids	NaOH	_	+	+	+
8.	Alkaloids	Dragendroff	_	+	+	+
9.	Phenols	Ferric chloride	+	+	+	+

Key: + =*positive,* -- =Absence *Source: Laboratory work, 2023*

Table 2 revealed the phytochemical compounds of *Senna didymobotrya* phytochemical such as alkaloid, flavonoids, anthraquinones, steroid, tannins, saponins, phenol, terpenoids, and glycosides, have been known to be biological active and those responsible for anti-HIV activities of the plants hence their use in traditional medicine. Some part of this plant extract is used in the treatments of fungal, bacteria, infections, hypertension, hemorrhoids, sickle cell anaemia, a rage of women diseases such as inflammation of fallopian tubes, fibroids, and backache to stimulate lactation and to induce uterine contraction and abortion.

Table 4: Result for RVS Test Before Treatment

One Month After Injecting The RVS	Result
RVS test	+

Source: Laboratory work, 2023

Source: Laboratory work, 2023

Figure 1: *RVS strip showing positive result before administration of the extract*

Table: 5 Result for RVS Test After Treatment

4 Days after treatment	Result
RVS test	-
RVS test after One week	-
RVS test after a month	-

Source: Laboratory work, 2023

Source: Laboratory work, 2023

Figure 2: *RVS strip showing negative result after administration of extract*

Conclusion

The presence of important pharmacological phytochemicals in the plant leaves is an indication of the diverse medicinal importance of *S. didymobotrya*. Phenols, steroids, saponins, flavonoids, glycosides, tannins, anthraquinones, alkaloids and terpenoids were found in the leaves of the plant. The research conducted revealed that the leaves extract of *Senna didymobotrya* would serve a good anti-HIV due to its high potential activity in the control of H.I.V. Finally, the study suggests the following recommendation which include:

- It is recommended that further research should carry out for scientific justification.
- It is recommended that future studies on the quantitative determination of the identification and phytochemical analysis *Senna didymobotrya* as medicinal plant.
- It is recommended that further studies in identifying the active component in *Senna didymobotrya.*

Conflict of Interest

The authors declare that they have no competing interests.

Acknowledgement

The authors are thankful to the institutional authority for completion of the work.

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