Characterization and Evaluation of Antifungal and Phytochemical Activities of *Senna Didymobotrya* Leave Extracts

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***Abstract:-*Conventional antifungal agents are expensive with numerous side effects. *Senna didymobotrya* plants are known to possess antifungal potential as extensively used by the people of central Kenya in the treatment of ringworm (*Tinea capitis*)The main aim of this study was to analyze the most expound bioactive compounds present in this plant's leaves crude extracts and determine its antifungal potency. Solvent extraction was done using water, methanol, chloroform, ethyl acetate and n-hexane solvents. The extracts were characterized for physical-chemical parameters, bio-metals, functional groups, phytochemicals and antifungal properties. Both water and methanol extracts were neutral while the rest of the extracts were slightly acidic. The extracts showed appreciable conductivities with methanol (123.05±2.88mS), water (73.43±34.85mS) and more solubility values at 25^0C. The infrared spectra of the compounds indicated presence of carbonyl groups, alcohols, organometallic compounds and halides. All the *S. didymobotrya* leaves extracts contained essential bio-metals in considerable concentrations. Flavonoids, phenolic compounds and alkaloids were the most pronounced phytochemicals present, especially in methanol and water extracts. The chloroform and ethyl acetate extracts had the highest of mycelial growth inhibitions in the leaves extracts, (16mm and 15mm respectively). In conclusion, water, methanol and chloroform extracts were found to be more suitable for medicinal applications.**

Key words: **Antifungal agents; *Senna didymobotrya*; Leaves extracts; *Tinea capitis***

I. INTRODUCTION

Fungal immunes are widely spread throughout the world (Johnson, 2017). Whenever they attack person's skin, it is extremely hard to kill them, as they have a potential to remain intact thus re-infecting the patient in the process of recovering (Johnson, 2017). These fungal infections are skin disorders brought about by several as well as specific germs, as the disorders do vary ranging from gentle to severe (Aly, 1996). Out of millions of existing distinct fungal species, only about 300 of them are known by people of having the ability of causing (Hawksworth, 2001) (Tedersoo, *et al.,* 2014). Fungal infections are frequently associated with a fungus that commonly thrive in specific environmental conditions, these fungi live outdoor on earth, plants and higher plants and also exists indoors on surfaces and human bodies (Tedersoo*, et al.,* 2014). A number of proposed causes for the increased fungal immunes are the utilization of immunosuppressive and antineoplastic agents, prosthetic devices and broad-spectrum antibiotics and grafts, as well as more advanced surgery. People's suffering from burns, neutropenia, HIV infection and pancreatitis are extensively predisposed to fungal immunes(Eggimann, *et al.,* 2003).

Popular categories of fungal skin immunes include; athlete's foot (*Tinea pedis*) that affects the feet; Yeast infections (*Candida albicans*) which commonly affects the toenail and diaper rash; Jock itch (*Tinea crusis*) which thrives in body's wet surfaces like butt-check, genitals, and inner thighs; Ringworm (*Tinea corporis*) which is associated with the fungi that thrive on no longer living body tissues, such as the dermis, hairs, and nails they are also known to cause jock itch and athlete's feet (Johnson, 2017). The ringworm (locally known as "mashilingi") also known as dermatophytosis or tinea, "ringworm" is a misnomer, since the infection is caused by a fungus, not a worm. Over-the-counter (OTC) medications used to treat the infection are in form of a powder, ointment or cream which include; Monistat and Vagistat for yeast infections (Holly, 2018); Miconazole (Desenex), terbinafine (Lamisil AT), clotrimazole (Lotrimini AF), butenafine (Lotrimium ultra), tolnaftate (Tinactin), Fluconazole (Diflucan) for treating ringworms as well as athlete's feet.

A large population of people in Africa depends on traditional medicine due to increase in resistance of the currently used antifungal, high cost and inaccessibility to antifungal agents especially in rural areas. On the other hand, medicinal plants are readily available, have little side effects and there is extensive local knowledge on herbal medicine among the communities (Rojas, *et al*., 2006) (Doughar, *et al.,* 2008). Several different plant organs like the barks, stems, fruits, seeds, leaves and tubers are used to prepare the herbal drugs (Mukherjee, 2002). Over 25% of the most widely used pharmaceuticals have a certain percentage of bio-active molecules derived from plants (Rischer, *et al.,* 2006). Plants produce a number of bioactive molecules for their protection

although the recent researches have revealed that, these chemicals possess medicinal properties helpful to humans.

S. didymobotrya, is a well-known plant for its medicinal properties and values have been globally explored by traditional herbalists (Nyamwamu, et al., 2015). *Senna didymobotrya* has shown synergistic activity with convectional antifungal agents against *C. albinos*(Hirasawa, et al., 2004). This emergence has led to more pronounced researches in order to unfold novel, broad spectrum, more potential antifungal agents in the plant.The plant has been used as a traditional medicine by the Kikuyu community of central Kenya in the treatment of skin related disorders among people and animals. In most cases, the plant sap was applied on animals' skin to remove infectious pests attacking the livestock. However, most studies have not fully characterized the plant to elucidate on the physical-chemicals, bio-chemicals and phytochemicals in the plants for its medicinal optimization. This research aimed at analyzing the bioactive compounds present in *S. didymobotrya* leaves extracts for antifungal agents. Elucidation of these agents will go a long way in helping to optimize on the plants ethnomedicinal ability for commercial gains.

II. MATERIALS AND METHODS

Experimental design

An independent measures design was undertaken to study the extracts of S. didymobotrya leaves. Solvent extraction method using five solvents i.e water, methanol, n-hexane, ethyl acetate and chloroform. Each of the extracts was analyzed for physical-chemical parameters, bio-metal concentration, functional groups, phytochemicals, separation by thin layer chromatography and antifungal activity. *C. albicans* fungi strains were used for antifungal studies.

The experiments were done at Maasai mara university, Kenya.

Materials

The chemicals and reagents were all obtained from Sigma-Aldrich. All chemicals and reagents used were lab grade. They include; Sulphuric acid, methanol, acetone, absolute ethanol, aluminium chloride, nitric acid, hydrochloric acid, ferric chloride, Mayers reagent, sodium bicarbonate,sodium acetate, sodium hydroxide pellets, sodium nitrate, oxalic acid, ammonia solution, chloroform. ethyl acetate, n-hexane, Dragendoff's reagent and potassium hydroxide flakes.

The analytical grade chemicals used include; potassium bromide (for IR analysis), zinc, copper, chromium, cobalt and iron standards for AAS analysis.

For antifungal studies, Potato Dextrose Agar (PDA) media (Sigma-Aldrich) was used. The studies strictly used sterile water and 70% ethanol as conventionally done according to antimicrobial studies guidelines. *C. albicans* fungi used was obtained from Sigma-Aldrich.

The following equipment were used; FT-IR (Shimadzu), AAS Spectrometer (PG-AAS-990) and pH meter (Hanna G-114).

Methods

Extraction of the antifungal extracts

Fresh *S. didymobotrya* leaves were obtained, washed to remove debris, and shade-dried for 6 hours before soaking in the respective solvents. A mass of 10g of the fresh leaves in 100ml of solvents was maintained. Thereafter, the leaves were crushed and squeezed using clean fibre to obtain the extracts. The extracts were filtered using Whatman no. 41 filter papers two-folds, before analysis. Enough extracts were obtained by repeating the above procedures severally.

Physical-chemical analysis of S. didymobotrya leaves extracts

The pH and electrical conductivity values of the extracts were obtained using a pH meter and conductivity meter respectively. Solubility analysis of the powder samples was carried out according to conventional methods using both a polar (distilled water) and non-polar (n-hexane) solvent.

Bio-metal concentration

For bio-metal analysis, the extracts were serially diluted 200-folds using 20ml aliquots distilled water and filtering using Whatman #42 filter paper at each dilution stage. The bio-metals were analyzed after formulation of calibration curve using standard salts prepared for each of the bio-metal analyzed. Table 1 below summarizes the conditions used during the bio-metal analysis.

Table 1: AAS conditions used to analyze the bio-metals

Bio-metal	Wavelength	Bandwidth	Lamp current	Flame	Sensitivity
Co	240.7nm	0.4nm	5.0ma	Air/Acetylene	0.05mg/L
Cu	324.7nm	0.4nm	5.0ma	Air/Acetylene	0.03mg/L
Fe	248.3nm	0.2nm	5.0ma	Air/Acetylene	0.05mg/L
Zn	213.9nm	0.4nm	4.0ma	Air/Acetylene	0.01mg/L

Conditions of Atomic Absorption Spectrometer for bio-metal analysis

Functional group analysis

For functional group analysis, the extracts were heated slowly at 60°C in a crucible cleaned with 2M ammonia solution until all the water was dried. The samples were then cast into pellets using potassium bromide pellet before analyzing for functional groups using IR Spectrometer.

Preliminary phytochemicals screening

Phytochemicals screening were conducted on the leaves extracts relying on the procedures proposed by(Nyamwamu, et al., 2015)

Alkaloids test

1ml of the extract was placed into a test tube followed by the addition 2ml of ammonia solution and left standing for 5 minutes. Then 2ml of chloroform were placed to the same sample's test tubes followed by thorough shaking. 1ml of the mixture was taken separately in two test tubes. The chloroform was evaporated by partially immersing the test tubes in a warm water bath followed by the addition of 2ml Mayer's reagent to one of the test tubes and the occurrence of a creamy colored precipitate indicated positive test for the alkaloids. To the next test tube, 3 drops of Dragendoff's reagent were added and the appearance of an orange-red precipitate was taken a positive test as well.

Flavonoids test

3 drops of dilute ammonia (NH_3) or sodium hydroxide solution were added to 1ml of *Senna didymobotrya* extracts. The appearance of a strong yellow solution, that turned colorless on addition of a few drops of dilute H_2SO_4 acid confirmed flavonoid compounds presence.

Saponins test

1ml of extracts were diluted with 20ml deionized water in a test tube and properly mixed by shaking for about 15 minutes. The appearance of a stable foam layer at the top of the test tube definitely indicated that saponins were present.

Steroids test

1ml of extracts was dissolved in 6ml of chloroform. 2 ml of the mixture were added to 1 ml of a homogeneous mixture of concentrated sulphuric acid and acetic acid by test tube wall side. The red coloration on the upper layer and the yellow-green fluorescence on the sulphuric layer appearance, that is, a blue green ring. Confirmed steroids present.

Tannins test

1ml of extracts in a test tube was heated to boil in a boiling water bath. 1ml 0.1% ferric chloride ($FeCl_3$) was mixed with the hot extract and the occurrence of a brown or a blue-blackcolor confirmedtannins present.

Test for terpenoids

1ml of extracts was mixed with 1 ml of chloroform ($CHCl_3$) in the test tubes then 1 ml of concentrated H_2SO_4 was cautiously added to the solution mixture for layer formation. The formation of a reddish-brown or reddish-violet colored interface indicated that terpenoids were present.

Test for anthraquinones

1ml of the sample extract was placed into a test tube followed by the addition 7.5 ml benzene solution. The test tube contents were thoroughly mixed. Then 3 ml of ammonia solution were added. The appearance of a violet color in the ammonia phase (lower phase) indicated the anthraquinones presence.

Test for glycosides

Salkowski's test was used for the determination of glycosides presence in these leave extracts. 2ml of leaves extracts were mixed with 1ml $CHCl_3$ as well as 2ml concentrated H_2SO_4. The appearance of a red-brown coloration indicated steroidal rings presence (glycone portions of glycosides).

Test for phenolic compounds

1ml of the extract was mixed with 2ml of deionized water. In this solution, 3 drops of 5% ferric chloride neutral solution were added. The occurrence of a dark green coloration confirmed phenolic compounds present.

Antifungal studies

C. albicans strain was used for antifungal analysis. All aseptic techniques were considered to minimize the contamination rates.

Media preparation

28.0g of PDA agar media was dissolved into 600ml of sterile distilled water in a media dispensing bottle. The mixture was gradually boiled to completely dissolve the media. Caution was taken not to break the media bottle by loosening the bottle stopper occasionally to avoid pressure build up. The media was then sterilized by autoclaving along with petri-dishes and all apparatus to be used at 121^0C and 15 psi pressure for 15 minutes. The media was allowed to cool to 45^0C before dispensing in sterile petri dishes. The media plates were allowed to cool, inverted and stored in the refrigerator at 4^0C for 24 hours.

Antimicrobial sensitivity tests

Sterile media plates were sub-divided into six equal parts using a marker pen and labelled accordingly. The test microbes were then spread aseptically on different media plates to prevent cross-contamination. Sterile octodiscs impregnated with different bio-slurry samples were then placed on the surface of the plates. The plates were then inverted and incubated at 37^0C for 24 hours. The inhibition zones were noted and recorded in millimeters.

Data Analysis

Data obtained from analysing the parameters conducted was subjected to statistical analysis. The degree of freedom value was maintained at 8 with 95% confidence level being used for

f-test analysis. The data was analysed using Ms Excel and Originlab statistical packages.

III. RESULTS AND DISCUSSIONS

Physical-chemical analysis of the extracts

pH is a crucial parameter in antifungal drugs due to the speciety of fungi strains with the environmental pH (Selvig and Aspaugh, 2011). Water and methanol extracts were found to be neutral while ethyl acetate, n-hexane and chloroform extrcts exhibited acidity. The acidic samples were clusterd at a similar pH value between 5.00±0.02 (chloroform) and 5.27±0.01 (n-hexane).Table 2 below summarizes the pH, electrical conductivity and solubility levels of the five extracts of *S. didymobotrya*leaves.

Table 2: Physical-chemical properties of *S. didymobotrya*leaves

Parameter	Samples				
	Water	Methanol	Ethyl acetate	n-hexane	Chloroform
pH	7.20±0.01	6.88±0.01	5.08±0.07	5.27±0.01	5.00±0.02
Conductivity (mS)	73.43±4.85	123.05±2.89	7.02±2.75	0.00±0.00	0.00±0.00
Solubility in water (g/100ml water at 25^0C)	0.40±0.00	0.41±0.01	0.10±0.00	0.01±0.00	0.39±0.01
Solubility in n-hexane (g/100ml n-hexane at 25^0C)	0.01±0.00	0.01±0.00	0.24±0.01	0.25±0.00	0.14±0.00

Physical-chemical parameters of various extracts of *S. didymobotrya* leaves

The results obtained indicate that *S.didymobotrya* leaves can have a range of pH values for broad-spectrum efficacies. Most studies conform that ethnomedicinal drugs should be neutral or with a pH value slightly deviating from neutrality (Mukherjee *et al*., 2010). Acidity in the extracts is attributable to presence of volatile acids and acidic ligands present in the leaves. It is thus likely from the results in table 2 above that the extracts containing chloroform would inhibit more fatty acids and acidic ligands. Cornet and Gaillardin, (2014); states that most fungi strains survive at acidic pH values. Variation of pH of antifungal drugs is thus crucial in the susceptibility of the fungi. Alongside pH, conductivity of extracts is paramount in determining the speciation of ions and by extension, the survival of fungi. Only water, methanol and ethyl acetate extracts depicted conductivity values. Lack of detection of conductivity in n-hexane and chloroform is due to the lack of polarity of these solvents. Methanol extracts had the highest conductivity (123.05±2.89mS) followed by water extracts (73.43±4.85mS) and ethyl acetate extracts (7.02±2.75mS). These findings imply more salts present in the extracts. Hay, (2017); found the growth of *Candida albicans* and *Tinea capitis* was inhibited at high conductivity values. The solubility of the extracts varied with the solvent of extraction used. Solubility of medicinal extracts is crucial due to efficacy of the drug in the human body or skin. Amy, (2018); reports that griseofluvin drug used against ringworm scalds are moderately soluble in water for them to be applicable on the skin surface and move freely within blood plasma. From the findings illustrated in table 2 above, the extracts were more soluble in water (especially for water, methanol and chloroform) as contrasted to n-hexane and ethyl acetate solvents. It is thus feasible to use polar solvents for extraction of these antifungal agents.

Concentrations of bio-metals

Light and heavy metals found in medicinal extracts can influence the usage of its extracts. Abundance of important bio-metals such as zinc is crucial. However, too much of the same can be toxic. The concentrations of five bio-metals analyzed in the *S. didymobotrya* leaves are illustrated in table 3 below.

Table 3: Bio-metal concentrations in *S. didymobotrya* leaves extracts

Bio-metals	Sample concentration (mg/Kg wet sample)				
	Water	Methanol	Ethyl acetate	n-hexane	Chloroform
Copper	27.34±0.00	20.82±0.00	20.82±0.00	14.72±0.00	24.72±0.00
Cobalt	2.04±0.00	48.48±0.00	15.62±0.00	72.26±0.00	25.82±0.00
Iron	57.14±0.00	9.52±0.00	109.52±0.00	61.90±0.00	61.90±0.00
Zinc	9.38±0.00	5.04±0.00	0.86±0.00	1.22±0.00	0.82±0.00
Chromium	34.78±0.00	24.96±0.00	37.20±0.00	34.78±0.00	39.62±0.00

Bio-metal concentration in the leaves extracts of *S. didymobotrya*

All extracts showed the potential of bio-metal abundance. Only n-hexane (14.72±0.00 mg/Kg wet sample) had less than 20mg/Kg of copper ions. Water and chloroform extracts had the highest concentrations of these ions. Saiba *et al.,* (2014);

found out that copper ions combined with gatifloxacins inhibit growth of fungi. The more concentrated the copper ions, the more effective the samples were on *Candida albicans* and *Aspergillus niger* fungi strains, which causes skin infections with more potency. Concentration of cobalt ions had great disparities in the extracts. n-hexane (72.26±0.00 mg/Kg wet sample) and methanol extracts (48.48±0.00 mg/Kg wet sample) were found to have the highest concentrations. Increase in cobalt ions has positive impacts on the antifungal properties of the extracts. Turecka *et al.,* (2018); found out that cobalt (iii) complexes perfectly with diamine chelate ligands against a broad spectrum of *Candida* species. Iron was found to be the most abundant metal analyzed in the extracts. Only methanol extracts (9.52±0.00 mg/Kg of wet sample) had less than 50mg/Kg of iron ions. Iron oxide nanoparticles have been adversely employed as antifungal drugs on their own (Arias *et al*., 2018). On the other hand, several iron chelators have been used alongside antifungal drugs to inhibit the effects of several fungi strains (Nazik *et al.,* 2015). Zinc concentrations were in the order of water extracts (9.38±0.00mg/Kg), methanol extracts (5.04±0.00mg/Kg), n-hexane (1.22±0.00mg/Kg), ethyl acetate (0.86±0.00mg/Kg) and chloroform (0.82±0.00mg/Kg) respectively.Chalcogens of zinc are known to have natural antifungal potential (Sardella *et al.,* 2017). Several studies have been successfully conducted on the antifungal efficacies of zinc oxide and zinc sulfide drugs. Chromium ions have mixed effects as medicinal extracts. Trivalent chromium has been found to chelate with other ligands and effectively inhibit growth of fungi. On the other hand, hexavalent chromium is very soluble and thus quite toxic when applied on the skin or taken orally. In this study, all ions had moderate chromium ions ranging between 34.78±0.00mg/Kg (water extracts) and 39.62±0.00mg/Kg (chloroform extracts) except methanol extracts whose concentrations (24.96±0.00mg/Kg) were found to be significantly different from the rest ($p \geq 0.05$, $n = 14$).

Functional group peaks of S. didymobotrya leaves extracts

All the spectra were found to have similar FT-IR spectra. The position of peaks in the peaks were concise with almost equal intensities. The FT-IR spectra of the leaves extracts of *S. didymobotrya* plant are illustrated in figure 1 below.

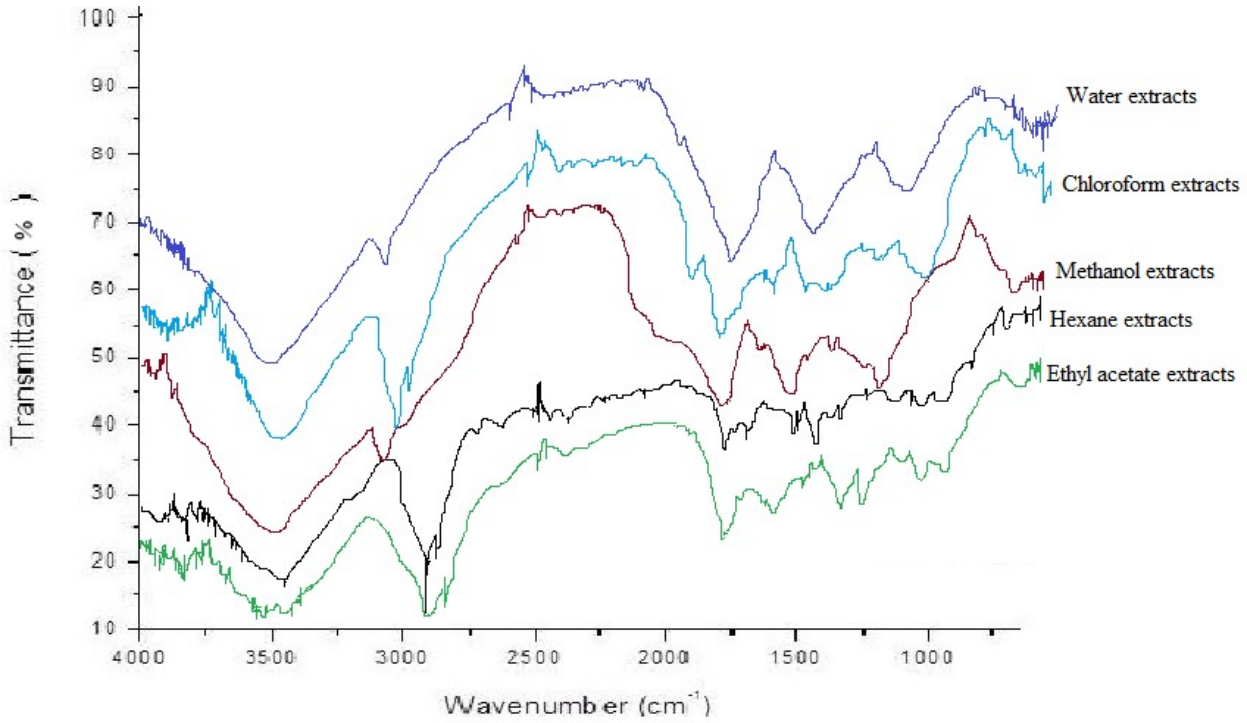

Figure 1; FT-IR spectra of *S. didymobotrya* leaves extracts

The FTIR analysis for *S. didymobotrya* leaves extracts extracted using different solvents deduced crucial functional groups which have to be present in the phytochemical compound structures. The spectra showed presence of carboxylic acids, aromatic compounds as well as halide and organometallic compounds. All spectra had broad peaks of O-H, hydrogen of an alcohol between 3448.72 cm^{-1} and 3487.30 cm^{-1}. There were also O-H stretches of the carboxylic acid ranging from 2854.65 (water),2924.09 (chloroform), 2924.65 cm^{-1} (methanol)and 3300 cm^{-1} (in all). The peaks at 2854.65 cm^{-1} and 2924.09 cm^{-1} depicted the presence of sp^3 hydrogen (-C-H) which always occurs at the range of 2850-3000 cm^{-1}.

There were several C=O stretches of a ketone (1705.07 (n-hexane),1720.50 (methanol),1735.93 (water) and 1743.65 cm^{-1} (chloroform), aldehydes (1720.50 cm^{-1} and 1735.93

cm^{-1}), esters(1735.93 cm^{-1} and 1743.65 cm^{-1}) and carboxylic acids (1705.07, 17.20.50 cm^{-1}). The C-O peak of carboxylic acid also appeared at 1242.16 cm^{-1} and that of an alcohol at 1072.42 cm^{-1}. There were overtone weak peaks of C-H of aromatic compounds at 1705.07, 1720.50 and 1735.93 cm^{-1}. Intense activities between 500cm^{-1} and 700cm^{-1} illustrated presence of organometallic compounds and halides.

Phytochemicals present in the extracts

The presence and intensity of phytochemicals present in *S. didymobotrya* leaves extracts were analyzed and summarized in table 4 below. From the table, methanol was proven to be the most suitable for extraction of these phytochemicals.

Table 4: Phytochemical compounds in *S. didymobotrya* leaves extracts

Phytochemicals analyzed	Samples				
	Methanol	Hexane	Ethylacetate	Chloroform	Distilled H_2O
Alkaloids Mayer's test Dragendoff's test	 +++ +++	 - +++	 +++ +++	 - +++	 - -
Flavonoids Dilute $NH_{3(aq)}$ Dilute $NaOH_{(aq)}$	 ++ ++	 ++ ++	 - -	 +++ +++	 - -
Saponins	+++	+	-	-	+++
Steroids	+++	+++	+++	++	+++
Tannins	-	-	-	-	+++
Terpenoids	++	-	-	-	+++
Anthraquinones	-	-	-	-	-
Glycosides	++	-	-	-	+++
Phenolic compounds	+++	+++	+++	-	+++

Key: +++More expound presence; ++Moderate presence; -Absence

Phytochemicals screened in the leaves extracts of *S. didymobotrya*

In regard to the results on the phytochemical compounds screening of the extracted leave extracts using various solvents, the presence of pharmaceutically active compounds was confirmed. These phytochemicals are largely recognized as pharmacological ingredients in the manufacture of pharmaceuticals as they either have antifungal potentials to several fungi strain (Suurbaar *et al.,* 2017). The alkaloids were extensively extracted by methanol, hexane, ethylacetate and chloroform as the Dragendoff's reagent test revealed more considerable positive results, wherein, the Mayer's reagent test responded positively on the methanol and ethylacetate leave extracts only. Gundel *et al*., (2018); showed that allosecurinin alkaloids were quite effective against fungi strains. On the other hand, Jose *et al.,* (2005); showed that flavonoids have the ability to inhibit growth of several fungi strains such as pre-symbiotic growth of *Gigaspora* and *Glomus*fungi.Flavonoids were revealed being more expound in the chloroform and moderately present in the methanol and hexane leave extracts. Steroids were confirmed present in all the extracts especially in methanol, hexane, ethyl acetate and water extracts. Saponins were confirmed more expound in the methanol and water extracts and in low amounts in hexane extract but in low amount. Tannins were only extracted by water. Terpenoids were extensively extracted by water and moderately by methanol which also tallied with the glycosides, though the crude leave extracts lacked anthraquinones. Phenolic compounds were revealed more expound in the methanol, hexane, ethyl acetate and water crude leave extracts.From the study, the leaves of *Senna didymobotrya* were found to contain appreciable quantity of pharmacological bioactive compounds such as; alkaloids, flavonoids, saponins, steroids, tannins, terpenoids, glycosides and phenolic compounds. This is in conformity with (Kitonde, *et al*., 2014) study on this plant's roots and studies carried out by(Nyamwamu, *et al*., 2015)(Korir, *et al*., 2012) on the *S. didymobotrya* barks which correctively depicted the presence of all these compounds.

Antifungal properties of S. didymobotrya leaves extracts

The antifungal activity of the leaves was found to range between moderate to strongly effective. However, only ethyl acetate and chloroform extracts strongly inhibited the growth of *C.albicans* fungi used. Methanol extracts were moderately efficient while water and n-hexane gave mild efficiencies. The inhibition zone diameters of the plant extracts against *C. albicans* fungi are illustrated by Figure 2 and table 5 below.

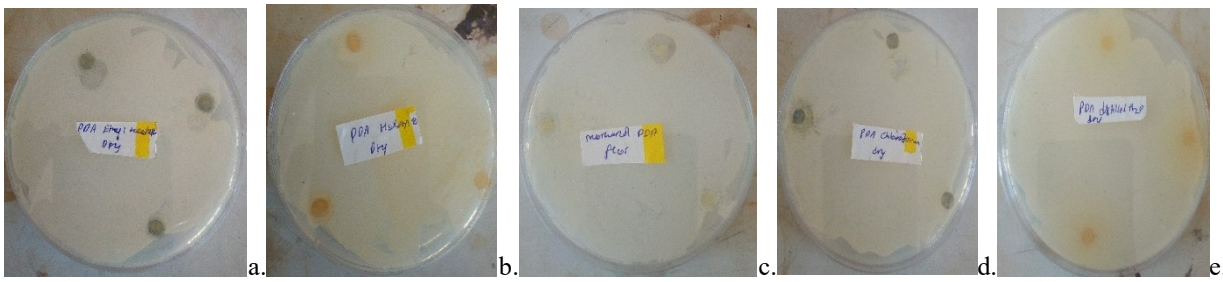

Figure 2; The inhibition zones of *S. didymobotrya* leaves extracts. (a) is ethyl acetate extracts, (b) is n-hexane extracts, (c) is methanol extracts, (d) is chloroform extracts and (e) is water extracts.

Table 5: Inhibition zone diameters of *S. didymobotrya* leaves extracts against *C. albicans* fungi

Inhibition zone	Samples				
	Water	Methanol	Ethyl acetate	n-hexane	chloroform
Diameters (mm)	9.00±0.00	11.00±0.00	15.00±0.00	6.00±0.00	16.00±0.00

(Tahiya, *et al.*, 2014) found out that the antifungal activity of medicinal extracts is dependent on the extraction solvent used. Chloroform and ethyl acetate extracts had strong inhibition activities citing potential to strongly fight ringworms and other fungal infections. Methanol and water extracts had moderate inhibitions while n-hexane had mild activity against the same fungi. The antifungal findings of this study, (using *S. didymobotrya* against the *Candida albicans*) tallied with those of(Hirasawa, *et al.*, 2004) and(Bhat, *et al*., 2007).

IV. CONCLUSION

There were major variances in the physical-chemical parameters of *S. didymobotrya* leaves extracts. The polar solvents i.e water and methanol had neutral pH and appreciable conductivities and solubilities at 25^0C unlike the extracts of chloroform, ethyl acetate and n-hexane. Bio-metal concentrations were quite uniform and moderate in all the test samples. Iron was found to be the most abundant specie while zinc ions were sparsely present in the extracts. There was concise resemblance in the infrared spectra with carbonyl groups, alcohols, aromatic compounds, organometallic compounds and halide peaks all vivid in the spectra. Flavonoids and alkaloids were abundant in the extracts whilst most of the other phytochemicals were present in small amounts. Water and methanol extracts had more phytochemicals compared to the other extracts. The antifungal studies revealed that chloroform and ethyl acetate extracts had the highest inhibition against *C. albicans* fungi.

The five extracts of *S. didymobotrya* leaves (water, methanol, chloroform, ethyl acetate and n-hexane) were all found to be suitable antifungal products. However, water, methanol and chloroform extracts showed more potential compared to ethyl acetate and n-hexane.

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CONFLICTS OF INTEREST

The authors declare to have no conflict of interest whatsoever.

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DATA AVAILABILITY STATEMENT

All data used is enclosed within the manuscript and any supplementary sheets attached.

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