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Phytochemical analysis and antimicrobial activity of *Senna didymobotrya* seed extracts

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ABSTRACT

Senna didymobotryaFresenis one of the 18 Senna species growing in Ethiopia. The plant is known for its value in traditional medicine. The present study was carried out on the phytochemical analysis and antimicrobial activity of seed extracts ofSenna didymobotrya. Seed was extracted with n-hexane, chloroform: methanol (1:4) and methanol through soxhlet apparatus to get crude extracts. The crude extracts were subjected to phytochemical test which shows the presence of tannins, saponins, terpenoids, phenols, alkaloids and steroidal rings. Chloroform: methanol (1:4) crude extract was subjected to column chromatography to get fraction 3. All extracts and fraction 3 were tested for antibacterial and antifungal activity. The maximum antibacterial activity was shown by the methanol crude extract to against Escherichia coli with an inhibition zone of 17.16mm. The maximum antifungal activity was shown by chloroform: methanol(1:4) crude extract against Aspergillus niger with an inhibition zone of 22.3 mm. The fraction 3 exhibited medium activity towards Staphylococcus aureus, Streptococcus pyrogenes and Escherichia coli (E. coli). These results have shown that Senna didymobotryaseed extracts had significant activity against all the organisms tested.

Key words: *Senna didymobotrya*, phytochemical analysis, antimicrobial activity

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INTRODUCTION

Plant-derived substances have recently become of great interest due to their versatile applications. Medicinal plants are the richest resource of drugs of traditional systems of medicine, modern medicines, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [1]. *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, Mauritius & South Africa. 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*Fresen is one of the 18 Senna species growing in Ethiopia [2]. The plant is known for its value in traditional medicine[3]. It is usually a several-stemmed shrub or small tree, 0.5- 5(-9) m tall. It is common in deciduous bushland, 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. 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 trisaccharideraffinose. It is commonly used as a stupefacient poison for fishing [4].

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 [5]. It is also used in the treatment of animal diseases such as removal of ticks)[6].

It is also used in women diseases such as inflammation of the fallopian tubes, fibroids and backache, to stimulate lactation, and to induce uterine contractions and abortion [6].Numerous works have been carried out on the chemistry and medicinal role of roots, barks, stems and leaves of *Senna didymobotrya*but few works have been reported on the chemistry as well as medicinal value of seed of *Senna didymobotrya*, therefore, this study was taken to evaluate phytochemicals analysis and antimicrobial activity of *Senna didymobotrya*seed extract.

MATERIALS AND METHODS

Collection and Identification of the Plant Material

Seeds of *Senna didymobotrya*were collected from Haramaya Eastern Ethiopia. The Botanical specimens of the plant were identified by Mr. AbeduruzakAbdulhahi and the voucher specimen was deposited at the herbarium of the Department of Plant Science, Haramaya University. After collection, the seeds were washed repetitively and air - dried in the shade to make it easily grind able.

Extraction of Seed

Air dried seeds of *Senna didymobotrya*were grounded by analytical mills (Western-Germany 5657) and packed in polyethylene bags to avoid entrance of air and any other mixing of surrounding material. 180 g a powdered seed of S. didymobotrya were extracted with n hexane, chloroform: methanol (1:4) and methanol for 8 h in Soxhlet apparatus. The extracts were filtered using whatman No. 1 filter paper and concentrated rotary evaporator under reduced pressure at 40°C. Chloroform: methanol crude extract was subjected to column chromatography to get fraction 3.Then all extracts were kept at 4^{o}C until analysis.

Phytochemical screening tests

The extracts of both MeOH and $CHCl_3$: MeOHphytoconstituents analyses for identification of bioactive chemical constituents were done using standard procedures [7-9].

Column chromatography:

Part of the crude extract (6g) was chromatographed on silica gel (230-400 mesh) using increasing amount of $CHCl_3$: MeOH in n-hexane as eluents. The purity of the fractions was monitored by TLC and UV lamp. The TLC plates were then visualized under UV lamp at 254 nm and 356 nm.

Antimicrobial assay

All extracts and fraction 3 were tested for antibacterial and antifungal activity. The antibacterial activities of all samples were tested against Gram positive bacterium *Staphylococcus aureus* (S. aureus), *Streptococcus pyrogenes*(SP) & Gram negative bacterium *Campylobacter jejuni* (CJ),*Escherichia coli* (E. coli) using MHA medium and the fungi, *Aspergillus niger* (A. niger) and *Aspergillus fumigates*, using PDA medium. All the microbial were obtained from Plant Pathology laboratory of the School of Plant Science, Haramaya University. Streptomycin was used as standard drug against bacteria whereas tilt was used against fungi.

Preparation of inoculums

The test bacterial strains were transferred from the stock cultures and streaked on Mueller Hinton plates and incubated for 24 hrs. Well separated bacterial colonies were then used as inoculums. Bacteria were transferred using bacteriological loop to autoclaved MHA that was cooled to about 45oC in water bath and mixed by gently swirling the flasks. The medium was then poured to sterile Petri plates, allowed to solidify and used for the biotest. For test fungi, mycelia plugs from stock cultures were transferred to PDA plates and incubated for 5-7 days. Then spores of *Aspergillus niger* were harvested by washing the surface of the colony using 10 mL sterile distilled water and transferred to 250 mL autoclaved PDA cooled to about 45oC in water bath. Likewise, mycelium of Aspergillus fumigates were washed with 10 mL sterile distilled water, macerated in a blender and the mycelia suspension was transferred to 250 mL autoclaved PDA cooled to about 45oC in water bath. The medium containing spore or mycelia suspension was poured to sterile; a plate allowed to solidify and was used for disk diffusion bioassay [10].

Preparation of test solution
The crude extract (0.2 g/mL) and fraction of F3 (0.06 g/mL) were dissolved in $CHCl_3$& MeOH (1:4) and the seed oil was used to test antimicrobial activities.

Testing for antifungal activity
Filter paper discs of 6 mm diameter placed in beaker were sterilized in an oven at 180^{o}C for 1 h. The crude extract and each of the concentration of the fraction were then pipetted to the sterile paper discs. 10 and 20 µL of the samples were pipette to the discs in three replications. The paper discs impregnated with the extract solution were then transferred using sterile forceps to PDA seeded with spore or mycelia suspension of test fungi as described under inoculums preparation above. The Petri dishes were incubated at 24^{o}C for 5-7 days. All the tests were performed in triplicate. The antifungal activity was evaluated by measuring the zone of inhibition against the tested organisms.

Testing for antibacterial activity
Sterilized paper discs were transferred to MHA plate's seeded with bacteria and incubated at 37^{o}C for 24 h. All the tests were performed in triplicate. The antibacterial activity was evaluated by measuring of the inhibition zone against the tested organism.

RESULTS AND DISCUSSION

Yield of Extracts
The yield of n-hexane extract is 5.26%. The yield of methanol crude extract is 14.53% and the chloroform: methanol crude extract is 11.08%.

Phytochemical Constituents
The methanol crude extract of *Senna didymobotrya* was found to contain tannins, saponins, terpenoids, flavonoids, phenols, alkaloids, steroidal rings but steroids were absent as shown in table 1. The phytochemical compounds detected such as saponins, tannins, flavonoids and alkaloids, have previously been reported to have antimicrobial and antioxidant activity[11]. This 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 be primarily due to the presence of either tannins or alkaloids. The most important use of alkaloid already known with its originality from plant is the use of alkaloid compound in the treatment of malaria[12]. The presence of saponins shows that it can have hypercholesterolemia, hyperglycemia, antioxidant activity [13]. Saponinimportance is also supported by other reports [14-15]. Flavonoids are used as antioxidants because of their ability to scavenge free radicals such as peroxide and hydroperoxide of lipid hydroxyl hence inhibiting oxidation that lead to degenerative diseases (Samatha et al., 2012)[16]. Additionally, Flavonoids activityis supported by other studies [17-19].

Table 1: Phytochemical screening results of methanol crude extract and chloroform: methanol Crude extract

No	Constituents	Methanol extract	Chloroform: Methanol extract
1	Steroids	-	+
2	Flavonoids	+	+
3	Phenols	+	+
4	Tannins	+	+
5	Terpenoids	+	+
6	Alkaloids	+	+
7	Saponins	+	+
8	Steroidal rings	+	+

+ = the presence of phytochemical constituents
- = the absence of phytochemical constituents

Antimicrobial Assay
The seed oil has no inhibition effect to against the tested bacteria but crude extract have highest inhibition effect to against the tested bacteria. The crude extract & their components are known to be active against a wide variety of microorganisms, including Gram-negative and Gram- positive bacteria. The methanol extract of *Senna didymobotrya* seed showed bacterial growth inhibition with *E.coli* being mostly inhibited than chloroform: methanol

extract. The commercial standard drug (Streptomycin) showed the greatest inhibition effect against both bacteria in both concentrations (10 µL and 20 µL) rather than the tested samples, the control (solvent) has no inhibition zone & was obtained not to have any effect or contribution to the test solutions so that all the measured values are purely for the respective solutes, analysis of variance while means presented are original values(Table 2& 3).

Table 2: Zone of bacterial growth inhibition (mm) for seed oil, methanol and $CHCl_3$: methanol (1:4) crude extract and isolated pure compound seed of *S. didymobotrya*

Sample	Zone of bacterial growth inhibition (mm)				
	Dose(µL)	*Staphylococcus aureus* (*SA*)	*Streptococcus pyrogenes*	*Campylobacter jejuni*	*Escherichia coli* (*E. coli*)
n-hexane extract	10	-	-	-	-
	20	-	-	-	-
MeOH crude extract	10	11.5 ± 0.12	4.5 ± 0.04	3.166 ± 0.02	12.80 ± 0.08
	20	17.16 ± 0.04	7.66± 0.08	6.33 ± 0.17	19.16 ± 0.25
$CHCl_3$:MeOH Crude extract	10	10.16 ± 0.17	4.66± 0.12	3.66 ± 0.02	12.50 ± 0.06
	20	12.8 ± 0.18	7.66± 0.14	6.62 ± 0.18	17.66 ± 0.08
Fraction-3	10	8.26 ± 0.31	2.16 ± 0.02	-	10.16 ± 0.3
	20	10.34 ± 0.22	3.46 ± 0.02	-	15.28 ± 0.26
Chloroform	10	-	-	-	-
	20	-	-	-	-
Methanol	10	-	-	-	-
	20	-	-	-	-
Streptomycin (antibiotic)	10	16.33 ± 0.18	17.5 ± 0.14	7.16 ± 0.11	21.20 ± 0.18
	20	22.00 ± 0.06	20.8 ± 0.12	8.66 ± 0.02	26.70 ± 0.21

Value represents mean of three replications ±SD; - stands for no inhibition

Table 3: Zone of fungal growth inhibition (mm) for methanol & chloroform: methanol crude extract, seed oil and isolated pure compound of seeds of *Senna didymobotrya*

Sample		Zone of fungal growth inhibition (mm)	
	Dose (µL)	*Aspergillusfumigates*	*Aspergillusniger*
n-hexane extract	10	-	-
	20	-	-
MeOH Crude extract	10	10.56 ±0.63	8.12 ± 0.70
	20	11.4 ± 0.59	9.13 ± 0.60
$CHCl_3$:MeOH Crude Extract	10	13.8 ± 0.03	14.3 ± 0.03
	20	19.6 ± 0.06	22.3 ± 0.03
Fraction-3	10	8.6 ± 0.06	12.8 ± 0.03
	20	17.3 ± 0.03	20.0 ± 0.00
Tilt (standard drug)	10	21.1 ± 0.01	28.8 ± 0.08
	20	27.6 ± 0.06	34.6 ± 0.06
Control (Solvent)	10	-	-
	20	-	-

Value represents mean of three replications ±SD; stands for no inhibition

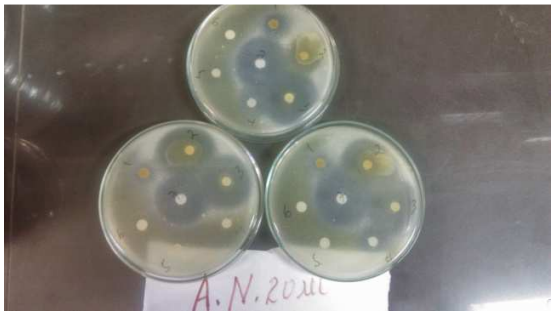

Figure 1. Antifungal activity of *Senna didymobotrya*extracts against *Aspergillus niger*(A.N.) and *Aspergillusfumigates* (A.F.)

The methanol extract and chloroform : methanol (1:4) crude extract of *Senna didymobotrya*produced a clear zone of inhibition with all test microorganisms. *Escherichia coli* (E. coli) was the most inhibited with a clear zone of inhibition of 19.16 ± 0.25 followed by *Staphylococcus aureus* (SA), *Streptococcus pyrogenes* (SP), and *Campylobacter jejuni*. Another study done by [20] indicated that fresh extract of *Senna didymobotrya*are used as an antiseptic against animal wounds which shows that it also contains ingredients that are able to act against microorganisms. This study therefore reveals that the phytochemicals extracted by methanol may be responsible for the wide anti microbial activity. These results have shown that *Senna didymobotrya*seed of crude extract had significant activity against all the organisms tested. The control *Streptomycin* also inhibited the organisms & solvent showed no inhibition at all. The maximum antifungal activity was shown (Figure1.)by chloroform: methanol(1:4) crude extract against *Aspergillus niger* with an inhibition zone of 22.3 mm and the minimum antifungal activity was shown by methanol crude extract against *Aspergillus niger* with an inhibition zone of 9.13 mm. With exception of seed oil all other samples show an increasing inhibition effect against the tested fungi from doses (10 to 20 µL) due to the concentration effect. The commercial standard drug (Tilt) shows higher inhibition zone against antifungal activity compared with all samples. The drug shows different inhibition effect for both concentrations (10 µL and 20 µL). The activity of S. didymobotrya plant against filarial vector was done by Nagappan, [21], which support biological activity of this plant.

CONCLUSION

Phytochemical analysis shows presence of various phytochemical. The presence of phytochemial make the seed useful for treating different disease and have a potential of providing useful medicine of human use. Among the various extracts tested (n hexane, chloroform: methanol, methanol and fraction 3) the methanol crude extract was found to have significant antibacterial activity while chloroform : methanol was found to have significant antifungal activity. Thus it show that S. didymobotrya seed has great potential as effective antimicrobial agent for medicine purpose. These extracts should be further investigating for identification of active constituents.

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