Fall et al., Afr J Tradit Complement Altern Med., (2017) 14 (4): 135-139
https://doi.org/10.21010/ajtcam.v14i4.16

PHYTOCHEMICAL SCREENING, ANTIMICROBIAL AND CYTOTOXICITY STUDIES OF ETHANOL LEAF EXTRACT OF *APHANIA SENEGALENSIS* (*SAPINDACEAE*)

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Abstract

Background: *Aphania senegalensis (Sapindaceae)* is commonly used in Senegalese traditional medicine to treat pain, inflammation, asthenia, bacterial and fungal infections.
The aim of this study was to determine the type of phytochemical constituents present in the ethanol leaf extract and its antimicrobial activity against selected bacterial and fungal pathogens.
Materials and Methods: The ethanol leaf extract of *A. senegalensis* was evaluated for its cytotoxic effect in the MTT assay against Vero cells. Flavonoids and tannins were the main constituents of the ethanol leaf extract.
Results: The extract inhibited the growth of the three fungal strains used in this study moderately with the lowest MIC obtained for *Candida albicans* (0.16 mg/mL). The extract also inhibited the growth of *Aspergillus fumigatus* and *Cryptococcus neoformans* with an MIC of 0.62 mg/mL. For bacterial pathogens, strong inhibition was obtained against *Enterococcus faecalis* **(**ATTC 29212) (MIC 0.08 mg/mL), while moderate inhibition was obtained for *Escherichia coli* (ATTC 25922) (MIC 0.16 mg/mL) and *Staphylococcus aureus* (ATTC 29213) (MIC 0.31mg/mL). The extract however did not inhibit the growth of *Pseudomonas aeruginosa* (ATTC 27853) at the highest concentration (2.5 mg/ml) tested. The ethanol leaf extract of *A. senegalensis* had a higher cytotoxicity than berberine used as the positive control (LC_{50} *2.67*±0.04 µg/mL and 9.99±0.54 µg/mL respectively). The best selectivity index values was obtained for *Enterococcus faecalis* (SI = 1.24), followed by *Escherichia coli* (SI = 0.62) for bacterial pathogens and C. *albicans* (SI = 0.62) for fungal pathogens.
Conclusion: The findings of this study suggest that the extracts may not be safe for use in animals infected by some pathogens.

Keywords: *Aphania senegalensis*, leaf, phytochemical, antimicrobial, cytotoxicity.

Introduction

Infectious diseases are a major public health problem in Africa. The prevailing economic crisis and lack of access to health care facilities, underscores these challenges. The cost of available pharmaceutical drugs, have further generated renewed interest in the use of medicinal plants to treat disease (Farnsworth et al. 1985). Apart from the high cost of antimicrobials, their potential toxicity, side effects and resistance development of pathogens against available drugs have further compounded the problem. The resistance against *Pseudomonas aeruginosa* is a good example (Stewart and Costerton, 2001).

Some bacteria are responsible for nosocomial infections and are naturally resistant to several antibiotics making the activity of these drugs administered at therapeutic doses unpredictable (Mah *et al.*, 2003). Among fungal infections, those due to *Candida*, especially *C. albicans*, are the most common. Their persistence particularly as opportunist infections in AIDS patients is alarming. Medicinal plants can therefore represent an alternative in the treatment of some infections (Nascimento *et al.*, 2000).

Aphania senegalensis Radlk ex Poir is a plant employed in Senegalese traditional medicine to treat various diseases such as bacterial and fungal infections, pain, inflammation and asthenia. The fruits of the plant are edible while the leaves are commonly used in traditional medicine (Figure 1). The analgesic and anti-inflammatory properties of leaf extracts of this plant have been previously established (Fall *et al.*, 2009). However, there is paucity of scientific data on the phytochemical constituents of leaf extracts and its potential antimicrobial properties as well as its cytotoxicity. This study was therefore undertaken to bridge these gaps.

https://doi.org/10.21010/ajtcam.v14i4.16

Figure 1: Fruit and leaves of *Aphania senegalensis* (*Sapindaceae*).

Material and Methods

Plant collection

Leaves of *Aphania senegalensis* were collected at the Botanical Garden of Faculty of Medicine, Pharmacy and Odontology, University Cheikh Anta DIOP, Dakar, Senegal. Voucher specimens numbered K1613 is deposited at the herbarium of the botanical garden of this faculty. Plant leaves were dried at room temperature in a ventilated room and grounded to a fine powder in a Brabender grinder (Model 2000 LAB Eriez®).

Phytochemical screening

Standard phytochemical analyses were carried out to test for the presence of the phytoconstituents (tannins, flavonoids, anthracenic glycosides, saponins, cardiotonic heterosides and alkaloids) in the prepared extract. Chemical tests were carried out on ethanol and aqueous extracts of the powdered specimens using standard procedures for the detection of saponins (foaming index), tannins (Stiasny test followed by ferric chloride test), flavonoids (Shibata's reaction), anthracenic glycosides (Borntraeger test), cardiotonic heterosides (Baljet, Kedde and Raymond-Marthoud reagents tests), alkaloïds (Bouchardat, Valser-Mayer and Dragendorff's reagent test), in order to identify the presence of phytochemical constituents (Harborne, 1998).

Extraction

Powdered leaf of *Aphania senegalensis* combined in a ratio of 1:10 using ethanol was decocted for 30 minutes as traditionally recommended and filtered through Whatman No. 1 filter paper. Ethanol was removed under reduced pressure using a rotary evaporator.

Cytotoxicity assay

Cytotoxicity of ethanol leaf extract of *Aphania senegalensis* was determined using a tetrazolium-based colorimetric assay (MTT assay) described by Mosmann (1983). Vero monkey kidney cells were harvested and centrifuged at 200×*g* for 5 minutes. The pellet was re-suspended in growth medium and seeded at a density of 2.4×10^3 cells/mL, using Minimal essential medium (MEM) (Highveld Biological, South Africa) supplemented with 0.1% gentamicin (Sigma) and 10% foetal calf serum (Highveld Biological, South Africa). After 24 h of incubation at 37°C, the medium on cells was removed and 200 µL of test plant extract or berberine chloride (Sigma) (positive control) at various known concentrations (quadruplicate dilutions prepared in growth medium) added. Cells were further incubated at 37°C in a 5% CO_2 incubator for 5 days. Thereafter, 30 µL MTT (stock solution of 5 mg/mL in phosphate–buffered saline) was added to each well and the plates incubated for a further 4 hours at 37°C. The medium was carefully removed by aspiration and the MTT formazan crystals dissolved by adding 50 µL of DMSO to each well. The absorbance, proportional to the amount of MTT reduced, was measured at 570 nm using a microplate reader (Versamax). The LC_{50} values were calculated as the concentration of plant extract resulting in a 50% reduction of absorbance compared to untreated cells from a linear regression equation. LC_{50} values are expressed as mean of three independent triplicate assays and values were expressed as mean of quadruplicate assays.

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Antimicrobial activity
Fungal and bacterial cultures

Fungal species *Aspergillus fumigatus*, *Candida albicans* and *Cryptococcus neoformans* were used. Fungal cultures were obtained from the fungal culture collection at the Microbiology Unit, Department of Veterinary Tropical Diseases (University of Pretoria, South Africa). Densities of fungal cultures used were: *A. fumigates*, $8.1x10^6$ cfu/mL; *C. albicans*, $2.5x10^6$ cfu/mL; and *C. neoformans*, $2.6x10^6$ cfu/mL.

For bacterial pathogens, two Gram positive bacterial organisms (*Staphylococcus aureus* (ATCC 29213), *Enterococcus faecalis* (ATCC 29212) and two Gram negative (*Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) were used in the study. These species are considered as the most important nosocomial pathogens (NCCLS, 1990). Bacterial cells were inoculated into fresh Müller–Hinton (MH) broth (Fluka) and incubated at 37°C for 14 hours prior to the screening procedures. Densities of bacterial cultures after incubation overnight, prior to testing were as follows: *Staphylococcus aureus*, 2.6×10^{12} cfu/mL; *Enterococcus faecalis*, 1.5×10^{10} cfu/mL; *Pseudomonas aeruginosa*, 5.2×10^{13} cfu/mL; *Escherichia coli*, 3.0×10^{11} cfu/mL.

Minimum inhibitory concentration (MIC) determination

For antifungal activity testing, the serial microplate dilution method using iodonitrotetrazolium violet as growth indicator developed by Eloff (1998) and modified by Masoko *et al*. (2005) was used while for antibacterial activity testing, the method described by Eloff (1998) was employed. MIC was described as the lowest concentration of the test extract inhibiting the growth of microorganisms. Amphotericin B was used as positive control and acetone as solvent control in the antifungal assay, while gentamicin was used as positive control and acetone as solvent control in the antibacterial assay. MIC values were expressed as mean of three independent triplicate assays.
The total activity (TA) of the extract was calculated by dividing from amount in mg extracted from one gram of the plant material by the MIC value (mg/mL). Total activity of an extract, gives an indication on the volume at which active constituents present in one gram of plant material can be diluted and still inhibit the growth of test organisms (Eloff, 2004). Selective activity (SI) of the extract was calculated by dividing the cytotoxicity LC_{50} in mg/mg by the MIC in mg/ml.

Results

Phytochemical screening

A mass of 19.9 g of dried extract was obtained after extraction of 200 g of powdered leaf material with ethanol representing a yield of 9.95%. Test for the presence of phytochemical revealed that tannins and flavonoids were the main constituents of the ethanol leaf extract of *Aphania senegalensis*. Negative reactions were obtained for the presence of anthracenic glycosides, cardiotonic heterosides, saponins (foaming index less than 100) and alkaloids.

Cytotoxicity

Berberine used as positive control had a higher LC_{50} than that of the extract (9.99±0.54 µg/mL *versus* 2.67±0.04 µg/mL respectively). This means that the extract was more toxic than the positive control.

Antimicrobial activity
Antifungal activity

The ethanol leaf extract of *Aphania senegalensis* inhibited the growth of the three fungal strains following exposure to extract for 24 h **(Table 1)**. By dividing the LC_{50} in mg/ml with the MIC in mg/ml the selectivity index could be calculated. This provides an indication of the safety of the extract. The lowest MIC was noted for *C. albicans* (0.16 mg/mL) with SI 0.62 while the extract inhibited the growth of *A. fumigatus* and C. *neoformans* at MIC 0.62 mg/mL with SI 0.16 and 0.13 respectively. The total activity of the extract for *C. albicans* (621.87 mL/g) was higher than that for *A. fumigatus* (160.48 mL/g) and *C. neoformans* (160.48 mL/g).

Table 1: MIC (mg/mL), total activity (mL/g) and selectivity index of tested samples on fungal strains.

Samples	Fungi								
	A. fumigatus			*C. neoformans*			*C. albicans*		
	MIC	TA	SI	MIC	TA	SI	MIC	TA	SI
Ethanol extract	0.62	160.48	0.16	0.62	160.48	0.13	0.16	621.87	0.62
Acetone	>2.5	---	---	>2.5	---	---	>2.5	---	---
Amphotericin B	2.5×10^{-6}	---	---	2.0×10^{-5}	---	---	2.5×10^{-6}	---	---

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Antibacterial activity

Table 2 represents the MIC average for each sample tested. In some cases the MIC was greater than 2.5 mg/mL the highest concentration used. The results indicated that ethanol leaf extract of *Aphania senegalensis* was active on *Enterococcus faecalis* (MIC = 0.08mg/mL and SI=1.24), *Escherichia coli* (MIC = 0.16mg/Ml and SI=0.62) and *Staphylococcus aureus* (MIC =0.31mg/Ml and SI=0.32). However the extract was not shown to inhibit growth of *Pseudomonas aeruginosa.* The total activity of the extract for *Enterococcus faecalis* (1243.75 mL/g) was greater than that for *Escherichia coli* (621.87 mL/g) and *Staphylococcus aureus* (320.97 mL/g).

Table 2: MIC (mg/mL), total activity (mL/g) and selectivity index (SI) calculated by dividing EC50 by MIC of samples tested on nosocomial bacteria.

Samples	Bacteria gram (+)						Bacteria gram (-)					
	E. faecalis ATTC 29212			*S. aureus* ATTC 29213			*E. coli* ATTC 25922			*P. aeruginosa* ATTC 27853		
	MIC	*TA*	*SI*	*MIC*	*TA*	*SI*	*MIC*	*TA*	*SI*	*MIC*	*TA*	*SI*
Ethanol extract	0.08	1243.75	1.24	0.31	320.97	0.32	0.16	621.87	0.62	>2.5	---	---
Acetone	>2.5	---	---	>2.5	---	---	>2.5	---	---	>2.5	---	---
Gentamicin	1.25	---	---	1.25	---	---	0.16	---	---	0.62	---	---

Discussion

The ethanol leaf extract of *Aphania senegalensis* had a better antimicrobial activity on bacterial pathogens than on fungal pathogens. The antibacterial activity of the extract was four times better than that of the positive control gentamicin against *S. aureus* and *E. faecalis*, the two Gram positive bacteria. In the case of *E. coli* the extract had the same activity (MIC=0.16 mg/mL) as gentamicin. The extract was however not active against *P. aeruginosa* at 2.5 mg/ml, the highest concentration tested.

The extracts had a much lower antifungal activity than the positive control amphotericin B. Higher activity of the extract was obtained against *C. albicans* (MIC=0.16 mg/mL).

The low SI values obtained for extract of this plant, indicates that with the exception of activity against *E. faecalis* (SI 1.24) the extract was more toxic to cells than to the microbial pathogens.

The main secondary metabolites identified in the leaf extract of *Aphania senegalensis* were polyphenols such as flavonoids and tannins (hydrolysed and condensed). These two phytochemical groups are known for their antimicrobial activity (Daglia, 2012). With the rise in antibiotic resistance around the world, it remains important to look for new antimicrobial molecules and active extracts containing polyphenols can be of interest in this regard. Polyphenols in combination with antimicrobial agents may be useful in antimicrobial therapy based on synergistic effects.

The extract was more toxic to Vero cells than the positive control. This finding was not surprising. In fact, Fall *et al*. (2011) have reported the sub-acute toxicity of an aqueous leaf extract of the same plant. In that study, the acute and sub-acute toxicity of aqueous leaf extract of *Aphania senegalensis* on male and female Wistar rats were evaluated and biochemical analysis following sub-acute toxicity study revealed significant increase in transaminase activity in treated groups. Histological examination of liver samples of the treated groups confirmed the presence of various liver lesions. A finding that is suggestive that a daily dose of 1000 to 2000 mg /kg of aqueous leaf extract of *Aphania senegalensis* may be hepatotoxic to humans following prolonged treatment.

Medicinal plants, widely used by African populations are presumed safe, a view that is reinforced by their secular use. In fact, the majority of the African population used medicinal plants to treat disease (Farnsworth *et al*., 1985).

However, adverse effects associated with the use of medicinal plants have been reported in the literature (Kane *et al.*, 1995). Therefore it is necessary to evaluate the toxicity of medicinal plants and to determine their threshold *in vivo*.

Conclusions

Ethanol leaf extracts of *Aphania senegalensis* containing polyphenolic compounds (flavonoids, condensed and hydrolysed tannins) has promising antifungal and antimicrobial activity justifying its use in folk medicine to treat some

bacterial and fungal diseases. Care should be taken if plant extracts are not used topically but taken internally due to the high cytotoxicity of the extracts. Further studies are underway to isolate active compounds from the plant. It is possible that such a compound may have lower toxicity, since toxicity may be related to the synergistic effect of the other compounds present in the extract.

References

1. Daglia, M. (2012). Polyphenols as antimicrobial agents. Curr Opin Biotechnol; 23 (2): 174- 81.
2. Eloff, J.N. (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med., 64: 711- 713.
3. Eloff, J.N. (2004). Quantifying the bioactivity of plant extracts during screening and bioassay-guided fractionation. Phytomed., 1: 370-71.
4. Fall, M., Boukandou, M., Fall, A.D., Cabral, M., Diatta, W., Gueye, P.M., Faye, M., Bakou, S.N., Mendes, V., Bassene, E., Diouf, A. (2011). Acute and sub-acute toxicity of the aqueous extract from *Aphania senegalensis* (Juss. ex Poir.) leaves in wistar rats. Dakar. Med., 56 (1): 216- 223.
5. Farnsworth, N.R., Akerele, O., Bingel, A.S., Soejarto, D.D., Guo, Z.G. (1985). Medicinal plants in therapy. Bul. World Health Org., 63 (6): 965- 981.
6. Harborne, J.B. (1998). Phytochemical methods. A guide to modern techniques of plant analysis. Springer Science & Business Media, 302 pages.
7. Kane, J.A., Kane, S.P., Jain, S. (1995). Hepatitis induced by traditional Chinese herbs, possible toxic components. Gut, 36 (1): 146- 147
8. Mah, T.F., Pitts, B., Pellock, B., Walker, G.C., Stewart, P.S., O'Toole, G.A. (2003). A genetic basis for *Pseudomonas aeruginosa* biofilm antibiotic resistance. Nature, 426: 306-310.
9. Masoko, P., Picard, J., Eloff, J.N. (2005). Antifungal activities of six South African *Terminalia* species (*Combretaceae*). J Ethnopharmacol., 99: 301- 308.
10. Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assay. J. Immunol. Methods, 65: 263- 271.
11. Nascimento, G.F., Locatelli, J., Freitas, P.C., Silva, G.L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. Braz. J. Microbiol., 31: 247- 256.
12. National Committee for Clinical Laboratory Standards. (1990). Performance Standards for Antimicrobial Disk Susceptibility Tests, 4th ed. Approved Standard. NCCLS Document M2-A4, Villanova, PA, USA.
13. Stewart, P.S., and Costerton, J.W. (2001). Antibiotic resistance of bacteria in biofilms. *Lancet*, 358, 9276: 135-138.