Phytochemical Screening and Antifungi Activity of Senna Alata Leaves Extract against Some Selected Fungi Isolate

***OLORUNNADO Gabriel Babatunde., OMEIZA Ojuwa Anthonia., MUHAMMED Karim Abdulrahim., IMAJI Mathew**

Kogi State Polytechnic Lokoja, Kogi State

Correspondence Author

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ABTSRACT

Senna alata also known as candle stick or bush candle is a very important medicinal plant due to the fact that,every part of the plant especially the leaves are medicinal. In this research, crude extract was prepared from *senna alata* leaves using ethanol, The crude extract was analysed for its phytochemical contents. The antifungal activities of the ethanol extract of *senna alata* leaves were also investigated against four different species of fungi namelly: *Aspergillus spp Rhizopus spp.peniciliumniger and Candida albican.* The result of the phytochemical analysis showed that *senna alata* leaves contain Tannin,Saponin, The fungi species were also tested with ketoconazole tablet (200mg) a stanadard drug as a positive control Alkaloid, Flavonoid, Steroids, Terpenoid, Phenol, Phobatanin and Glycoside were present in the extract. The ethanolic crude extract of *senna alata* was found to inhibit the growth of the entire organism tested with a certain diameter that is proportional to the concentration of the crude extract. The experiment was repeated five times for each of the fungi and the mean with standard deviation was calculated using spss. The extract with the concentration of 25% have a growth inhibition diameter of 5.23±0.17mm when tested with *aspergillus niger,* 4.12±0.11mm with *Rhizopus spp*, 9.18±0.20mm with *penicilium niger* and 10.15±0.03mm with *Candida albican.* The extract with concentration of 50% shows growth inhibition diameter of 11.00±0.15mm when tested with *Aspergillus niger*, 10.22±0.25mm with *Rhizopus spp*,14.11±0.80mm with *penicilium niger,* and 14.21±0.23mm with *Candida albican*. Finally, the extract with the concentration of 100 % have a growth inhibition diameter of 20.30±0.10mm with *Aspergillus spp,*26.15±0.11mm with Rhizopus spp, 30.20±0.12mm with *penicilium spp* and 25.10±0.10mm with *Candida albican.* The results obtained from the various concentration of the crude extracts with the same concentration show no significant different($P<0.05$) and are dose dependant. The fungi culture tested with a standard drug as positive control was found to be more effective. The result from both the antifungal property and the phytochemical screening revealed that the leaves of *senna alata* show some sign of antifungal activity

Key words: Antifungal, Inhibition diameter, *Senna Alata*, Extracts, Organism.

INTRODUCTION

The use of herbal medicine as alternative form of therapy has increased tremendously over the years and across the world, This is due to the increased resistance of pathogens to conventional antimicrobial drugs (Ria,2018). According to WHO (2000) an estimated 60% of individuals in the developing countries believe completely in ancient medicines for their health care. Herbs products development depend mostly on the local botanical flora . In Nigeria, there are wide varieties of medicinal plants distributed in the different geoecological regions of the country (Ria, 2018). *Senna alata* also known as candle stick is called 'Asunrun Oyinbo' in Yoruba language 'Ogalu' in igbo language (Yadau *et al* ,2014) . It is also refered to as ring worm shrub or craw craw plant in in some part of Nigeria (seaforth, 2012) .The genus senna mill originates from the Arabic name 'sana' and belong to the subtribe 'cassieae' , sub family 'Caesalpinioidea', Family 'Leguminosae' and order 'Fabales' (Yadau *et al* , 2014). It had been reported that extract from the leaves can

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be mixed with local gin, time or oil or used alone for the of skin diseases and some eruptive diseases (Palanichany*, et al*., 2011). Also the leaf extract mixed with lanolin was used by European doctors in West African to treat ‘ dhobic itch’ and also the extract alone are taken as purgative, laxative, abortifacient or deconction to induce labour (Sawyer,2012). Researcher like Verpoorte*et al.,* 1982, Benjamin *et al* ., 2011, Palanchanny*et al* 2010, Aladesanmi *et al* 1991 and Olorundare*et al* 1992 all reported the antibacterial actitvity of *senna alata* leaves extract. Rao (2016) , Benjamin (2011) and Palanichany *et al*.,(2011) revealed the presence of Anthraquinones, Steroids, Flavonoids and Saponin in order to ascertain the claimed chemical composition and antibacterial properties of *Senna alata.* This research was put forward on an activity guiding the study of the plant with the view to characterizing the antibacterial agents present in *senna alata* leave.

METHODOLOGY

Invitro experiment study was carried out to evaluate the antimicrobial effects and phytochemical screening of *senna alata* against some selected fungal isolates. All the reagent used are of high analytical grade. They include 0.1Mole Hydrochloric acid. 1.25% Sulphuric acid solution, Ethanol, Sabrose Dextrose Agar, Phenolpthalein indicator, 0.1M Sodium hydroxide, 40% Formaldehyde Solution, 1.25% Sodium hydroxide Solution, Ferric chloride, Chloroform and Distilled water. The equipments and apparatus used include Water bath, Muffle furnace, Oven Beakers, Volumetric flask, Whattman filter paper, Spatula, Measuring cylinder, Stirring rod, Funnel, Heating mantle, Cotton wool, Sample bottles, Flat bottom flask, Mortar and pestle.

Sample Collection and Preparation

Senna alata were obtained from a bush in felele, Lokoja axis in Kogi State, It was identified by Dr Namadi Sunusi, Horticulture Department, Ahmadu Bello University Zaria. The voucher number is ABUH0981, It belong to the family; *Fabaceae Senna Alata*. The leaves were air dried for 15 days. It was pounded to powder using Mortar and Pestle. The powdered sample of the leaves was kept in an air tight container at room temperature prior to commencement of the analysis.

Sample Extraction

Crude extract of *senna alata* leaves was prepared by weighing 20g of *senna alata* leaves powder into a beaker. This was soaked in 400ml Ethanol for 72 hours with vigorous shaking in orbital shaker series. It was then filtered, the filtrate was concentrated by evaporating it in water bath evaporator.it was stored in a clean sample container in a refrigerator.

Acute Toxicity Study

The oral median lethal dose (LD50) of the extracts was determined using experimental rats accoding to the method of Lorke, 1983

Determination of Phytochemical Contents in *Senna Alata* Leaves Extract

Test for Phobatannins

The method used is according to the method described by Trease (1989). 2ml of the extract was diluted with 2ml distilled water. It was then agitated in a test tube for 5 minutes. About 0.1cm layer of foam indicates the presence of Phobatamins

Test for Saponin

The method adopted is the method according to Mbatchou (2012). 2ml of the extract was diluted with 2ml of distilled water. It was shaken for 5 minutes. The formation of about 1cm of foam which persist indicates the presence of saponin.

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Test for Flavonoids

2ml of 10% sodium hydroxide was added to 2ml of the extract in a test tube. An intense yellow colour was formed which turned colourless upon addition of 2ml of hydrochloric acid. This indicates a positive test for flavonoids

Test for Alkaloids

2ml of 10% hydrochloric acid was added to 2ml of the extract in a test tube followed by addition of 1ml Dragendroff's reagent. An orange precipitate indicates the presence of alkaloids.

Test for Steroids

2ml of the extract was dissolved in 10ml of Chloroform and then 10ml of concentrated Sulphuric acid was added by the side of the test tube. Red color was observed at the upper layer while the Sulphuric layer turned yellow with green fluorescence.

Test for Terpenoids

2ml of the extract was mixed with 2ml Chloroform and 1ml of concentrated Tetraoxosuphate (vi) acid was carefully added to form a layer. A clear upper layer and lower layer with redish brown interphase indicates the presence of Terpenoid

Test for Glycosides

2ml Acetic acid was added to 2ml of the extract. The mixtiure was cooled in a cold water bath . 2ml of concentrated tetraoxosulphate (vi) acid was added Color development was noticed from blue to bluish green.

Test for Phenols

To 2ml of the sample extract, 2ml of 1% Ferric chloride was added. The formation of deep blue black coloration was observed which indicates the presence of phenol.

Test for Tannin

To 2ml of the extract 4ml of water with drops of Ferric chloride was mixed. Green precipitate was observed. This indictates the presence of Tannin.

Determination of AntiFungal Effect of the Crude Extract on *Aspergillus spp, Rhizopus spp, Penicilium spp,* and *Candida albicans.*

Three different samples of the extract containing 100%, 50%, and 25% of the extract were prepared. The test organism used are *Aspergillus spp, Rhizopus spp, Penicilium spp*, and *Candida albicans* .These were clinically isolated and obtained from a parasitological laboratory in a medical center lokoja. Microbial load of 1.0×10^6 cfu of the tested organism were obtained by serial dilution using the standard methord of dilution by Raynolds Jackie, 2005. With the aid of a sterile swab, each of the fungi species were inoculated into an appropriety prepared Sabrose Dextrose Agar A sterilized cork borer (5mm in diameter) was used to make a hole on each of the agar plates. Each holes were filled with the different concentration of the leaves exrract using sterilized pastuer pipette .The process described above was repeated with the fungi isolate culture but with a solution of a standard drug ketoconazole tablet (200mg) The Plates were incubated at 37°C for 18hours. The diameter of the zone of inhibition around each hole was measured and used to determine the microbial susceptibility of the extract at different concentration

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Acute Toxicity

No sign of toxicity or mortality observed even up to a dose of 5000 mg/kg of the aqueous extracts The result of the acute toxicity studies . the oral LD50 of each of the extracts was then taken to be > 5000mg/kg

Phytochemical Contents and The Antifungal Effect of Senns alata Leaves Extracts

The results of the phytochemical screening of senna alata leaves is shown in table 1 below. The result revealed that it contained significant amount of Tannins, Phenols, Alkaloids, Saponins, Flavonoids, Phlobatamins and trace amount of Steroids while Glycoside was not detected. Theses secondary plant meatabolites are very important compounds because they contribute positively to traditional medicine.

Table 1 Phytochemical Analysis of *Senna alata* leaves

Phytochemical parameters	composition
Tannins	+++
Saponins	++
Alkaloids	+++
Flavonoids	++
Phlobatamins	+++
Steroids	+
Terpenoids	++
Glycosides	-
Phenol	+++

Key

Slightly present = +

Moderately Present = ++

Highly present = +++

Absent = --

The results of the ethonolic extract of *senna alata* tested on *Aspergillus niger, Rhizopus spp, Penicilium spp* and *Candida a*lbicans revealed that *senna alata* crude extract have antifungal activity and is dose dependent .Table 2 below showed the growth inhibition diameter of *senna alata* leaves extract on the tested organism at different concentration. Also, table 3 revealed the growth inhibition of ketoconazole solution, a standard drug as a positive control. The zone inhibition diameter of the different fungi culture was found to be dose dependant with 10% concentration having the highest zone of inhibition. *Penicilium spp* is the most susceptible of all the tested fungi however, comparing the zone of inhibition at 10% there are no significant differen t($p<0.05$) down the column between *Rhizopus spp, Penicilium spp* and *Candida a*lbicans but there is a significamt different with *aspergillus spp* .comparing the zone of inhibition of the crude extract at 10% with the one of the positive control (ketoconazole) threr is significant different between the values.

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Table 2 Zone of growth inhibition of *Senna alata* leaves extract on tested organism

Test Organism	100%	50%	25%
Aspergillus niger	**20.30±0.10^{a}mm**	**11.00±0.15^{a}mm**	**5.23±0.17^{a}mm**
Rhizopus spp	**26.15±0.11^{b}mm**	**10.22±0.25^{a}mm**	**4.12±0.11^{a}mm**
Penicillium spp	**30.20±0.12^{c}mm**	**14.11±0.80^{b}mm**	**9.18±0.20^{b}mm**
Candida albican	**25.10±0.10^{b}mm**	**14.21±0.23^{b}mm**	**10.15±0.03^{b}mm**

Data are pesented as mean ± SD (n=6) of the inhibition diameter of the crude extracts. Mean values having different alphabet as superscript down the column are significantly different at $p<0.05$

Table 3: Zone of growth inhibition of ketoconazole dissolved tablet solution on tested organism

Test Organism	100%	50%	25%
Aspergillus niger	**35.51±0.211^{a}mm**	**22.00±0.100^{a}mm**	**12.43±1.201^{a}mm**
Rhizopus spp	**39.14±0.201^{b}mm**	**21.14±0.244^{b}mm**	**11.21±0.221^{b}mm**
Penicillium spp	**37.42±0.130^{c}mm**	**25.21±1. 300^{c}mm**	**12.36±0.211^{a}mm**
Candida albican	**40.15±0.214^{d}mm**	**25.55±0311^{c}mm**	**8.55±0.21^{c}mm**

Data are pesented as mean ± SD (n=6) of the inhibition diameter of ketoconazole **. Mean values having different alphabet as superscript down the column are significantly different at $p<0.05$**

DISCUSSION

All tested microorganism showed susceptibility to *senna alata* extract at concentration of 25%, 50%, 100% . The extract at the concentration of 25% was observed to have growth inhibition diameter of 5.23±0.17mm with *Aspergillus spp*, 4.12±0.11mm with *Rhizopus spp*, 9.18±0.20mm with *Penicilium spp* and 10.15±0.03mm

with *Candida albicans*. The extract with concentration of 50% have an inhibition diameter of 11.00±0.15mm with *Aspergillus spp,* 10.22±0.25mm with *Rhizopus spp*, 14.11±0.80mm with *Penicilium spp* and 14.21±0.23mm with *Candida albicans.* Also, the extract with with 100% concentration have a growth inhibition zone of 20.30±0.10mm with *Aspergillus spp*, 26.15±0.11mm

with *Rhizopus spp*, 30.20±0.12mm with *Penicilium spp* and 25.10±0.10mm with *Candida albicans.*there is no significant different between this values ($p<0.05$). The research is in agreement with the work of Raji *et al*., 2015 and Gurera *et al* 2020., Gurera and his group carry out a research on Antifungal Activity of *Senna Alata, Senna Bicapsularis a*nd *Pityrogramma Calomenalos*. In their research they find out that *Pityrogramma Calomenalos* showed the highest antifungal activity and next is *senna alata* hence antifungal property exhibited by *Senna aalata* is very significant. The antifungal activities of *senna alata* could be probably because of the presence of phytochemicals like Alkaloids, Tannins Phenols and Saponin. Saponin is bitter, insoluble in water and exhibit coagulating activity. It also posseses hemolytic activity. Flavonoids known as nature biological response modifiers protect plant from pathogens attack. it exhibitanti ulcer, anti hepatotonix. Anti allergic, anti viral and anti inflammatory activities (Hauptmann, 2010). They are good antioxidants capable of scavenging ROS duo to the presence of phenolic hydroxyl group (Hauptmann, 2010). Alkaloids, Tanins and Flavonoids showed medical activity against pathogens and are used in the treatment of various diseases (Verpoorte,*et al* 2012). The results of the phytochemical screening of *senna alata* agrees with the

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work of enzo 2007., he reported the presenceof Saponin, Steroids, Terpenoids and trace amountof phenol in *senna alata* leaves extract hence the phyto compounds of the plant exhibit various biological activity and are of pharmaceutical importance.

however all kind of metabolites detected in this study are well known to have significant inhibition action against fungi such as vaginal candidiasis, ring worm, valley fever, nail infection, Candida auris etc. Some of the limitation of this research is lack of adequate finance and also the minimum inhibitory concentration could not be determined . more also, the method used for the phytochemical analysis could not indicate the quantity of each phytochemicals in figures, phytochemicals were quantified base on the intensity of their physical properties

CONCLUSION

The results of the research revealed that, ethanol leaves extract of *Senna alata* show invittro activity against *Aspergillus spp, Rhizopus spp, Peniciliumspp*and *Candida a*lbicans.. It also revealed that its antifungal property is dose dependent hence can serve for effective antifungal agent. The extract is rich in phytochemical components and exerted appreciable antimicrobial activities therefore further investigation is required using more species of fungi . Following the observation in the results and its activities.

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