http://dx.doi.org/10.4314/bajopas.v12i1.99S

Bayero Journal of Pure and Applied Sciences, 12(1): 663 - 667
ISSN 2006 – 6996

PHYTOCHEMICAL EVALUATION AND IN VITRO ANTIMICROBIAL ACTIVITY OF *Senna alata* LEAVES EXTRACT

***Amina Muhammad[1], Elinge C.M[2], Isah Musa[1] and Abbas Umar**
[1]Department of Microbiology. Kebbi State University of Science and Technology.
[2]Department of Pure and Applied Chemistry. Kebbi State University of Science and Technology. Aliero Kebbi State.
*Corresponding author: aminamuhammad243@gmail.com

ABSTRACT
The use of Senna alata to treat wide range of microbial diseases in traditional medicinal practice, coupled with instant growing demand of plant based drugs call for this research, which aimed at evaluating the phytochemical content and antimicrobial effect of the ethanolic leaf extract of Senna alata. The extract was subjected to phytochemical screening using standard procedures, and the antimicrobial activity against three (3) bacterial isolates (Escherichia coli, Staphylococcus aureus and Pseudomonas aerugenosa) and two (2) fungal isolates, (Candida Albicans and Microsporum canis.) were tested using Kirby bauer disc diffusion method. Phytochemical screening shows the presence of alkaloids, saponins, steroid, flavonoids, tannins, terpenoids and glycosides in the plant extract. The ethanolic extract of Senna alata showed antibacterial activity with zone of inhibition diameter of 15mm, 10mm and 7.0mm on Escherichia Coli, Staphylococcus aureus and Pseudomonas aeruginosa respectively, and antifungal activity of 16mm and11mm on Microsporum canis. and Candida albicans respectively all at 80mg conentration. The minimum inhibitory concentration (MIC) ranged between 10mg and 80mg, minimum bactericidal concentration (MBC) ranged between 20mg and 80mg. The results provided evidence that leaf of Senna alata has the potential that can be harnessed to produce drugs that can be used to treat ailments caused by these pathogens.
Key: Senna alata, Antimicobial activity, Ethanolic extract, Phytochemical

INTRODUCTION

The spontaneous search for new drugs by Humans particularly medicinal plants brings a continuous use of traditional medicine by about 80% of the world population to treat their common illnesses since pre-historical time (Ikegbunam *et al.,* 2013). Different medicinal plants were found to be useful to cure different bacterial and fungal diseases. Medicinal plants are rich source of antibacterial agents. Although they produce slow recovery, the use of medicinal plants as therapeutic agents is becoming popular due to their lesser side effects and low microbial resistance (Ola, *et al*., 2013)

The antimicrobial activity of plant extracts results from the compounds known as secondary metabolites. Thus, the combined activities of the secondary products present in the plants provide the beneficial effects of the plants material. These secondary products include alcohols, phenols, glycosides, tannins and saponins which are able to physiologically act on the body (Muhammad and Yakubu, 2014). Many years of the trial and error using traditional medicine as therapeutic agents brings about the Knowledge on plants uses and selection of the desirable, efficient, and the most successful plant present in the immediate environment at a given time (Tsobou *et al.,* 2015).

Senna alata (*Cacia alata*), is a shrub found in different areas of West Africa. It is widely used in Nigeria and Ghana for the treatment of different ailments including; dysentery, stomach disorder and helminthes infections. Also the decoction of fresh leaves of *Senna alata* is used to treat bronchitis, athma and skin infections including ringworm (Doughari and Okafor, 2007). It is also used to treat urinary tract infections and snake bites among others (Somchit *et al.,* 2003). The prevalence use of the plant for treatment of different ailments brings the urgent need to research on its efficacy against some selected isolates.

This research work aimed at determining the antimicrobial activity of *Senna alata* leaves on some selected clinical isolates.

MATERIALS AND METHODS

Sample collection: The fresh leaves of *Senna alata* were collected from Zuru local government area of kebbi state. They were identified and authenticated at the Department of plant science and biotechnology, Kebbi State University of Science and Technology Aliero.

Sample preparation: The leaves were washed and shade dried at room temperature for 7 days. After which they were pounded with clean mortar and pestle to powder form which was then parked into clean air tight containers and stored for further analysis**.** 50g of the powdered sample was extracted with 200mL of ethanol for 3 days using Soxhlet extractor. The extract obtained were concentrated in water bath at 40ºC (Gurma *et al*., 2019).

Phytochemical screening

The samples were examined to detect the presence the following compounds: Alkaloids, saponins, glycosides, flavonoids, anthraquinones, tannins, terpenoid and cardiac glycoside using standard laboratory techniques according to Abba *et al*., (2009) ; Harbone, (1973).

Alkaloids: about 0.5g of the extract was stirred in 5ml of 1% aqueous hydrocholoric acid (HCl) on a steam bath, allowed to cool and filtered. 1ml of the filtrate was treated with few drops of Mayer's reagent and to another 1ml of the filtrate, a few drops of Dragendorff's reagent was added. Precipitate with white turbidity with either of the reagents was taken as the presence of alkaloid.

Saponins: 0.5g of the extract was mixed with water in a test tube and shaken. Frothing which persist on warming was taken as positive saponing.

Glycosides: 2.5ml of 50% H_2SO_4 was added to $5cm^3$ of the extract in a test tube, the mixture was heated in boiling water for 15mn, cooled and neutralized with 10% NaOH, 5ml of fehling's solution and boiled for 15mn. A brick red precipitate indicates the presence of glycosides.

Flavonoids: to alcoholic solution of the extract, magnesium powder and few drops of concentrated HCl were added and formation of orange, pink, red, to purple color indicates the presence of flavonoids.

Steroids: $5cm^3$ of the extract was poured in $2cm^3$ chloroform and $2cm^3$ H_2SO_4 was added carefully to form a low layer. A reddish brown color at the interface indicates the presence of steroid ring.

Anthraquinones: $5cm^3$ of the extract was shaken with $10cm^3$ of benzene and $5cm^3$ of 10% ammonia solution was added to the mixture and shaken. The presence of pink, red or violet color in the ammoniacal (lower) phase indicates the presence of anthraquinones.

Tannins: 5% Ferric chloride solution was added drop by drop into $2cm^3$ of the extract. Condensed tannins gives dark green color and hydrolysable tannins give blue-black color

Cardiac Glycoside: To the extract $2cm^3$ of 3.5% ferric chloride solution was added and allowed to stand for one minute, $1cm^3$ of concentrated H_2SO_4 was carefully poured down the wall of the testube so as to form a layer. A redish-brown ring in the interface was taken as cardiac glycoside positive.

Bioassay

Test organism/ Inoculum standardization

The bacterial and fungal isolates used were obtained from microbiology department Kebbi state university of science and technology Aliero. The organisms were authenticated using standard tests, the bacterial cultures were sub cultured, gram stained and biochemical tests were carried out for confirmation, while the fungal isolates were sub cultured and identified. The isolates were then prepared for antimicrobial activity as follows:

A loop full of the test organism was taken from an overnight broth culture and suspended directly in 5ml of saline, and the resulting microbial suspension was diluted until the turbidity matched 0.5 McFarland standard estimated to be equivalent to approximately $5x10^7$ CFU/ml. Inoculation of the media for susceptibility test was made within 30mn of adjusting the inoculums (Omogbai and Eneh, 2011).

Determination of antimicrobial activity

Antimicrobial activity of the plant extract was tested using disc diffusion method as described by Nair *et al.,* (2005) at different concentrations (i.e. 20, 40, and 80 mg/ml). Muller Hinton and Sabouraud Dextrose Agar (SDA) were prepared according to the manufacturer's instructions and the plates were seeded with appropriate test organisms (*Escherichia coli, Staphylococcus aureus, Microsporum spp.* and *Candida albicans*). Discs of 6 mm diameter were prepared from Whatman filter paper No. 1 and sterilized. The discs were then impregnated with the extracts of varying concentration. Ciprofloxacin and fluconazole were used as controls for antibacterial and antifungal extracts respectively. The bacterial plates were incubated at 37°C for 24 hrs and the fungal plates for 5-7 days, after which the zones of inhibitions were measured.

Determination of Minimum Inhibitory Concentration (MIC)

This was determined using the broth dilution techniques according to Oyeleke and manga (2008). Nine (9) test tubes were used for each sample, each containing 5mls of nutrient broth. 5mls of 80mg, of the extracts was added to 5mls of nutrient broth, 5mls was then transferred to the next test tube containing 5mls of nutrient broth, this dilution continued until the 9^{th} test tube was reached. 5mls was taken from the 9^{th} test tube and discarded. 0.2ml of standardized bacterial culture was inoculated in to each of the nine test tubes and the content was thoroughly mixed. Another two (2) test tubes were used as controls The first test tube served as control for the sterility of the media, containing 9mls of nutrient broth and the second test tube containing 5mls of nutrient broth and 0.2ml bacterial suspension serve as control to test the viability of the organism. Tubes were incubated at 37^0C for 24hours. The lowest concentration of the formulation that produces no visible bacterial growth when compared with the control was taken as the MIC. The same procedure was repeated for the fungal isolates using Potato Dextrose Agar and incubated for 3 days.

Determination of Minimal Bactericidal Concentration (MBC)

Tubes showing no visible growth from the MIC test were sub cultured in to nutrient agar plates and potato dextrose agar plates for bacteria and fungi respectively and incubated at 37^0C for 24hours – 3days. The lowest concentration of the formulations yielding no growth was recorded as minimal bactericidal concentration (MBC) (Omogbai and Eneh, 2011).

RESULTS

The phytochemical screening of *Sena alata* leaves extract revealed that Alkaloid, cardiac glycoside, glycoside, saponin, steroid, tannins and saponoids were present in varying concentrations while Anthraquinone was not detected from the extract.

Table 1: Phytochemical constituents of *Sena alata* leaves extract

Phytochemical Components	**Remarks**
Alkaloid	**++**
Antraquinone	N.D
Flavonoid	**+**
Glycoside	**+**
Saponin	**++**
Steroid	**+**
Tannins	**++**
Terpenoid	**++**

Key: ++ : Highly Detected; **+:** Slightly Detected; N**.**D: Not Detected

Antibacterial activity of *Sena alata* leaves extract showed that highest zone of inhibition was exhibited against *Escherichia coli* 15mm and the least seen in *Pseudomonas aeruginosa* 7mm

Table 2: Antibacterial activity of *Sena alata* leaves extract on the test isolates

Bacterial isolates	**Positive control (mg)**	**Negative control**	**Zone of inhibition (mm) at diff concentrations**		
			20mg	**40mg**	**80mg**
Escherichia coli	30	-	-	11.0	15.0
Staphylococcus aureus	25	-	-	7.0	10
Pseudomonas aeruginosa		-	-	-	7.0

Key: - no effect; mg= milligram; mm= millimeter

The antifungal activity of *Sena alata* leaves extract is indicated in table 3. With *Microsporum canis* showing the highest zone of inhibition of 16mm, followed by *Candida albicans* 11mm

Table 3: Antifungal activity of *Sena alata* leaves extract on the test isolates

Fungal isolates	**Positive control**	**Negative control**	**Zone of inhibition (mm) at diff concentrations**		
			20mg	**40mg**	**80mg**
Microsporum canis	30	00	10	12	16
Candida albicans	22	00	6	9	11

Key: - no effect; mg= milligram; mm= millimeter

Table 4 below indicates the MIC and MBC of the tested organisms with different concentrations of 80mg - 20 mg in MIC and 80mg – 40mg in MBC

Table 4: Minimum inhibitory concentration and Minimum bactericidal concentration of *Sena alata* leaves extract

Microbial isolates	**MIC**				**MBC**			
	80	40	20	10	80	40	20	10
Escherichia coli	-	-	+	+	-	+	+	+
Staphylococcus aureus	-	+	+	+	+	+	+	+
Pseudomonas aeruginosa	+	+	+	+	+	+	+	+
Microsporum canis	-	-	-	+	-	-	+	+
Candida albicans	-	-	+	+	-	+	+	+

Key: - = no growth; + = turbid; mg = milligram; MIC= minimum inhibitory concentration; MBC: minimum bactericidal concentration

DISCUSSION

From the result of this research *Microsporum Canis* has the higest zone of inhibition followed by *E. coli* while the *pseudomonas aeruginosa* has the least zone size all at 80mg/mL concentration of the extract. The susceptibility of *Microsporum canis* and *E. coli* to the extract of this plant indicated the effectiveness of this plant on the treatment of ring worm and urinary tract infection respectively. And increasing the concentration of the extract may bring about its effectiveness on the unsusceptible isolates such as *pseudomonas aeruginosa.*

The *Senna alata* ethanolic leaves extract was screened for the presence of phytochemical components and its antimicrobial activities against some bacterial isolates (*Escherichia coli* and *Staphylococcus aureus*) and fungal isolates (*Microsporum canis* and *Candida albicans)* using disc diffusion method. The result of the phytochemical screening reveals the presence of Tannins, Alkaloid, Glycosides, steroids, Favonoids and terpenoids. This work is in line with the work of Doughari and Okafor, (2007); and Somchit *et al.,* (2003) whose research also indicates the presence of these bioactive compounds.

The ethanolic leaves extract of Senna *alata* shows varying invitro antimicrobial activities against the tested isolates. The activity may be attributed to the presence of active phytochemical components present in the leaves of *Senna alata.* These compounds have been reported to inhibit bacterial growth and to serve as the second line of defence to plants against bacterial and fungal infections. Thus, the variation may be due to the presence of most secondary metabolites at varying concentrations in the extract as suggested by previous reports (velanganni *et al*., 2010).

This work is in line with the work of Khan *et al*, (2001) which reported that the methanolic extract of *Senna alata* showing broad spectrum of activity against wide range of bacteria and fungi, among which are *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* ; and Doughari and Okafor, (2007) who reported the susceptibility of the plant extract agaist some of the test organisms.

CONCLUSION

It can be concluded from the research that the leaves extract of *Senna alata* have some promising effect against the tested organisms most especially if the concentration have been increased, and its' use to treat different illness, specifically fungal infection of the skin (ring worm) is now justified.

It is therefore recommended that further researches should be done to test the toxicity of the plant to acertain its safety when taken orally since it is now found to be active against *E. coli*, as it is widely used as topical drug.

REFERENCES

Abba, D.I., Yakubu, S.E., and Olonitola, O.S. (2009). Phytochemical analysis and antibacterial activity of some powdered herbal preparations marketed in Kaduna Metropolis. *Science World Journal.* **4(1)**:23-26.

Chesbrough, M .(2009).*District laboratory practice in tropical countries, second edition*. Cambridge University press New-york. **Pp**. 66-70.

Doughari, J.H. and Okafor, B. (2007). Antimicrobial activity of *Senna alata* Linn. *East and Central africa Journal of pharmaceutical science.* **10 Pp. 17-21**

Gurma, A.G., Shamsuddeen U. and Dahiru A.T. (2019) Phytochemical screening and antibacterial activity of ethanolic leaf extracts of *Terminalia catappa* on some clinical bacterial isolates. *Nigerian journal of microbiology*, 33(2): 4539 - 4544

Ikegbunam, N.M., Okpota, O.O., Ukwo, M.C., and Esimone, C.O. (2013) Authentication of the antimicrobial activity of some indigenous herbal remedies used in the treatment of typhoid and urinary tract infections in Anambra State, Nigeria*. International Journal of Pharmacy and Pharmaceutical Science* **5**(**2**):0975-1491

Khan, M.R.U., Kihara, M., and Omoloso, A.D. (2001). Antimicrobial activity of *Cassia alata*. *Fitoterapia.* **72 Pp 561-564.**

Muhammad, U-K., and Yakubu, A. (2014). Preliminary antibacterial screening of a traditional medicinal formulation used as medicine in Sokoto, Nigeria. *International Journal of DevelopmentResearch**4***(**2**):209-210

Nair, R., Kalariya, T. and Chanda, S. (2005). Antibacterial activity of some selected Indian medicinal flora. *Turkish Journal of Biology*. 29: 41-47.

Ola, I.O., Omomowo, I.O., Aina, D.A., Majologbe, N.O., and Oladipo, E.K. (2013). Bacteriological evaluation and antimicrobial sensitivity test of common herbal concoctions in Ogbomoso metropolis. *African Journal of Microbiology Research*. **7**(**48**):5479-5483.

Omogbai, B.A., and Enah, T.O. (2011). Antibacterial activity of *Dacaryodes edulis* seed extracts on food–borne pathogens. *Bayero Journal of Pure and applied Science* **4**(**1**):17-21.

Oyeleke, S., B. and Manga, B.S. (2008). Essentials of LaboratoryPracticals in Microbiology. *Tobest pub, Minna*. **Pp** 36**–** 70.

Somchit N.M., Reezal I., Elysha N. and Mutalib, A.R. (2003). Invitro antimicrobial activity of ethanol and water extracts of *Cassia alata*. *Journal of ethnopharmacology*. **84 Pp. 1-4.**

Tshobou, R., Mapongmetsem, P., Voukeng, K.I. and Vandamme, P. (2015). Phytochemical Screening and antibacterial activity of medicinal plants used to treat Typhoid fever In Bamboutus division, west Cameroon. *Journal of Applied Pharmaceutical Science.* **5**(**06**):034-049.

Velenganni, J., Kadamban, D., and Tangavelou, A.C. (2011). Phytochemical screening and antimicrobial activity of the stem of *Mallotus philippensis* (LAM) *MLUELL,ARG.VAR.PHILIPPENSIS(Euphorbiaceace*). *Intemational Journal of Pharmacy and Pharmaceutical Science* **3**(**2**):160-163.