

Phytochemical Screening and Antibacterial Activity of Methanol Leaf Extract of *Senna obtusifolia* L. against *Staphylococcus aureus* and *Salmonella typhi*

Kona H.D¹., Yusuf, I¹., Bashir, I^{1,2}., Adamu, Z¹., Aduku, S.I¹., Saidu, H.M., Musa, A. and Adam M.U².

¹Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria

²School of Basic Sciences, Maryam Abacha American University of Nigeria, Kano, Nigeria.

*Corresponding Author: ismailbashir533@gmail.com

ABSTRACT

This study aimed at investigating the phytoconstituents of methanol leaf extract of *Senna obtusifolia* and their antibacterial activities against *Staphylococcus aureus* and *Salmonella typhi*. Leaves of *Senna obtusifolia* were collected around Kufaina, Zaria, and pure cultures of test organisms were obtained from the Department of Veterinary Microbiology, Ahmadu Bello University, Zaria. The leaves were shed, dried at room temperature, then powdered with a pestle and mortar. Muller-Hinton Agar and Peptone water were used to determine whether the extract inhibited growth or killed the test bacteria. The leaf extract showed presence of Saponins, Tannins, Flavonoids, Cardiac glycosides, Steroids, and Alkaloids. However, Phenols, Triterpenes, and Anthraquinones were not detected. The extract showed remarkable antibacterial activity against the organisms, with *Salmonella typhi* exhibiting three zones of inhibition at concentrations of 125 mg/mL, 250 mg/mL, and 500 mg/mL of the extract. While *Staphylococcus aureus* exhibited two zones of inhibition at concentrations of 250 mg/mL and 500 mg/mL. At the concentrations of 250mg/ml and 500mg/ml, *Staphylococcus aureus* were killed completely, while *Salmonella typhi* was only killed at the highest concentration of 500mg/ml, with no turbidity of the testing colonies. This study therefore confirmed the presence and inhibitory effect of the phytochemicals of *Senna obtusifolia* on the test organisms (*S. aureus* and *S. typhi*) and recommended pharmaceutical industries to consider using the leaves of *Senna obtusifolia* in developing new medicines/herbal formulations that could treat bacterial infections. Further research also should be done to ascertain the presence or absence of other beneficial or toxic phytochemicals to decide their safety for consumption as food.

Keywords: Phytochemicals, *Senna obtusifolia*, Antibacterial Activity, *Staphylococcus aureus*, *Salmonella typhi*, leaf extract.

Introduction

Senna obtusifolia is commonly known as American sicklepod, cassia, Chinese senna (ILDIS, 2005), French Cassie Puante, Arabic, Panwar, Hausa, Tafasa (Komlan, 2010). It is an annual plant growing to a height of 1.5 to 2.5m (Holm *et al.*, 1997). It belongs to the family Fabaceae, subfamily caesalpinoideae, and grows on well-drained fertile soil across the tropics, especially coastal forest countries (Chauhary *et al.*, 2007) and (Irwin and Barneby, 1982). It is native to tropical America and the southeastern United States and is currently found on five continents with a pantropical distribution (Steckel, 2021). The plant is widespread throughout tropical Africa except for Madagascar (Ajayi, 2014). In Sudan, it is mainly found in clay pans and wetlands in the central and southern regions (Hussain, 2017).

The young leaves of *Senna obtusifolia* serve as a source of vegetables (Paster, A. 2007) in Africa and the plant is cultivated for this purpose in Senegal, Ghana, Cameroon, and Ethiopia (Smith, 1987). Its leaves and seeds are moderate sources of protein (Ingweye *et al.*, 2010) with acceptable amino acid composition (Augustine *et al.*, 2017), carbohydrate content in the seed (Ingweye *et al.*, 2010; Augustine *et al.*, 2018) and in the leaf (Bake *et al.*, 2016). *Senna* seeds and leaves are high in fiber (Tarimbuka *et al.*, 2017) and low in fat (Umar *et al.*, 2017). Since antiquity, humans have been using plants to treat infectious diseases, and some of these traditional medicines are still included as part of the habitual treatment of various maladies. Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential, as they contained what we currently characterize as medicinal properties was well accepted.

The use of *S. obtusifolia* in traditional medicine is due to the presence of several phytoconstituents such as anthraquinones, naphthopyrones, xanthenes, lactones, sterols, triterpenoids, saponins, tannins, alkaloids and flavonoids which are responsible for the following pharmacological effects: anti-inflammatory, anti-allergic, antiseptic, anthelmintic, antipyretic, diuretic, carminative, purgative, antidiabetic, antimicrobial, antioxidant, hepatoprotective, neuroprotective, anti-Alzheimer's disease, antiplatelet aggregation, larvicidal activities and insecticidal effect (Smith, 1987).

The Phytochemical screening of the leaves of *Senna obtusifolia* revealed the presence of some phytoconstituents, including saponins, tannins, alkaloids, and flavonoids are present in the acetone extracts; tannins, alkaloids, and flavonoids are found in the methanol extracts; and alkaloids and flavonoids are found in the water extract. All the extracts demonstrated antimicrobial activity against the test bacteria, with the acetone extract demonstrating the highest activity while the water extract demonstrated the least activity (Doughari et al., 2008).

The young tender leaf of *S. obtusifolia* are used as vegetables in Cameroun, Niger, Nigeria, Ghana, Ethiopia, Senegal, Benin and Burkina Faso (Gueye, Ndiaye et al., 2018). In Chad and other parts of Africa, the leaves are made into Kawal through in solid-state fermentation process and used in sauces eaten with cereal couscous (Abakar et al., 2019).

S. obtusifolia is reported to be an important medicinal plant with multiple uses. The seeds are used to treat eye problems; they lower cholesterol and blood pressure, prevent the formation of atherosclerotic plaques in the arterial wall, and have laxative and antibacterial effects (Naik, 2016). Roasted and boiled in the form of tea, the seeds are used against diarrhea, tremors, and urinary tract infections and the leaf extract possesses a broad spectrum of activity against bacteria and fungi (Doughari et al., 2008). The seeds of *S. obtusifolia*, although famous in traditional medicine, also play an important role in feeding. Roasted and ground, they are used as a coffee substitute in China (Mao et al., 2018).

Additionally, they are the source of cassia gum, a food additive commonly used as a thickener (Doughari et al., 2008).

The leaves, stem, root, and seeds are used to cure a wide range of diseases, especially in traditional Chinese medicine, with significant antioxidant, anti-inflammatory properties (Park 2021). The plant is used for skin diseases, and ringworm and it has been found to possess antimicrobial activity in India (Rakib et al., 2018). It is used in several African countries in traditional medicine. In Burkina Faso, the leaves are used against stomachache, malaria, arterial hypertension, jaundice, and the stem against cough, and malaria (Nanema et al., 2013). In Nigeria, the seeds, leaves, and roots of the plant are used by traditional medicine practitioners as mild laxative drugs for children and pregnant women (Sudi et al., 2011). In Benin, it is used by communities to heal malaria (Dance et al., 2010). Leaves are also used as a decoction febrifuge and for the treatment of scorpion stings, gingivitis, dysentery, and diarrhea (David, 2002).

Therefore, it is imperative to investigate the phytochemicals and antimicrobial activity of the leaf extract of *Senna obtusifolia* against some pathogenic bacteria and generate data for the development of modern medicine. This is because, diseases caused by microorganisms are the world's leading human and animal killers, of which the majority of these diseases are of bacterial origin (Yakubu et al., 2014). Infectious diseases remain the topmost cause of death all over the world (Sarmah et al., 2017).

The study aimed to investigate the phytochemical composition and antibacterial activity of methanol leaf extract of *Senna obtusifolia* against *Staphylococcus aureus* and *Salmonella typhi*. This is to confirm the presence or absence of phytochemical constituents of the methanol leaf extract of *S. obtusifolia* and to evaluate the antimicrobial activity of methanol leaf extract of *S. obtusifolia* against *Staphylococcus aureus* and *Salmonella typhi*.

Materials and Methods

Study Area

The study was conducted at the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Science, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.:- N 11 93.30552(LAT), E7973910.10556.

Sample Collection

The *Senna obtusifolia* plant was collected around Kufaina, Samaru, and Sabongari Areas of Zaria. The plant materials were identified and authenticated in the herbarium unit of the Department of Botany, Ahmadu Bello University, Zaria and assigned with a voucher specimen number: ABU06836.

The test organisms, *Staphylococcus aureus* and *Salmonella typhi* used for this study were obtained from the Department of Veterinary Microbiology, Ahmadu Bello University, Zaria.

Senna obtusifolia Leaf Sample Preparation

The leaves of *S. obtusifolia* were thoroughly washed with distilled water, sheddried at room temperature, then powdered by pestle and mortar to facilitate the extraction of the phytochemicals from the leaves.

Extraction Procedure

One hundred grams (100 g) of the *Senna obtusifolia* leaf powder was weighed using an electric weighing balance (KERN EMB 200-2). The powdered sample was extracted using 500 mL of 80% methanol, then covered with aluminum foil and allowed to stand for 48 hours at room temperature with occasional shaking. After 48 hours, the solution was filtered using Whatman No. 1 filter paper to remove plant residues. The filtrate was evaporated to dryness using a hot air oven set at 40–45°C to avoid degradation of heat-sensitive compounds. The resulting crude extract was then placed into a clean, labeled sample vial and stored in a refrigerator at 4°C for further analysis.

Phytochemical Screening

Phytochemical screening was carried out using the crude extract to detect the presence of secondary metabolites (phenols, anthraquinones, flavonoids, tannins, alkaloids, saponins, glycosides, steroids, and triterpenes) present in the plant using standard methods as described by Evans and Trease (1989).

Test for Tannins (Ferric chloride test)

Five drops of ferric chloride solution were then added to the 1 ml of the extract; formation of the blue-black precipitate indicated the presence of tannins.

Test for Alkaloids (Dragendoff's test)

The extract of 0.5g was put into a test tube and dissolved in distilled water; three drops of Dragendoff's reagent were added to the extract. The appearance of a precipitate indicated the presence of an alkaloid.

Test for Phenols

To 1 mL of the leaf extract, 2 mL of distilled water, followed by a few drops of 10% ferric chloride, was added. Absence of blue color formation indicates the absence of phenols.

Test for Flavonoids (Shinoda Test)

The extract of 0.5g was dissolved in a test tube using about 2 cm³ of 50 % methanol, and the mixture was heated. A few chips of magnesium and 5 drops of concentrated hydrochloric acid were added to the mixture. An orange coloration indicated the presence of flavonoids.

Test for Anthraquinones (Modified Borntrager's Test)

The extract of 1 g was dissolved in a test tube using 5 cm³ of distilled water. The solution was then boiled with 5 cm³ of 10% sulphuric acid for 3 minutes. This process hydrolyzed the glycosides to give rise to the aglycones, which were soluble in hot water only. The solution was filtered hot, and the filtrate was cooled and extracted with 5cm³ of benzene. The benzene layer was filtered off and shaken gently with half its volume of 10 % ammonia solution; lack of color change indicated the absence of anthraquinones.

Test for Saponins (Frothing Test)

The extract of 0.2g was dissolved in distilled water in a test tube. The mixture was shaken vigorously for 30 seconds and was allowed to stand for 20 minutes. A honeycomb-like structure that foamed for 30 minutes indicated the presence of saponins in the extract.

Test for steroids and triterpenes (Lieberman-Buchard test)

Extract (0.5g) was dissolved in a test tube, and 1 cm³ of acetic anhydride was added to the extract. Concentrated sulphuric acid (1 cm³) was carefully added to the mixture along the side of the test tube.

No immediate appearance of purple color indicated the absence of triterpenes, but the late appearance of a blue or blue-green coloration in the test tube was indicative of steroids.

Test for Cardiac Glycosides

The extract of 0.5 mL, 2 mL of glacial acetic acid, and a few drops of 5% ferric chloride were added. This under underlayered with 1 mL of concentrated sulphuric acid. Formation of a brown ring at the interface indicates the presence of cardiac glycosides.

Antibacterial Screening using Agar well diffusion Method

The antibacterial activities of the leaf extract of *S. obtusifolia* were determined against *Staphylococcus aureus* and *Salmonella typhi* using Mueller Hinton Agar (MHA) and Peptone Water. All media were prepared according to the manufacturer's recommendations.

The inoculum of both bacteria was subjected to a sensitivity test using the Agar well diffusion method. 0.5g of the extract was weighed and introduced onto the surface of MHA in a sterile Petri dish and labeled accordingly. A sterile cork borer 5mm was used to produce five 5 mm wells at an equal distance in the inoculated agar. The wells were filled with different concentrations of the leaf extract as 31.25, 62.5, 125, 250 and 500mg/ml and were incubated at 37°C for 24 hours and zone of inhibition diameter distance formed around the wells and measured in millimeters (mm) using transparent ruler and the growth inhibitory effect of the plant leaf extract was recorded.

Minimum Inhibitory Concentration (MIC) of the leaf extract against Test Microbes

MIC of the extract was carried out on the inoculum that was sensitive to the extract and was determined using the broth dilution method. Mueller Hinton agar was the medium used as the growth medium for the test inoculum. The medium was sterilized at 121°C for 15 minutes, poured into sterile petri dishes, and allowed to cool and solidify. McFarland's turbidity standard scale number 0.5 was prepared to give a turbid solution., Two-fold serial dilutions of the extract were prepared by adding 2ml of 1g of the extract into a test tube; thus producing concentrations of 500mg/mL, 250mg/mL, 125mg/mL, 62.5mg/l and 31.25mg/L.

Exactly 0.5 ml of 0.5 McFarland equivalent standards of test organisms was introduced into the test tubes and incubated at 37 °C for 24 hours. After incubation the test tubes were observed for growth, after which the test tube of the broth was observed for turbidity (growth) the lowest concentration of the broth, which shows no turbidity was recorded as the Minimum Inhibitory Concentration of the extract as following the procedure of Ahmad and Beg (2001).

Minimum Bactericidal Concentration of the Extract against Test Microbes (MBC)

Minimum Bactericidal concentration (MBC) was carried out to determine whether the test organism was killed or only its growth was inhibited. Mueller Hinton agar was prepared, sterilized at 121°C for 15 minutes, poured into sterile petri dishes, and allowed to cool and solidify. The content of the MIC in the serial dilution were sub cultured onto the prepared medium, the incubation was made at 37°C for 24 hours, after which the plate of the medium were observed for colony growth and the MBC are the plates with lowest concentration of the plant extract without colony growth or The MBC of the extracts was recorded as the lowest concentration of the extract that had less than 99% growth on Mueller Hinton agar plates (Ahmed et al., 2001).

Statistical Analysis

The result is expressed as the mean standard error of mean (SEM). Confidence interval of mean determined, and 95% differences in the mean growth inhibition were tested by analysis of variance (ANOVA)

Result

The result of the phytochemical analysis as presented in Table 1 indicates that the chemically active substances, such as Alkaloids, Saponins, Tannins, Flavonoids, Steroids, and Cardiac glycosides, were present in the methanol leaf extract, while Triterpenes, Phenols, and Anthraquinones were absent.

The results of the antibacterial activities (zone of inhibition, the Minimum inhibitory Concentration MIC and of the Minimum Bactericidal Concentration MBC) of the methanol leaf extract of *S. obtusifolia* against *S. aureus* and *S. typhi* extract are presented in Table 2 and in Plates 1 to 4.

Table 1: The occurrences of phytochemicals in methanol leaf extract *Senna obtusifolia*

S/N	Phytoconstituents	Analysis of the extract
1	Alkaloids	+
2	Saponins	+
3	Flavonoids	+
4	Steroids	+
5	Tannins	+
6	Cardiac glycosides	+
7	Triterpenes	-
8	Phenols	-
9	anthraquinones	-

Key: (+) Indicate the presence of phytoconstituents; (-) Indicate the absence of phytoconstituents

Table 2: Antibacterial activities of methanol leaf Extract of *S. obtusifolia* against *S. aureus* and *S. typhi*

Conc. of Extract (mg/ml)	Zone of Inhibition (mm)		Minimum Inhibitory Concentration (MIC)		Minimum Bactericidal Concentration (MBC)	
	<i>S. aureus</i>	<i>S. typhi</i>	<i>S. aureus</i>	<i>S. typhi</i>	<i>S. aureus</i>	<i>S. typhi</i>
500	17.00	21.00	-	-	-	-
250	14.00	17.00	-	-	-	+
125	0.00	13.00	+	-	+	+
62.5	0.00	0.00	+	+	+	+
31.25	0.00	0.00	+	+	+	+

Key: (-) = No growth or no turbidity; (+) = turbidity or growth of colonies

Results of the zone of inhibition in Table 2 shows significant differences for the zone of inhibition among the concentrations of both the *S. aureus* and *S. typhi*. The Minimum inhibitory Concentration (MIC) was observed on *S. aureus* at a low concentration of 250mg/ml. However, the MIC of *S. typhi* was observed at a low concentration of 125mg/ml. At a concentration of 31.25mg/ml and 62.5mg/ml, the highest turbidity (Growth) was observed in both the test organisms. However, at the concentrations of 250 and 500mg/ml, no turbidity was observed in both.

On the other hand, the Minimum Bactericidal Concentration (MBC) of the extract against test organisms shows that, at the concentration of 250mg/ml, the MBC was observed on *S. aureus*, while the MBC of *S. typhi* was observed at the highest concentration of 500mg/ml. Heavy colony growth was observed in both the test organisms at a concentration of 31.25mg/ml, 62.5mg/ml, and 125mg/ml. At concentrations of 250 and 500mg/ml of *S. aureus* showed no visible growth, while at a concentration of 500mg/ml of *S. typhi* showed no visible growth.



Plate 1: Zone of inhibition of *S. aureus*



Plate 2: Zone of inhibition of *S. typhi*



Plate 3: Turbidity of the *S. aureus*



Plate 4: Turbidity of the *S. typhi*

Discussion

The screening of *Senna obtusifolia* in this study confirmed the presence of phytochemical compounds that prove to have medicinal importance. The result of methanolic extracts shows the presence of some phytochemical constituents such as Tannins, alkaloids, saponins, and many more, as I have mentioned in the results. Tannins react with protein to provide a typical tanning effect. Medically, this is important for the treatment of abdominal worms, arthritis, and gout. Tannins also have an important role, such as hastening the healing of a wound, treating diarrhea, stomachache, and conjunctivitis. This report is aligned with the report of ILDIS (2005).

The methanol leaf extract demonstrated the presence of Saponin, which is responsible for its anti-yeast, anti-fungal, antimicrobial, and anti-inflammatory activities. This is aligned with the report of Sparge *et al.* (2004).

This study shows the presence of Flavonoids, which also exhibit various medicinal properties, including antioxidant, anticancer, and antimicrobial activities. This also aligns with the work of Muhammad (2022). Cardiac glycosides were detected in the methanol leaf extract, where it is also detected in the work of Trease and Evans (2002).

Interestingly, the secondary metabolites that were screened occurred in different proportions in the leaves of *Senna obtusifolia*. This satisfied one of the major aims of this study, which was to check the occurrence of the different compounds.

The methanol leaf extract of *Senna obtusifolia* does not detect Phenolic compounds, contradicting the work of Koga *et al.* (2021) which demonstrated the presence of phenolic compounds, as it has strong antioxidant, antimalarial, and antiviral properties. The result also showed that the methanol leaf extract of *S. obtusifolia* possessed antimicrobial potential against *Staphylococcus aureus* and *Salmonella typhi*, In which the *S. aureus* showed the activities in some of the concentrations that inhibited the establishment of the microbes in 250mg/ml and 500mg/ml concentration and this highlighted that the microbes were still present but inhibited from growing in the two concentrations.

These findings showed that any concentration of *S. obtusifolia* above 250mg/ml can inhibit the growth of microbes while the concentration below 250mg/ml cannot inhibit their growth so also for *S. typhi* showed that three concentrations can inhibited the microorganisms, 125mg/ml, 250g/ml and 500mg/ml, Hence the *S. obtusifolia* can inhibited the growth of *S typhi* in a smaller concentration than in *S. aureus*. This report aligns with the report of Lutterodt *et al.* (1999). Where, in most plants, materials are known to be bactericidal, pesticidal, or fungicidal.

This research also showed the activities of methanol leaf extract of *S. obtusifolia* against the gram positive and gram negative (*S. aureus*) and (*S.typhi*) respectively, the Minimum Bactericidal Concentration (MBC) was observed on *S. aureus* in the following concentration 250mg/ml and 500mg/ml, while in *S. typhi*, it was observed at the highest concentration of 500mg/ml. This demonstrated that any concentration of *S. obtusifolia* below 250mg/ml and 500mg/ml for *S. aureus* and *S. typhi* cannot kill the organism; rather, they can reduce their establishment, and likewise, any concentrations mentioned above can suppress the organisms completely from growing. This showed that most of the MIC values were lower than the MBC values, indicating that the extract could be bactericidal in action. The higher the concentration, the higher the activity of the extract against the bactericidal species. The findings of this research aligned with the work of Nascimento *et al.*, 2000.

Conclusion

This study revealed the presence of secondary metabolites such as saponin, tannins, flavonoids, cardiac glycosides, steroids, and alkaloids, in the methanol leaf extract of *Senna obtusifolia*. These phytochemicals are likely to suppress the growth of *Staphylococcus aureus* and *Salmonella typhi* used in this study. The study proved that the leaf extract of *S. obtusifolia* exhibits antibacterial activities against *S. aureus* and *S. typhi*. Based on these findings, it is recommended that, Pharmaceutical Industries should consider using *Senna obtusifolia* in developing new medicines/herbal formulations that could treat bacterial infections. More research should be carried out to ascertain the presence or absence of other beneficial or toxic phytochemicals, hence to decide their safety for consumption as food, which will also help the body system to fight bacterial infections.

References

- Abakar, I.L., Tidjani, A., Taale, E., Hissein, O.A., Tankoano, A. & Aly, S. (2019). Traditional Technologies and Probiotic Properties of Bacillus Strains Isolated from Kawal -A Chad Traditional Fermented Food Condiment. *Journal of Food Technology Research*, 6, 57-71.
- Ahmad, I. & Beg, A.Z. (2001). Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multidrug-resistant human pathogens. *Journal of Ethnopharmacology*, 74, 113–123.
- Ahmed, M.M.M., El Hag, F.M., Wahab, F.S. & Salih, S.F. (2001). Feeding strategies during dry summer for lactating desert goats in a rainfed area under tropical conditions. *Small Ruminant Research*, 39, 161– 166.
- Ajayi, C.F., Funso, A., Farmers, A. & Lujoba. (2014). Laxative activities of *Cassia sieberiana* and *Senna obtusifolia*. *African Journal of Traditional, Complementary and Alternative Medicines*, 11, 44.
- Augustine, C., Khobe, D., Babakiri, Y., Igwebuikwe, J.U., Joel, I., John, T. & Ibrahim, A. (2020). Blood parameters of Wistar albino rats fed processed tropical sickle pod (*Senna obtusifolia*) leaf meal-based diets. *Translational Animal Science*, 4, 778–782.
- Bake, G.G., Atoyebi, O.A., Abdulkarim, I.A., Adam, A. & Sadiku, S.O.E. (2016). Evaluation of varying inclusion levels of toasted sickle pod (*Senna obtusifolia*) seed meal in the practical diet of catfish (*Clarias gariepinus*) fingerlings in a concrete tank. *International Journal of Fisheries and Aquatic Studies*, 4(3), 458–463.
- Chaudhary, I.A., Sadiq, S., Lakhani, M., Baig, S., Qureshi, M.F.H. & Shah, M. (2007). Seroprevalence of hepatitis B and C among the healthy blood donors at Fauji Foundation Hospital, Rawalpindi. *Pakistan Journal of Medical Sciences*, 23, 64–67.
- David, G.C. (2002). Cognition and cultural transmission of Tzeltal Maya medical plant knowledge. Retrieved from <http://www.wiu.edu/users/dge101/disabs.html>
- David. (1982). Cognition and cultural transmission of Tzeltal Maya medical plant knowledge. In: H.S. Irwin & R.C. Barneby; *Senna obtusifolia* (L.). *Memoirs of the New York Botanical Garden*.
- Doughari, A.H., El-Mahmood, A.M. & Tyoyina, I. (2008). Antimicrobial activity of leaf extracts of *Senna obtusifolia* (L.). *African Journal of Pharmacy and Pharmacology*, 2, 007–013.
- Evans, W.C. (2000). *Trease and Evans Pharmacognosy* (14th ed.). London: W.B. Saunders Company Ltd., 195–445.
- Gueye, M., Ndiaye, C. & Diop, C.M. (2018). Nutritional potential of two leafy vegetables, *Leptadenia hastata* Decne and *Senna obtusifolia*, consumed in Senegal. *Food and Nutrition Sciences*, 9, 77–85.
- Holm, L.G., Pancho, J.V., Herberger, J.P. & Plucknett, D.L. (1979). *A Geographical Atlas of World Weeds*. New York: John Wiley and Sons, 391 pp.
- Hussain, I. (2017). *Cassia obtusifolia* (Sicklepod Seed). In: *Unconventional Oilseeds and Oil Sources*. Elsevier, 101–113.
- ILDIS. (2005). Genera *Cassia* and *Senna*. *International Legume Database and Information Service*. <https://ildis.org/cgi-bin/Araneus.pl?version~10.01&LegumeWeb&tno~603&genus~Senna&species~alata>. Accessed January 2021
- Ingweye, J.N., Kalio, G., Ubuja, J. & Umoren, E.P. (2010). Nutritional evaluation of wild sicklepod (*Senna obtusifolia*) seeds from Obanliku, South-Eastern Nigeria. *American Journal of Food Technology*, 5, 1–12.
- Irwin, R.C. & Barneby. (1982). *Senna obtusifolia* (L.). *Memoirs of the New York Botanical Garden*.
- Irwin, R.C. & Barneby. (2021). Getting sleepy? *Weed Technology*, 35, 1052–1058.
- Koga, R.C.R., Santos, A.V.T.L.T., Sarquis, R.S.F.R. & Carvalho, J.C.T. (2021). *Bauhinia guianensis* Aubl., a plant from the Amazon biome with promising biologically active properties: A systematic review. *Pharmacognosy Reviews*, 15(29), 82–90.
- Komlan, F., N'Danikou, S., Dansi, A. & Ambrose-Oji, B. (2010). *Traditional Vegetables in Benin*. Institut National des Recherches Agricoles du Bénin, Imprimeries du CENAP, Cotonou. ISBN: 978-99919-334-4-3. DOI:10.13140/RG.2.1.1803.1121

- Lutterodt, G.D., Ismail, A., Basheer, R.H. & Baharudin, H.M. (1999). Antimicrobial effects of *Psidium guajava* extracts as one mechanism of its antidiarrhoeal action. *Malaysian Journal of Medical Sciences*, 6(2), 17–20.
- Mao, R. (2018). Genetic diversity and population structure assessment of Chinese *Senna obtusifolia* L. by molecular markers and morphological traits of seed. *Acta Physiologiae Plantarum*, 40, 12.
- Naik, B. (2016). Evaluation of in vitro antimicrobial activity of extracts from *Cassia obtusifolia* L. and *Senna sophera* (L.) Roxb against pathogenic organisms. *Journal of Applied Pharmaceutical Science*, 6(4), 83–85.
- Nanema, R. K., Kiebre, M., Traore, R. E., Nerbewendé, S., Hamed, O. M., Ali, B. L., Boureima, S., Pauline, K. & Mahamadou, S. (2013). Local nomenclature and uses of *Senna obtusifolia* (L.) in Burkina Faso. *Journal of Applied Biosciences Review*, 160, 16438–16453.
- Nascimento, G.G.F., Locatelli, J., Freitas, P.C. & Silva, G.L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian Journal of Microbiology*, 31, 247–256.
- Paster, A., Woltering, D., Nikiema, D., Senbeto, J., Fatondji, J. & Ndjeunga. (2007). *Acta Horticulturae (ISHS)*, 752, 299–302.
- Rakib, M. (2018). A review of phytochemical and biological studies on *Cassia obtusifolia* Linn. in the folklore medicine of eastern Uttar Pradesh. *World Journal of Pharmaceutical Research*, 7, 191–201.
- Sharma, M.S. & Choudhary, P.R. (2017). Effect of fenugreek seeds powder (*Trigonella foenum-graecum* L.) on experimentally induced hyperlipidemia in rabbits. *Journal of Dietary Supplements*, 14(1), 1–8.
- Smith, J. (1987). Copper is nutritive and supports cardiovascular integrity. In: Hemphill, D.D. (Ed.), *Proceedings of 21st Annual Conference on Trace Substances in Environmental Health*, Columbia, 499–513.
- Steckel, L.E. (2021). Getting sleepy? *Weed Technology*, 35, 1052–1058.
- Sudi, I.Y., Ksgbiya, D.M., Mulu, E.K. & Clement, A. (2011). Nutritional and phytochemical screening of *Senna obtusifolia* indigenous to Mubi, Nigeria.
- Tarimbuka, L.I., Yusuf, H.B. & Wafar, R.J. (2017). Response of weaner rabbits fed toasted sickle pod (*Senna occidentalis*) seed meal. *Asian Journal of Advanced Agricultural Research*, 1, 1–7.
- Thompson, L.U., Rea, R.L. & Jenkins, D.J.A. (1983). Effect of heat processing on hemagglutinin activity in red kidney beans. *Journal of Food Science*, 48, 235–236.
- Trease, G. & Evans, W. (2002). Phytochemicals. In: *Pharmacognosy* (15th ed.). London: Saunders Publishers, 42–393.
- Umar, A., Abubakar, M., Muhammad, B. & Sir, S.M. (2017). Replacement values of treated *Senna obtusifolia* leaf meal for *Moringa oleifera* leaf meal in the diets of growing Yankasa sheep. *Journal of Animal Production Research*, 29, 347–358
- Yakubu, J.M., Ehiowemwenguan, G. & Inetianbor, J.E. (2014). Microorganisms associated with mutilated *Naira* notes in Benin City, Nigeria. *International Journal of Basic and Applied Science*, 3(1), 9–15.