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# Antimicrobial potential and phytochemical screening of leaves and fruits of *Solanum thorvum* (swartz). A medicinally important plant

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**ABSTRACT**

The present study designed for antimicrobial potential and phytochemical screening of leaves and Fruits of *Solanum torvum* (Swartz) belongs to the family Solanaceae it is an Important Medicinal Plant. The plant has been used in the folklore system of medicine for the treatment of Asthma, Diabetes and hypertension. To evaluate the antimicrobial potential activity, hydrogen peroxide radicals scavenging activity, reducing power, the total phenolic and flavonoids contents, and antioxidant and antifungal activities of methanol, ethanol and water extracts of leaves and fruits of *Solanum thorvum.*(Swartz). Methanol, ethanol and water extracts were evaluated against four Gram positive and Gramnegative bacterial isolates (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Bacillus subtilis*) and two fungal strains (*Aspergillus fumigatus* and *Aspergillus flavus*). Methanol extract at different concentrations was tested for antimicrobial potential and phytochemicals were determined by using spectrophotometric method.
The total phenolic content was (40.859±0.017) mg gallic acid/g in the leaves of *L. camara*, while the total flavonoids were (53.112±0.199) mg/g dry weight. Methanol leaves and fruits extract of *Solanum thorvum.*(Swartz) showed maximum antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* and was also effective against other bacterial strains as compared to ethanol and aqueous extracts of leaves and fruits. The methanol leaf extract of *Solanum thorvum.*(Swartz) exhibited significant inhibition (71%) and (66%) against *Aspergillus fumigatus* and *Aspergillus flavus* respectively.
The methanol extract of the *Solanum thorvum.*(Swartz) leaves and fruits effective against selected bacterial and fungal strains. Its phytochemical contents have broad antimicrobial properties and the plant might be a novel source of antimicrobial drug.

**Keywords:** Methanol, ethanol, Antimicrobial, Phytochemicals *Solanum thorvum*

## INTRODUCTION

Medicinal plants have always been used to relieve and cure human diseases (Szopa *et al.,* 2017). Currently, the development of microbial resistance to antibiotics and the toxicity of synthetic antioxidants have led researchers to exploit the plant world in order to search for effective natural molecules that are free of any adverse effects (Silva *et al.,* 2016).

*Distemonanthus benthamianus (D. benthamianus)* H. Baill (Leguminosae) is a tree distributed in tropical Africa, its bark powder associated with that of red wood (padouk) is used traditionally against skin conditions. It is also administered in enemas for diarrheal diseases (Raponda *et al.,* 1967). This species is rich in phenolic compounds such as oxyayanine, cyanine and alkaloids (Aiyegoro *et al.,* 2008). Certain compounds derived from *D. benthamianus* have anti-antiadrenergic, antioxidant, antitumor and contact dermatitis effects (Yousaf *et al.,* 2013). *Solanum torvum* Sw (S**.** *torvum)* (Solanaceae) is a slender shrub, its fruits and leaves can fight series of microbial diseases. The heated leaves of *S. torvum* are applied to cutaneous infections (Silva *et al.,* 2016). *S. torvum* is rich in phytochemicals such as steroidal saponins, steroidal alkaloids and phenols (Chang *et.al.* 2007). The antimicrobial, antiaggregant, analgesic, anti-inflammatory and cytotoxic activities of this plant have been described by (Yousaf *et al.* 2013) Microbial infections are diseases caused by the development of bacteria or yeasts, some of which are pathogenic (Rahal *et al.,* 2014) In addition to microbial infections, free radicals are implicated in the etiology of a large number of pathologies that are now considered to be one of the major public health problems (Koech *et al.,*2014)

However, plants have an anti-radical and antimicrobial potential that would allow them to play a beneficial role in terms of preventive action, which is very important for human and animal health (Aiyegoro and 2010). The purpose of this work is to determine the medicinal properties of stem bark extracts of *S. torvum* Sw in Gabon by evaluating the phytochemical constituents as well as the antimicrobial and antioxidant activities of the extracts of these plant.

## MATERIAL METHODS

**Plant material :** The Leaves and fruits of *S. torvum* Sw. was made according to traditional medicinal use. Plant samples were collected in Department of Botany Govt. Degree College Mahabubabad in Sept 2019. Identification of the species was carried out at the National Herbarium of the Institute of Pharmacopoeia and Traditional Medicine. The identification numbers of *S. torvum* Sw. were Bourobou 255,respectively.

**Treatment of plant material:**
The plant samples were freeze-dried, powdered, kept at ambient temperature, and protected from light. Each sample (20 g) was mixed with 250 ml of suitable solvents [water (100%); water-acetone (30:70, v/v); water-ethanol (30:70, v/v)]. The water extracts were boiled for 60 min. All the extracts were filtered and concentrated. The concentrates were lyophilized and stored in sterile vials at 4°C. *Chemical products* Butylated hydroxyanisole (BHA), 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), ethanol, sulfuric acid, hydrochloric acid, sodium chloride, Folin-Ciocalteu, gallic acid and ascorbic acid (vitC) were from Sigma-Aldrich (StLouis,MO,USA).

**Preliminary photochemical study**
Each extract was tested for the presence of flavonoids, coumarins, tannins, total phenolics, saponosides, triterpenoids, alkaloids and anthracenoids as described by Aiyegoro *et al.,*2013

**Quantitative photochemical analysis**

**Total phenol content**
To determine the total phenol content, the Folin-Ciocalteu method was used (Hossain *et al.,* 2013). Absorbance was measured at 735 nm. All experiments were performed in triplicate and the phenolic compounds were expressed in gallic acid equivalents (GAE).

**Total flavonoid content:**
The aluminum trichloride method was used to determine the flavonoid content and the absorbance was measured at 435 nm. The flavonoid content was expressed in quercetin equivalent (QE) (Angkawijaya2014)

**Tannin content:**
The reference method by Sima-Obiang *et al* was used to determine the tannin content (Ngoua-Meye-Misso 2018). Absorbance was measured at 525 nm and tannic acid was used as a standard. The tannin contents were

expressed in mg of tannic acid equivalent (TAE)/100g of extract.

**Proantho cyanidins content:**
The determination of proanthocyanidins was carried out by the HCl-Butanol method (Blois 1958) Absorbance was read at 550 nm and apple procyanidin was applied as standard. Proanthocyanidin levels were expressed in apple procyanidins equivalent (APE).

**Antioxidant activity assay DPPH assay:** The measurement of the anti-radical activity was conducted according to the method of Blois as described by Brand-Williams *et al* 2017 with some modifications. The principle of this method is based on the measurement of the free radical scavenging of diphenyl picryl hydrazyl (DPPH) dissolved in methanol. The addition of an antioxidant in a solution of DPPH leads to a discoloration of the latter which is directly proportional to the antioxidant capacity of the added product. This discoloration can be followed spectrophotometrically by measuring the decrease in absorbance at 517 nm. It provides a convenient way to measure the antioxidant activity of *D. benthamianus* and *S. torvum* extracts. DPPH solutions were incubated for 30 min in the absence (control) or in the presence of increasing concentrations of plant extracts. Vit C and BHA were used as references.

At the end of the incubation period, the absorbance at 517 nm was read and the antioxidant activity was calculated according to the following formula: %Radical scavenger activity = [(Absorbance of DPPH – Absorbance of sample) / Absorbance of DPPH] x 100
**ABT S method:** The ABTS test is based on the ability of an antioxidant to stabilize the $ABTS^{\bullet}$ radical by transforming it into ABTS. A mixture of ABTS solution (7 mM) and potassium persulfate (2.4 mM) was incubated for 12 h in the dark at room temperature until formation of the ABTS radical complex ($ABTS^{+}$). To 60 µL of extract, 2.94 mL of $ABTS^{\bullet+}$ solution were added. The mixture was incubated at 37 °C for 20 min in the dark. Vit C and BHA were used as references. After incubation, the absorbance was measured in a spectrophotometer at 734 nm. The percent inhibition (PI) was calculated by the following method:

**Percentage inhibition**= $[(A_0 - A)/A_0]$ x100 where, $A_0$ is the absorbance of ethanol, A is the absorbance of sample extractor standard.
**Microorganism test:** *Microorganisms used in this study included* Escherichia coli (*E. coli*) 0157 ATCC, *E. coli* 105182 CIP, *Listeria innocua* (L. innocua) LMG 135668 BHI, *Staphylococcus aureus* (S. *aureus*), ATCC 25293 BHI, *Enterococcus faecalis* (*E. faecalis*) 103907 CIP, *Bacillus cereus* (*B. cereus*) LMG 13569 BHI, Shigella dysenteriae (*S. dysenteriae*) 5451 CIP, *Pseudomonas aeruginosa* (*P. aeruginosa*), *Salmonella enterica* (*S. enterica*), *Salmonella typhimurium* (S. typhimurium), *Shigella flexneri* (*S. flexneri)*, *S. dysenteriae*, *Neisseria gonorrhoeae* (N. gonorrhoeae), *E. coli*, *E. faecalis, S. aureus, Klebsiella pneumoniae* (*K. pneumoniae)*, *Acinetobacterbaumannii (A.baumannii*),. Gentamicin, ampicillin and tetracycline were used as positive controls for the bacterial strains tested.

**Antibacterial sensitivity test:**
The diffusion method was used to study the susceptibility of microorganisms to plant extracts. Bacteria and fungi were respectively grown in Muller Hinton and Sabouraud broths. Each culture was then suspended in a solution of sodium chloride (NaCl, 0.9%) to a turbidity equivalent to that of the standard Mac Farland 0.5. The extracts were diluted in dimethylsulfoxide at 100 mg/mL. Each extract (10 µL) was loaded onto each filter paper disc.

The agar was suspended in distilled water, heated to complete dissolution and autoclaved at 121 °C and poured into Petri dishes. Disks were placed on cultures and antimicrobial activity was estimated after incubation at 37°C for 24h by measuring the inhibition diameter.
The relative percentage inhibition (RPI) of the plant extracts relative to the positive control (Gentamicin) was calculate dusing the following formula
RPI=100x(X-Y)/(Z-Y)
Where X is the total zone of inhibition of the plant extract, Y is the total zone of inhibition of the solvent and Z is the total zone of inhibition of the standard drug (Gentamicin).

**Minimum inhibitory concentrations (MICs), minimum bactericidal concentrations (MBCs) and minimum fungicidal concent rations (MFCs)**
MICs, MBCs and MFCs were determined by the microdilution technique. Briefly, the nutrient broth was dispensed into the wells of a microplate. One hundred microliters of extracts were added to the first well of one row and double dilution was performed in other wells. Ninety microliters of nutrient broth and 10 µL of inoculum were added to the wells. A concentration range of the extract of 0.004 9 to 5 mg/mL was obtained.

The plates were gently shaken and incubated at 37 °C for 24 h; the inhibition was evaluated by the absence of turbidity in the wells.

To determine MBCs and MFCs, 100 µL of each well showing no visible growth were collected and seeded in agar plates containing agar. The plates were incubated at 37 °C for 24-48 h and the number of colonies was counted.

The action of an antimicrobial on a microorganism can be characterized with several parameters such as MIC and MBC or MFC. According to the MBC/MIC or MFC/MIC report, antimicrobials with MBC/MIC ratios of 1 are considered to be microbicides; while those with the MBC/MIC ratio as 2 or greater are considered to be bacteriostatic or fungistatic.

**Statistical analyses**

The experimental results were expressed as mean ± standard deviation. All measurements were replicated three times. The data were analyzed by the univariate ANOVA test followed by the Dunnet/Tukey test for multiple comparisons and determination of significance rates. Values of $P < 0.05$ were considered statistically significant.

**RESULTS**

**Phytochemical screening**

Phytochemical screening of extracts was performed to detect major chemical groupsshows that total phenols, total flavonoids, proanthocyanidins, anthracenosides and coumarins were abundant in the crude extracts of *D. benthamianus* and *S. torvum*.

The total phenolic, total flavonoids, total tannins and total proanthocyanidins contents of *D. benthamianus* and the total phenolic content ranged from (660.2 ± 4.3) to (2 760.7 ± 5.2) mg GAE/100 g of extracts. The water-ethanol extract of *D. benthamianus* had the highest phenolic content and the water extract of *S. torvum* was the lowest in phenolic compounds. The results of the total flavonoids did not show a significant difference between *D. benthamianus* and *S. torvum* extracts. The amount of tannin was highest in the water-ethanol extract of *D. benthamianus* [(1 350.8 ± 9.0) mg TAE/100 g extracts]. *3.2. Antioxidantactivities*.

**Sensitivity test of extracts:**

Screening of antimicrobial properties of six samples showed that all extracts of *D. benthamianus* and *S. torvum* had antimicrobial activities . The antimicrobial activity of the two plants studied varied from one extract to another. In fact, *B. cereus* LMG 13569 BHI and *S. dysenteriae* were most sensitive among all microbial strains studied. Extracts of *S. torvum* had the higher inhibition diameters compared to extracts of *D. benthamianus*. Several microbial strains such as *B. cereus* LMG 13569 BHI, *S. dysenteriae* 5451 CIP, *S. dysenteriae, N. gonorrhoeae* and *E. faecalis* were more sensitive on the majority of crude extracts compared to standard (gentamicin, tetracycline, ampicillin).

**Table 1: Phytochemicals present in aqueous and ethanolic extract of** *S. torvum*

| Sl.No | Phytochemical | Results | |
|---|---|---|---|
| | | Aqueous extract | Ethanolic extract |
| 1. | Anthocyanin | - | - |
| 2. | Diterpenes: Copper acetate test | + | ++ |
| 3. | Steroids | + | ++ |
| 4. | Cardial Glycosides: Keller-Killani test | +++ | _ |
| 5. | Tannin: Lead acetate test | ++ | _ _ |
| 6. | Lead acetate test $FeCl_3$ | + | _ |
| 7. | Flavonoid Alkaline Reagent Test | +++ | + |
| 8. | Phlobatannins | - | - |
| 9. | Phytosterol: Salkowski's test | + | + |
| 10. | Alkaloids Wagner's reagent | +++ | + |
| 11. | Phenols: $FeCl_3$ test | +++ | + |
| 12. | Leucoanthocyanin | - | - |
| 13. | Coumarin Test | | |
| 14. | Saponin: Foam test | +++ | + |

## DISCUSSION

Traditional healers make use of medicinal plants to treat microbial diseases without any scientific basis. This experimental study was used to evaluate the antioxidant and antimicrobial potential of plant extracts rich in phenolic compounds (water-acetone, water-ethanol and water extracts of *S. torvum)*. Phytochemical screening in this study revealed the presence of a few secondary metabolites in the stem bark of *D. benthamianus* and the fruits of *S. torvum*. The work of Mounguengui *et al*. also showed the presence of tannins and flavonoids in the extracts of *S. torvum* The qualitative study of *S. torvum* highlights secondary metabolites in the six extracts studied. Phenolic compounds are active substances that may have biological or pharmacological activities Angiolella *et al*. (2017) also reported that phenolic compounds have antibacterial, antioxidant and anticancer effects. Therefore, the use of *S. torvum* fruit in traditional medicine could be attributed to the high content of phenolic compounds. This content contributes to the antioxidant power of the plant. These antioxidants can act according to two major mechanisms, either by transfer of hydrogen atom or by electron transfer. In the present study, two methods were used to demonstrate the antioxidant activity of the crude extracts of *S. torvum*. Thus, the capacity of the water-ethanol and water-acetone extracts to reduce the free radicals DPPH and ABTS is greater than that of the aqueous extract. The results of our study on the antioxidant activity of *S. torvum* extracts are compatible with the work of Mounguengui *et al* 2006 . However, Kumar *et al* 2007 demonstrated that water extracts of *S. torvum* had a high antioxidant capacity compared to methanol extracts. Antioxidant activity can be directly related to the amount of phenolic compounds present in various extracts[1]. The antimicrobial activity of the crude extracts of *S. torvum* was evaluated by two methods (diffusion and microdilution). The results obtained in this study show that the water-ethanol and water-acetone extracts of both plants have a great inhibitory effect on the growth of all bacterial and fungal strains tested. These observed activities are also explained by the results of the chemical analysis of plants which reveal the presence of phenolic compounds whose antimicrobial properties have already been demonstrated The antimicrobial activity of the stem bark of fruits of *S. torvum* varies from one extract to another and from one microorganism to another

These results support Evina *et al*. which showed the antimicrobial activity *S. torvum* extracts against several Gram-positive and Gram-negative bacteria. This variability of inhibition may be due to the resistance capacity linked to the bacterial groups or to the nature of the compounds present in the plant extracts. The work of Lalitha *et al* 2001 on antimicrobial activity of *S. torvum* also corroborates with the results of our study.

## CONCLUSIONS

The methanol extract of the *Solanum thorvum.*(Swartz) leaves and fruits effective against selected bacterial and fungal strains. Its phytochemical contents have broad antimicrobial properties and the plant might be a novel source of antimicrobial drug.

**Conflicts of interest:** The authors stated that no conflicts of interest.

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