



## Research Article

# Phytochemical and *In-vitro* Antibacterial Analysis of Crude Ethanol Leaf Extract and *n*-Butanol Partitioned Portion of *Cadaba farinosa* Forsskal (Capparaceae)

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## ABSTRACT

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Pathogenic resistance has seen no major breakthroughs since the quinolones of the 1960s, despite over fifty new antimicrobial drugs reported by WHO in 2019. Methicillin-resistant *Staphylococcus aureus* (MRSA) alone caused over 100,000 deaths in 2019. Developing countries still suffer high mortality rates due to bacterial infections increasingly resistant to existing drugs. This study investigates the phytochemical content and antibacterial efficacy of extracts from *Cadaba farinosa* Forssk as a potential source of non-resistant drugs. Phytochemical screening revealed the presence of alkaloids, anthraquinones, carbohydrates, cardiac glycosides, flavonoids, glycosides, saponins, and tannins. The antibacterial activity of the crude ethanol leaf extract (CEE) and *n*-butanol partitioned portion (NBU) was tested against three Gram-positive and five Gram-negative bacteria using standard antibiotics (Ciprofloxacin and Streptomycin) for comparison. Both extracts showed dose-dependent inhibition of all tested bacteria, outperforming standard drugs. Notable results included *Corynebacterium diphtheriae* susceptibility of 14±0.9mm and 20±0.0mm for CEE and NBU at 20mg/hole and 40mg/hole, respectively, compared to 11.4±0.01mm Ciprofloxacin and 11.6±0.01mm Streptomycin at 5µg. Similar patterns were observed for *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Shigella dysenteriae*. Minimum inhibitory concentrations were 5 mg/ml for both extracts. The antibacterial activity is attributed to the phenolic content, affecting microbial cells through enzyme inhibition and protein inactivation. This study supports traditional medicinal uses of the plant and its potential for developing novel antibacterial agents against drug-resistant pathogens.

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## INTRODUCTION

Antimicrobial agents are substances (i.e. antibiotics, antiviral agents, fungicides, parasiticides, e.t.c) produced with clear intention of inhibiting growth or killing of microbes. Likewise, other substances like antiseptics, disinfectants, other pharmaceuticals and some natural products are also included amongst antimicrobial agents. However, when microbes (i.e. bacteria, fungi, parasites and viruses) become resistant to antimicrobials which they were initially susceptible to, then AMR (antimicrobial resistance) has occurred (United Nations Environment Programme, 2022). AMR today has unanimously been recognized as one of the existential threats to both animal and human health (OIE, 2020). However, diseases caused by pathogens have been documented for so many years in history. Such example was the one which decimated a third of the Greeks in what's called "Athen's Plaque" during the Peloponnesian war in 430 BC (Huremovi'c, 2019). Another example was that of *Mycobacterium tuberculosis* which is the bacteria responsible for spreading tuberculosis (TB) disease had infected ten million in 2020 with fatality of one and half million and drug-resistant strains have developed accounting for half a million infections yearly (EPHA, 2022). Another report by WHO, (2022) was that of COVID-19 (corona virus disease) when it swept (across the globe) lives of over fourteen million in 2019. That awakened scientists all over the world, the importance of finding effective drugs against microbes for effective healthcare. Resistance to antimicrobial agents is a major global public health problem. In the developing countries, bacterial infections are still the main cause of deaths (Iwu *et al.*, 1999). Despite the progress made in the understanding of microorganisms and their control in industrialized nations, incidents due to drug resistant microorganisms and the emergence of hitherto unknown disease-causing microbes, pose enormous public health concerns (Iwu *et al.*,

1999). Some of the major pollution sources affecting antimicrobial resistance (AMR) includes poor sanitation; sewage and waste effluent; effluent and waste from pharmaceutical industries; effluent and waste from healthcare facilities; use of antimicrobials and manure in crop production; releases, effluent and waste in animal production (United Nations Environment Programme, 2022). The search for new antibacterial compounds with improved activity to replace those that have become inactive is therefore necessary. Traditional healers use many plants to treat diseases that appear to be of bacterial aetiology and plants may afford valuable antimicrobial compounds (Cowan, 1999). Over 75% of the antibacterial drugs in clinical use are of natural origin and most of them are obtained from fungal sources (Newman *et al.*, 2003). A renewed interest in plant-based antimicrobials has arisen some decades ago, but still plant based antimicrobials are poorly explored. Screening of plant extracts for antimicrobial activity has shown that higher plants represent a potential source of new anti-infective compounds. Plant derived antimicrobial compounds might inhibit bacteria through different mechanisms than conventionally used antibiotics, and could therefore be of clinical value in the treatment of infections caused by resistant microbes (Eloff, 1998). Only a small fraction of the known plant species of the whole world have been evaluated for the presence of antimicrobial compounds including *Cadaba farinosa* Forsskal to which very little or no reports on antibacterial or antimicrobial activities have been reported, and thus it is necessary to increase the efforts in collecting and screening plants for the development of novel and environmentally safe antimicrobial agents (Stein, 2005). Despite reports on the important ethnomedicinal values of *Cadaba farinosa*, this is one of the pioneer studies in finding its antibacterial efficacy with a view to further go into isolation of effective

bioactive compound(s) using bioassay guided isolation protocol.

## METHODOLOGY

### Collection, Identification and Preparation of Plant Material

Fresh leaf samples of *Cadaba farinosa* were collected from Maiduguri Metropolitan Council Area of Borno State, Nigeria. The plant specimen was identified and authenticated at the Herbarium section of the Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria which corresponded with that of voucher specimen number V/No: 2744. The leaf was air-dried under shade for several days and pulverised into fine powder for extraction.

### Extraction of Plant Material

The air-dried ground powdered leaf material was extracted exhaustively with 70 % ethanol using cold maceration method for several days with occasional shaking. The crude ethanol leaf extract was concentrated to dryness on water bath at 50° C to give dark gummy mass coded CEE – crude ethanol leaf extract of *Cadaba farinosa* and marc. The gummy mass was dissolved in 500 ml distilled water and partitioned with ethyl acetate (EAE) to give dark green mass. The aqueous portion was further partitioned with *n*-butanol (NBU) to give dark brown mass and an aqueous (residual) portion. The CEE and NBU served as working sample for the antibacterial investigations of the plant.

### Phytochemical Screening

A little quantity each of the CEE and NBU were subjected to qualitative phytochemical screening to test for the presence of alkaloids, anthraquinones, carbohydrates, cardiac glycosides, flavonoids, glycosides, saponins and tannins as described by Brain and Turner, (1975); Silva *et al.*, (1998); Trease and Evans, (1983); Trease and Evans, (1996); Trease and Evans, (2002).

## Determination of Antibacterial Activities

### Microorganisms and media used for the study

The Gram positive bacteria used in this study were *Corynebacterium diphtheriae*, *Staphylococcus aureus* and *Streptococcus pneumoniae*, while Gram negative bacteria were *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Shigella dysenteriae*. These organisms were clinical laboratory isolates obtained from the Department of Medical Microbiology, University of Maiduguri Teaching Hospital, Maiduguri, Borno State-Nigeria. Standard susceptibility antibiotic discs were: Ciprofloxacin (5 µg/disc) and Streptomycin (5 µg/disc) produced by Oxoid Ltd., Hampshire, England. The powdered nutrient agar and nutrient broth medium were obtained from a chemical store in Maiduguri, Nigeria and prepared according to manufacturer's guidelines LDP (Laboratory Diagnostic Product) UK, Middlesex HA88BP, UK Batch = NUT (Nutrient Agar) A0401. Cultures were maintained on agar slants and refrigerated at 4°C until use.

### Antibacterial Assays

#### Antibacterial susceptibility test

The preliminary antibacterial activity of CEE and NBU on the test bacteria were evaluated using the hole-in-plate disc diffusion technique as described by Forbes *et al.*, (1990); Vlietinck *et al.*, (1995) and Usman *et al.*, (2007a, b). Therefore, CEE and NBU were prepared in two different stock concentrations of 200 mg/ml and 400 mg/ml prepared by dissolving 2.0 g and 4.0 g respectively into 10 ml each of distilled water to afford working concentrations of 20 and 40 mg/hole respectively.

#### Spray plate technique

A young colony of the bacterial isolates was aseptically obtained using a sterilized wire loop. It was readily emulsified in sterile peptone water in about 2 ml. The solution was sprayed on a sterile nutrient agar jell plate, where the excess was drained off (This process was repeatedly and

independently performed for all organisms to avoid contamination).

Briefly, a cork-borer of 2 mm in diameter was used to punch 3 holes per Petri dish. The borer was sterilized in methanol-lit flame each time after punching 3 holes per organism in a Petri dish. A total of 16 dishes for CEE were coded E1 having 0.2 ml and E2 having 0.1 ml for each of the 8 organisms. The same was carried out for NBU coded N1 for 0.2 ml per hole and N2 for 0.1 ml for each of the 8 organisms summing the total to 32 Petri dishes. They were incubated overnight in an oven at 35°C.

The microorganisms were maintained on agar slants. Inoculates were prepared by subjecting the test organisms in nutrient broth and incubated for 24 hours at 35°C. After incubation, the broth cultures were diluted to 1:1000 for Gram-positive bacteria and 1:5000 for the Gram-negative bacteria. One millilitre of the diluted cultures was inoculated into 19 ml sterile molten nutrient agar (48°C) and poured into sterile Petri dishes. These were swirled gently and allowed to solidify. Afterwards, holes of 2 mm diameter were bored into the solidified and inoculated nutrient agar plates using sterilised cork borer. All the holes were filled with equal volumes of 0.1 ml (20 and 40 mg/hole) of crude extract, the concentration was thereafter varied depending on the level of purity of the substances. Reference antibiotics used were Ciprofloxacin (5 µg/disc) and Streptomycin (5 µg/disc). These standard discs were placed on already bacteria-inoculated nutrient agar plate. An hour was allowed for the extract to diffuse into the agar. Thereafter, plates were then incubated overnight at 35°C for bacterial strains. At the end of the incubation period, inhibition zones were recorded in millimetres as the diameter of growth-free zones around the bored holes using a transparent metre rule. The CEE, NBU and standard antibiotics were independently tested in triplicates (Table 2). Diameters of zones of inhibition  $\geq 10$  mm

exhibited by plant extractives was considered active (Zwadyk, 1972; Usman *et al.*, 2007a).

#### **Inoculation of Petri dish**

Peptone water each was transferred into 8 test tubes after rinsing and cleaning with peptone water. A micron wire loop (inoculation loop) was heated (red hot) in methanol-lit flame and all the various microorganisms were transferred into the test tubes containing peptone water using the loop. Each test tube was then shaken and poured into corresponding coded Petri dish. The same process was done repeatedly into other Petri dishes containing same coded specie of organism until finally disposed. A total of 32 Petri dishes were coded for the 8 microorganisms having 0.2 ml per hole = 40 mg and 0.1 ml per hole = 20 mg for the two (2) extractives.

#### **Determination of Minimum Inhibitory Concentration (MIC)**

The MIC was determined using the nutrient broth dilution technique as described by Sidney *et al.*, (1978) and Vollekova *et al.*, (2001). The minimal inhibitory concentration value was determined for the microorganisms that were sensitive to the extracts under study. The microorganisms were prepared as described earlier. Each extract was first diluted to the highest concentration (100 mg/ml) in distilled water and then two-fold serial dilution of the crude extract was then made to a concentration ranging from 0.780 mg/ml to 200 mg/ml using nutrient broth and then inoculated with 0.2 ml suspension of test organisms. The concentration was then varied depending on the extracts and level of activity. The extracts were inoculated with 1 ml suspension of the organisms and incubated (thereafter) at 35°C for bacterial strains (Tables 3). MIC was considered the lowest concentration where no visible turbidity was observed (in the test tubes).

#### **RESULTS**

Preliminary phytochemical examinations of CEE and NBU revealed the presence of alkaloids, anthraquinones, carbohydrates, cardiac

glycosides, flavonoids, glycosides, saponins and tannins (Table 1).

**Table 1:** Preliminary phytochemical screening of crude ethanol leaf extract and *n*-butanol partitioned portion of *Cadaba farinosa*

S/No	Group Constituents	Extractive/Inference	
		CEE	NBU
1.	Alkaloids	+	+
2.	Anthraquinones	+	+
3.	Carbohydrates	+	+
4.	Cardiac Glycosides	+	+
5.	Flavonoids	+	+
6.	Glycosides	+	+
7.	Saponins	+	+
8.	Tannins	+	+

**Key:** CEE = crude ethanol leaf extract; NBU = *n*-butanol partitioned portion; + = present

The antibacterial evaluation of CEE and NBU exhibited a wide range of activities on both Grams' strain bacteria under study (Table 2).

**Table 2:** Activity spectrum of crude ethanol leaf extract and *n*-butanol partitioned portion of *Cadaba farinosa* against some selected Gram-positive and Gram-negative bacteria

S/No.	Microorganisms	Concentrations/Diameters of inhibition zone (mm)					
		20 (mg/hole)		40 (mg/hole)		5 µg/disc	
		CEE	NBU	CEE	NBU	CIP	STP
1.	<i>Corynebacterium diphtheriae</i>	14.0±0.9	14.0±0.3	20.0±0.0	19.0±0.9	11.4±0.01	11.6±0.01
2.	<i>Staphylococcus aureus</i>	15.0±0.7	13.0±0.3	20.0±0.7	20.0±0.7	11.8±0.00	11.3±0.01
3.	<i>Streptococcus pneumoniae</i>	14.0±0.3	12.0±0.0	20.0±0.7	18.0±0.7	11.0±0.00	11.4±0.00
4.	<i>Escherichia coli</i>	13.0±0.3	15.0±0.3	19.0±0.7	20.0±0.3	00.7±0.00	11.2±0.01
5.	<i>Klebsiella pneumoniae</i>	15.0±0.3	14.0±0.6	20.0±0.7	18.0±0.0	00.9±0.00	11.8±0.00
6.	<i>Pseudomonas aeruginosa</i>	13.0±0.3	13.0±0.0	18.0±0.7	20.0±0.7	11.6±0.00	11.6±0.00
7.	<i>Salmonella typhi</i>	14.0±0.3	15.0±0.7	19.0±0.9	19.0±0.3	11.1±0.00	09.0±0.06
8.	<i>Shigella dysenteriae</i>	16.0±0.6	15.0±0.6	18.0±0.7	17.0±0.7	NT	NT

**Key:** Data are presented as mean±SEM (n = 3); CEE = crude ethanol leaf extract; NBU = *n*-butanol partitioned portion; CIP = ciprofloxacin; STP = streptomycin; NT = Not tested

The Minimum Inhibitory Concentrations (MIC) of both CEE and NBU against the active microorganisms was conducted (Table 3) and both extracts inhibited the growth of the pathogens at 5 mg/ml.

**Table 3:** Minimum Inhibitory Concentrations of crude ethanol leaf extract and *n*-butanol partitioned portion of *Cadaba farinosa* against some Gram-positive and Gram-negative bacteria

S/No.	Microorganisms	Extracts	Concentration (mg/ml)		
			10	5	2.5
1.	<i>Corynebacterium diphtheriae</i>	CEE	$\pi$	+	+
		NBU	-	$\pi$	+
2.	<i>Staphylococcus aureus</i>	CEE	-	$\pi$	+
		NBU	-	$\pi$	+
3.	<i>Streptococcus pneumoniae</i>	CEE	$\pi$	+	+
		NBU	$\pi$	+	+
4.	<i>Escherichia coli</i>	CEE	-	$\pi$	+
		NBU	-	$\pi$	+
5.	<i>Klebsiella pneumoniae</i>	CEE	-	$\pi$	+
		NBU	-	$\pi$	+
6.	<i>Pseudomonas aeruginosa</i>	CEE	$\pi$	+	+
		NBU	$\pi$	+	+
7.	<i>Salmonella typhi</i>	CEE	$\pi$	+	+
		NBU	-	$\pi$	+
8.	<i>Shigella dysenteriae</i>	CEE	-	$\pi$	+
		NBU	$\pi$	+	+

**Key:** CEE = crude ethanol leaf extract; NBU = *n*-butanol partitioned portion;  $\pi$  = minimum inhibitory concentration; - = non-turbid; + = turbid

## DISCUSSION

The CEE, NBU and standard antibiotics were found to be active against all selected species of Gram-positive and Gram-negative tested bacteria (Tables 2 and 3). The activity could be attributed to secondary metabolites present in CEE and NBU as alkaloids, flavonoids, saponins and tannins (most especially the phenolics have been reported to possess antibacterial, anticancer and antiviral activities (Ayoola *et al.*, 2006; Ayoola *et al.*, 2008 and Savithramma *et al.*, 2011). The mechanisms thought to be responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydroxyl groups or through more non specific interactions with proteins (Mason and Wasserman, 1987) often leading to inactivation of the protein and loss of function. Probable targets in the microbial cell are surface exposed adhesions, cell wall polypeptides and membrane-bound enzymes. Phenols may also render substrates unavailable to

microorganisms (Stern *et al.*, 1996). Isolation of phenolics such as 2-(3,4-Dihydroxy-phenyl)-3,5,7-trihydroxy-chromen-4-one known as Quercetin and 5, 7-Dihydroxy-2-(4-hydroxy-phenyl)-3-methoxy-chromen-4-one (Astragalins) from the same extractives have been reported earlier by the same author (Tijjani *et al.*, 2017; 2018). Therefore, the observed activities may not be unrelated to flavonoids (e.g. astragalins, kaempferol, quercetin, rutin, etc), alkaloids (eg cadabicine, cadabicine diacetate, cadabicine methyl ether, thiazolidine, etc), phenolics (eg isoorientin, isoquercetin,  $\alpha$ ,  $\beta$ -dihydroferulic acid, etc), etc that have been isolated from this plant. However, *Cadaba farinosa* has been reported to contain large number of active constituents like alkaloids, sugar, carbohydrates, glycoside, protein, amino acid, flavonoid, saponin, tannins, phenolic compounds, gums, mucilage and steroids (Telrandhe and Uplanchiwar, 2013).

The CEE and NBU were significantly active against tested microorganisms: *Corynebacterium diphtheriae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella dysenteriae*, *Staphylococcus aureus* and *Streptococcus pneumoniae*. The trend of the broad-spectrum concentration-dependent activities were in line with the earlier works of Usman *et al.*, (2009, 2018) who conducted similar study on varieties of medicinal plants with similar phytochemical contents and inhibitory effects against some pathogenic bacteria of same strains. The plants reported by the authors were *Bauhinia rufescens*, *Aristolochia albida*, *Calotropis procera*, *Ziziphus spina-christi*, *Punica granatum* and *Waltheria indica*.

The antibacterial activity of any compound against Gram-positive bacteria is partly due to their ability to reach the site of action. In bacteria, various enzymes especially components of energy-converting systems such as electron transport chains (ETC) and ATPases are embedded in the plasma membrane. The ETC is a chain of specialized complex molecules (redox agents) which form a conducting path for electrons. The inner and outer surfaces of the membrane are hydrophilic, whereas the interior is hydrophobic, so lipophilicity of a compound affects its movement into the membrane lipid bilayer portion. Once inside the lipid bilayer

portions, the compound may inhibit the ETC perhaps by interfering with the redox reactions (Franks and Leib, 1986).

The relative resistance of Gram-negative bacteria compared to Gram-positive resides in the outer membrane of Gram-negative bacteria acting as a permeability barrier so that uptake of the compounds in the cell is reduced (Russell and Chopra, 1996; Russell, 2003).

This study showed most of the activities resides in the polar part of the extract. More so, no significant variation is seen in the data obtained for the inhibitory effects against the test microorganisms by the CEE and NBU. Therefore, in this case, it can be summarised that partitioning with non-polar or mid-polar solvent (*n*-hexane and ethylacetate) had no significant masking effect since the *n*-butanol partitioned portion had showed similar effects with crude ethanol leaf extract.

#### CONCLUSION

The *in vitro* antibacterial assay of CEE and NBU using Mueller-Hinton agar (well) diffusion and broth dilution (MIC and MBC) susceptibility techniques exhibited broad spectrum bioactivity against all bacterial species tested; thus, lending credence to the vast ethnomedicinal uses of the plant. The activities observed could be attributed to the presence of some of the phytochemicals detected which have hitherto been associated with antibacterial activities.

#### REFERENCES

- Amber, R. (1990). *A Studies on the Chemical Constituents of Cadaba Farinosa* (Doctoral thesis, University of Karachi, Pakistan).
- Ayoola, G.A., Sofidiya, T., Odukoya, O. and Coker, H.A.B. (2006). Phytochemical screening and free radical scavenging activity of some Nigerian medicinal plants. *Journal of Pharmaceutical Science and Pharmaceutical Practices*. 8:133-136.
- Ayoola, G.A., Coker, H.A.B., Adesegun, S.A., Adepoju-Bello, A.A. Obaweya, K. Ezennia, E.C. and Atangbayila, T.O. (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. *Tropical Journal of Pharmaceutical Research*. 7:1019-1024.
- Brain, K.R. and Turner, T.D. (1975). The Practical Evaluation of

- Phytopharmaceuticals (pp. 140-154). *Wright Science Technica. Bristol.*
- Burkill, H. M. (Ed.) (1985). *The Useful Plants of West Tropical Africa* (Volume 1, families A-D, pp. 319-320). Great Britain: White friars.
- Cowan, M.M., (1999). Plant products as antimicrobial agents: *Clinical Microbiology Reviews*, 12(4):564-582.
- Eloff, J.N. (1998). Which Extraction should be used for the Screening and Isolation of Antimicrobial Components from Plants. *Journals of Ethnopharmacology*, 60:1-8.
- Franks, N.P. and Leib, W.R., (1986), Partitioning of long chain alcohols into lipid bilayers: Implications for mechanism of genera. In *Proceedings of National Academy of Science* (83:5116-5120.)
- EPHA-European Public Health Alliance (2022). An overview of antimicrobial resistance. The Health Burden, Science, Surveillance, Policy Developments and Areas of Future Action, pp. 4-6.
- Forbes, B.A., Sahm, D.F. and A.S. (1990). Trevino, In: *Diagnostic Microbiology: Bailey and Scotts Baron, E.J. & Finegold, S.M. (eds.)*. (pp. 171-194) St. Louis: Mosby Co.
- Huremović, D. (2019). Brief history of pandemics (Pandemics Throughout History). *Psychiatry Pandemics*, pp. 7–35.
- Iwu, M.W., Duncan, A.R. and Okunji, C.O. (1999). New Antimicrobials of Plant Origin. In: Janick, J. (ed). (pp. 457-462) *Perspective on New Crops and New Uses*. A. S. H. S. Press, Alexandria, V.A.
- Kirtikar, K. R. and Basu, B. D. (1999). *Indian Medicinal Plants* (pp. 259-261). Dehradun: International Book Distributors.
- Mason, T.L. and Wasserman, B.P. (1987). Inactivation of red beet betaglucan synthase by native and oxidized phenolic compounds. *Phytochemistry*, 26:2197-2202.
- Newman, D.J., Cragg, G.M. and Snadar, K.M. (2003), Natural products as a source of new drugs over a period of 1981–2003, *Journal of Natural Products*, 66:1022-1037.
- Murray, C. J., Ikuta, K. S., Sharara, F., Swetschinski, L., Aguilar, G. R., Gray, A., Han, C., Bisignano, C., Rao, P., Wool, E., Johnson, S. C., Browne, A. J., Chipeta, M. G., Fell, F., Hackett, S., Haines-Woodhouse, G., Hamadani, B. H. K., Kumaran, E. A. P., McManigal, B., Agarwal, R., Akech, S., Albertson, S., Amuasi, J., Andrews, J., Aravkin, A., Ashley, E., Bailey, F., Baker, S., Basnyat, B., Bekker, A., Bender, R., Bethou, A., Bielicki, J., Boonkasidecha, S., Bukosia, J., Carvalheiro, C., Castañeda-Orjuela, C., Chansamouth, V., Chaurasia, S., Chiurchiù, S., Chowdhury, F., Cook, A. J., Cooper, B., Cressey, T. R., Criollo-Mora, E., Cunningham, M., Darboe, S., Day, N. P. J., Luca, M. D., Dokova, K., Dramowski, A., Dunachie, S. J., Eckmanns, T., Eibach, D., Emami, A., Feasey, N., Fisher-Pearson, N., Forrest, K., Garrett, D., Gastmeier, P., Giref, A. Z., Greer, R. C., Gupta, V., Haller, S., Haselbeck, A., Hay, S. I., Holm, M., Hopkins, S., Iregbu, K. C., Jacobs, J., Jarovsky, D., Javanmardi, F., Khorana, M., Kissoon, N., Kobeissi, E., Kostyanev, T., Krapp, F., Krumkamp, R., Kumar, A., Kyu, H. H., Lim, C., Limmathurotsakul, D., Loftus, M. J., Lunn, M., Ma, J., Mturi, N., Munera-Huertas, T., Musicha, P., Mussi-Pinhata, M. M., Nakamura, T., Nanavati, R., Nangia, S., Newton, P., Ngoun, C., Novotney, A., Nwakanma, D., Obiero, C.W., Olivas-Martinez, A., Olliaro, P., Ooko, E., Ortiz-Brizuela, E., Peleg, A. Y., Perrone, C., Plakkal, N., Ponce-de-Leon, A., Raad, M., Ramdin, T., Riddell, A., Roberts, T., Robotham, J. V., Roca, A., Rudd, K. E., Russell, N., Schnall, J., Scott, J. A. G., Shivamallappa, M., Sifuentes-Osornio, J., Steenkeste, N., Stewardson, A. J., Stoeva, T., Tasak, N., Thaiprakong, A., Thwaites, G., Turner, C., Turner, P., Doorn, H. R. van, Velaphi, S., Vongpradith, A., Vu, H., Walsh, T., Waner, S., Wangrangsimakul, T., Wozniak, T., Zheng, P., Sartorius, B., Lopez, A. D., Stergachis, A., Moore, C., Dolecek, C. and Naghavi, M. (2022). Global burden



- of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet* 399:629–655
- OIE-World Organisation for Animal Health (2020). OIE Standards, Guidelines and Resolutions on Antimicrobial Resistance and the use of antimicrobial agents. pp. 1-5.
- Russell, A.D. and Chopra, I. (1996). Understanding Antimicrobial Action and Resistance, 2<sup>nd</sup> ed. Ellis Horwood, Chichester, UK.
- Russell, A.D. (2003). Similarities and differences in the responses of microorganisms to biocides. *Journal of Antimicrobials and Chemotherapy*, 52:750-763.
- Savithamma, N., Linga, M.R. and Suhulatha, D. (2011). Screening of medicinal plants for secondary metabolites. *Middle-East Journal of Scientific Research*. 8: 579-584.
- Sidney, M. F., William, J.M. and Elvyn, G.S. (1978). Bailey and Scott's Diagnostic Microbiology (pp. 385-403). C.V. Mosby, St. Louis; USA.
- Silva, L.G., Lee, I.S. and Kinghorn, D.A. (1998). Special problem with the extraction of plants. In: R.J.P. Cannell (Ed). Natural products isolation (pp 343-64). New Jersey, Humana Press Inc. 999, Riverview Drive, Suite 208, Totowa: USA.
- Stein, T. (2005). *Bacillus subtilis* Antibiotics: Structures, Syntheses and Specific Functions. *Molecular Microbial*, 56:845-857.
- Stern, J.L., Hagerman, A.E., Steinberg, P.D. and Mason, P.K. (1996). Phlorotannin–protein interactions. *Journal of Chemical Ecology*, 22:1887-1899.
- Telrandhe, U.B. and Uplanchiwar, V. (2013). Phyto-Pharmacological Perspective of *Cadaba farinosa* Forsk. *American Journal of Phytomedicine and Clinical Therapeutics*, 1:011-022.
- Tijjani, M.A., Magaji, M.G., Yaro, A.H., Hanwa, U.A., Hassan, H.S., Pateh, U.U., Sule, M.I. and Bukar, L.I. (2017). Analgesic and Anti-Inflammatory Studies on Crude Ethanol Extract of *Cadaba Farinosa* Forssk Leaf in Mice and Rats. *Journal of Pharmacy and Bioresources*. 14(2): 1-7.
- Tijjani, M.A., Magaji, G.M., Yaro, A.H., Hassan, H.S., Pateh, U.U., Sule, M.I. and Bukar, L.I. (2017). Quercetin from *n*-Butanol Soluble Fraction of Crude Ethanol Leaf Extract of *Cadaba farinosa* Forssk. *Chemistry Research Journal*, 2(5):270-274.
- Tijjani, M.A., Musa, Y.M., Hanwa, U.A., Egwu, E.C., Usman, H. and Bukar, L.I. (2018). Astragalin from *n*-Butanol Soluble Fraction of Crude Ethanol Leaf Extract of *Cadaba farinosa* Forssk. *Biological and Environmental Sciences Journal for the Tropics*, 15(1):59-63.
- Tijjani, M.A., Magaji, M.G., Yaro, A.H., Usman, H., Muhammad, A., Bello, S.S., Egwu, C.E. and Chellube, Z.M. (2022). Behavioural studies on crude Ethanol leaf extract of *Cadaba farinosa* Forssk. in mice. *Infectious Diseases and Herbal Medicine*. 3:222.
- Tijjani, M.A., Usman, H., Muhammad, A., Magaji, M.G., Yaro, A.H., Hassan, H.S., Pateh, U.U., Sule, M.I. and Inuwa, L.B. (2022). Anticonvulsant Studies on Ethanol Leaf Extract of *Cadaba farinosa* Forssk. in Experimental Models. *Tropical Journal of Natural Product Research*. 6(8): 234-245.
- Trease, G.E. and Evans, M.C. (eds). (1983). Textbook of Pharmacognosy. 12th ed. Balliere Tindall. London.
- Trease, G.E. and Evans, M.C. (eds). (1996). Textbook of Pharmacognosy. 15th ed. Balliere Tindall. London.
- Trease, G.E. and Evans, M.C. (eds). (2002). Textbook of Pharmacognosy. 14th ed. Harcourt Brace and Company Asia Pte. Ltd. Singapore.
- United Nations Environment Programme (2022). *Environmental Dimensions of Antimicrobial Resistance: Summary for Policymakers*.
- Usman, H., Musa, Y.M., Ahmadu A.A. and Tijjani, M.A. (2007a). Phytochemical and Antimicrobial Effects of *Chrozophora senegalensis*. *African Journal of Traditional, Complementary and Alternative Medicines*, 4(4):488-494.

- Usman, H., Abdulrahman, F.I. and Ladan, A.A. (2007b). Phytochemical and Antimicrobial Evaluation of *Tribulus terrestris* L. (Zygophyllaceae) Growing in Nigeria. *Research Journal of Biological Sciences*, 2(3):244-247.
- Usman, H., Abdulrahman, F.I., Kaita, H.A. and Khan, I.Z. (2009). Antibacterial Assays of the Solvents Partitioned Portions of Methanol Stem Bark Extract of *Bauhinia rufescens* Lam. [Leguminosae-Caesalpinioideae]. *Pacific Journal Science & Technology*, 10(2):857-867.
- Usman, H., Tijjani, M.A., Hassan, A. and Aji, Z.B. (2018). Comparative Phytochemical and *In vitro* Antimicrobial Activities of the Leaf Extracts of Two Medicinal Plants Growing in North-East, Nigeria. *Journal of Herbmед Pharmacology*, 7(2):61-67.
- Vlietinck, A.J.L., Vanhoof, L., Totte, J., Lasure, A., Vanden berghe, D., Rwangabo, P.C. and Mvukiyumwami, J. (1995). Screening of Hundred Rwandese Medicinal Plants for Antimicrobial and Antiviral Properties. *Journal of Ethnopharmacology*, 46:31-47.
- Vollekovà, A., Kòst'álovà, D. and Sochorová, R. (2001). Isoquinoline Alkaloids from *Mahonia aquifolium* stem bark is active against *Malassezia* spp. *Folia Microbiologia*, 46:107-111.
- WHO (2019). Antibacterial agents in clinical development: an analysis of the antibacterial clinical development pipeline (No. ISBN 978-92-4- 000019-3).
- WHO (2022). 14.9 million excess deaths associated with the COVID-19 pandemic in 2020 and 2021. URL <https://www.who.int/news/item/05-05-2022-14.9-million-excess-deaths-were-associated-with-the-covid-19-pandemic-in-2020-and-2021>.
- Zwadyk, P. (1972). Enteriobacteriaceae in Zinsser Microbiology (pp. 20-32). 20th ed. George Thiene Verlag, Stuttgart, Germany.