

Medicinal Potentials of Virgin Plants: the Case of *Beloperone Asclepiadea* Nees (Acanthaceae)

*Hafsat N. Dankadai, *Greatman C.O. Okafor

*Department of Chemistry, Baze University, Plot 686 Cadastral Zone C00,
Kuchigoro, Behind National Judicial Institute,
Abuja, Nigeria.

Abstract:- Shade-dried *Beloperone asclepiadea* Nees (whole plant) was extracted by Microwave Assisted Extraction and Cold Maceration using n-hexane, dichloromethane, ethyl acetate and methanol in order of increasing polarity. Phytochemical analysis was run on the extracts followed by antibacterial and antifungal evaluations. Phytochemical screening revealed the presence of glycosides, tannins, flavonoids, saponins, cardiac glycosides, steroids/triterpenoids and alkaloids in the methanol extract. Ethyl acetate and dichloromethane extracts showed the presence of all except flavonoids and saponins respectively while only glycosides, cardiac glycosides and steroids/triterpenes were detected in n-hexane extracts. The extracts showed antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, *Streptococcus pyogenes*, *Candida albicans* and *Aspergillus niger* with zones of inhibition (ZOI) in the range 30.0±0.0 mm and 11.0±0.0 mm except for dichloromethane extract which showed no activity against *S. pyogenes*, *S. typhi* and *C. albicans*. Inhibition against *S. typhi* (ethyl acetate extract) and *E. coli* (methanol and n-hexane extract) have the most competitive ZOI values of 30±0.0 mm compared to 32.0 mm and 40.0 mm respectively for ciprofloxacin at 50 µg/mL. The strongest antifungal ZOI's are 18.0±0.0 mm against *C. albicans* (ethyl acetate extract) and 16.5±0.5 mm against *A. niger* (hexane extract) compared to 30.0 mm at 50 µg/mL by the standard drug, terbinafin. Relatively very low minimum inhibitory concentrations (MIC's) of 1.5625 mg/mL and 3.125 mg/mL were observed respectively for activity against *S. aureus* (N-hexane extract) and *E. coli* (ethyl acetate and methanol extracts) while the lowest antifungal MIC of 12.5 mg/mL was recorded against *C. albicans* (ethyl acetate extracts). Minimum bactericidal/fungicidal (MBC/MFC) values of 25 mg/mL were observed for *S. aureus* (n-hexane extract) and *E. coli* (methanol and ethyl acetate extracts) while 50 mg/mL were recorded for *S. typhi*, *B. subtilis*, *S. pyogenes* and *C. albicans* by various solvent extracts. These results taken together, confirm medicinal potency of *B. asclepiadea* against diseases caused by *S. aureus*, *E. coli*, *S. typhi*, *B. subtilis*, *S. pyogenes* and *C. albicans* (in that order) and strongly indicate the presence of bioactive compounds of high therapeutic value.

Key Words: Antimicrobial, bioactive, phytochemical, medicinal plants, minimum bactericidal/fungicidal concentration, minimum inhibitory concentration, virgin plants, zones of inhibition.

1. INTRODUCTION

Since prehistoric times, humans have used natural products, such as plants, animals, microorganisms, and marine organisms, as traditional medicines to alleviate and treat diseases, with the use of plants as medicines traceable to as far back as at least 60,000 years (Shi *et al.*, 2010 &

Fabricant, 2001). Beyond the positive therapy from ethnic medicines obtained by trial and error people also consumed poisonous plants, which led to vomiting, diarrhea, coma, or other toxic reactions and even death, all experiences being handed down to succeeding generations who become more and more medicinally enriched.

Traditional or ethnic medicine (ethno-medicine), also known as, or that metamorphosed into complementary and alternative medicine, still plays key medicinal roles in many countries today (Abdullahi, 2011). It deploys natural products in its crude or semi crude forms, mostly from plants, based on such information inherited from ancestral lineages. It is fast blossoming into orderly regulated systems of medicine in their various forms and despite certain defects, remain a valuable storage area of human medicinal knowledge (Alves & Rosa, 2007; Fabricant, 2001).

Ethnic herbal medicines, the major contributors to traditional medicinal practice, have been urbanized over the past thousands of years with respect to methods of preparation, selection of herbs, identification of medicinal materials and the most suitable time for obtaining different plants. Their role in the development of new drugs, affordability, fewer side effects, easy accessibility, natural healing and also dissatisfaction with the results from orthodox pharmaceuticals have kept them in constant use (Bandaranayake, 2006). Herbalists provide drug leads and information on therapeutic potentials of plants which save energy and cost. Today countless active compounds critical to modern 'western' medicine have been extracted from plants, some following their traditional uses, others not. These include drugs such as *codeine*, *morphine*, *quinine*, *quinidine*, *digoxin*, *vincristine*, *vinblastine* and *atropine* among many others. It is estimated that 60% of anti-tumour and anti-infectious drugs already on the market or under clinical trial are of natural origin (Yue-Zhong, 1998).

Use of herbal medicines even in developed countries has expanded sharply in the latter half of the twentieth century (Blumenthal *et al.*, 1998 & World Health Organization (WHO), 1999) following their unique chemical diversity which results in diversity in their biological activities and drug-like properties. A single herb or formula may contain many phytochemical constituents such as alkaloids, terpenoids, flavonoids, tannins, glycosides, saponins, steroids etc, and these chemicals may function alone or in combination with one another to produce the desired pharmacological effect (Parasuraman, 2014). The

cumulative aftermath of the foregoing is that, by and by, plants increasingly remain the bedrock of human medication and medicinal solutions.

Historically, a lot of diseases had no cure until drugs were eventually developed from plants. Up until 1820 malaria still had no cure until *quinine* was isolated from the bark of the cinchona tree (a plant) and has been used as one of the most effective treatments for malaria to date (Achan *et al.* 2011). Cancer was the second leading cause of death in the world after cardiovascular diseases. There was no known cure to cancer until the anticancer drug *Paclitaxel (Taxol)* was derived from the bark of the “pacific yew” tree (still a plant) was discovered in 1962 and was first tested in patients in 1984 (Slichenmyer *et al.*, 1991). It is therefore logical to believe that the cure to today’s (and possibly tomorrow’s) incurable diseases (like AIDS, Alzheimer’s, Arthritis, Cystic fibrosis, Diabetes, Ebola virus, Hepatitis B, Asthma, Parkinson’s disease, Poliomyelitis etc) may rest with un-researched plants!

Another critical area of concern is the issue of resistance developed by microbes with time to hitherto effective drugs. *Penicillin* (17) was successfully developed to treat bacterial infections until microbes started resisting it (Spellberg & Gilbert, 2014). *Quinine* was known to be one of the most effective drugs for malaria, but resistance was reported in the 1980s (Bunnag *et al.*, 1996) and as of 2006, it was no longer used as the best treatment for malaria. *Praziquantel* is one of the few drugs known to treat schistosomiasis and the parasites are beginning to become resistant (Knobler *et al.*, 2003). *Artemisinin* another plant based game-changer of an antimalarial has started to show resistance by microbes as of July 2016. Also the last resort for gonorrhoea “*cephalosporin*” has been confirmed to show resistance by microbes in at least 10 countries (WHO, 2018). This brings to fore the need for fresh discoveries and new drug options to tackle microbial resistance. Alternative antimicrobials are urgently needed (Mandal *et al.*, 2009). There are about 250,000–500,000 existing plant species and only a tiny proportion has been scientifically researched for bioactivities (Ngo *et al.*, 2013). Another estimate insists there are about 2.5 million species of higher plants and the majority of these have not yet been examined for their pharmacological activities (Cox *et al.*, 2010). Therefore, there is great potential for fresh discoveries from *virgin* plants, to provide answers to today’s unanswered medical questions. Phytochemicals from these plants could offer huge potential in deriving useful information about chemical structures and new types of action related to new drug development.

Virgin plants are plants harvested and used traditionally to treat diseases without their pharmacological effects being studied and without enough information about dosage, effects and risk factors. A lot of virgin plants have been used successfully to treat illnesses. Only a tiny fraction of the existing plant species have been scientifically researched for bioactivities since 1805, others are being used traditionally while the rest are yet to be discovered (Yuan *et al.*, 2016). Many of the medicinal plants whose pharmacological activities provided answers to medicinal questions at various times in the history of disease control,

drug resistance and response, from analgesic *codeine*, antimalarial *quinine*, antihypertensive *reserpine* to anticancer *taxol*, were plants that were at one time considered ‘virgin’. Remedy for development of drug resistance by microbial strains and solution to other subsisting medical challenges worldwide will likely reside in the ‘bosom’ of virgin plants, whose ethno-medicinal potentials may not be known (Pooja & Tannaz, 2017). Virgin plants are most likely the reservoir for antimicrobials against today’s incurable diseases and drug resistant strains.

Beloperone asclepiadea Nees (Acanthaceae) is a virgin plant that is widespread in the tropical and sub-tropical regions. As far as I know its medicinal potentials have not been investigated despite great medicinal value of plants in the same genera. Phytochemical, antibacterial and antifungal analysis will be done on extracts of the whole plant from four solvents of different polarities. The medicinal potency of the different plant will be assessed by comparing the sensitivity zone of microbial inhibition, of different solvent extracts with those of standard antimicrobial drugs. Further medicinal value will be estimated from the minimum inhibitory concentrations and the minimum bactericidal/fungicidal concentrations of each extract against sensitivity strains. Information from these assays may be the key to providing answers to the contemporary medical question. Positive results will hasten the isolation of implicated bioactive phyto-constituents and may ultimately lead to new drug discovery and formulation.

2. MATERIALS AND METHODS

2.1 Collection and identification of plant materials

Beloperone asclepiadea was harvested in July 2019 from the local forests of Ekeh Ayeje area in Edumoga District, Okpokwu Local Government Area of Benue State Nigeria. The plant material was identified by Mr. Namadi Sunusi of the Herbarium of the Department of Biological Sciences, Faculty of Life Sciences, Ahmadu Bello University, Zaria.

2.2 Preparation and extraction of plant material

The plant material (2.5kg) was shade-dried and pulverized, after which it was separated into two batches. The first batch was distributed into glass bottles, covered with n-hexane, tightly closed and subjected to Microwave-Assisted Extraction (four rounds of three minutes each, after leaving to cool) with the microwave set at defrost. Each jar was then exhaustively ‘washed’ in sequence with four selective solvents in order of increasing polarity (n-hexane, dichloromethane, ethyl acetate and methanol). The combined extracts for each solvent were concentrated and left to dry to constant mass.

The second batch was distributed into glass bottles covered with n-hexane and subjected to cold maceration for 72 hours with frequent agitation. The extracted materials were then washed exhaustively with n-hexane. The combined extracts were concentrated and left to dry to constant weight. The same procedure was repeated in

sequence using dichloromethane, ethyl acetate and methanol after drying the marc from the preceding solvent.

2.3 Qualitative phytochemical screening

The extract from each solvent was subjected to phytochemical screening for the presence glycosides, tannins, flavonoids, saponins, cardiac glycosides, anthraquinones, alkaloids, steroids and triterpenoids using the standard procedures (Ciulei, 1982; Banu & Cathrine, 2015).

2.4 Culture media and microbes

Antimicrobial screening was done to check the activity against the following microorganisms: (bacteria) *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, *streptococcus pyogenes*; (Fungi) *Candida albicans* and *Aspergillus Niger*. Growth media used were prepared according to manufacturer's instructions and include: Nutrient agar (Acoumix™), Nutrient broth (HKM), Sabourand Dextrose agar (Titan Biotech), Mueller Hinton agar (Titan Biotech), Sabourand Liquid Medium and Normal saline. They were sourced from the Department of Pharmaceutical Microbiology Ahmadu Bello University, Zaria, Kaduna State, Nigeria. Ciprofloxacin (anti-bacterial) and Terbinafin (anti-fungi) were used respectively as positive antibacterial and antifungal controls.

2.5 Sensitivity test for antimicrobial activity by zone of inhibition (ZOI)

The Agar Well Diffusion method (Singleton, 1981, Ghatage *et.al*, 2014) was adopted. 20ml each of sterile Mueller Hinton Agar prepared according to manufacturer's instruction were poured into sterile Petri dishes and allowed to harden. 2ml of each culture standardized with 0.9% normal salines (McFarland turbidity standard) were swept over each dish and excess discarded. Equidistant wells of about 8mm diameter were bored into the seeded agar using flamed cork borer and labeled. Each well was filled with 100µL of the appropriate extract at 100mg/mL, lower concentrations (50,25,12.5mg/mL) and standard drugs at 50µg/mL. The plates were loaded in duplicate and left for one hour to allow for proper diffusion before

incubating upright in the dark. After incubating for 24 hours (at 37°C) for bacteria and 72 hours (at 30°C) for fungi they were observed for growth and the diameters of any resulting zones of inhibition formed around wells measured in millimeter scale using a transparent ruler.

2.6 Minimum inhibitory concentration (MIC)

The Broth Dilution methods (Cleudson *et.al*, 2007) were used. The following concentrations: 100, 50, 25, 12.5, 6.25, 3.125, 1.5625, 0.78125, 0.390625 and 0.1953 mg mL⁻¹ of plant material were prepared by two-fold serial dilution in Muller Hinton agar (for bacteria) and Sabourand Dextrose agar (for fungi) petri dishes. Sterile micro filter paper discs for each of the six organisms were placed on the solidified agar and 10µl of each standardized culture was then added and left for an hour to diffuse. The loaded dishes were incubated in the dark at 37°C for 24 hours (bacteria) and 30°C for 72 hours (for fungi). They were then observed for visible growth. The lowest concentration at which no detectable bacterial or fungal growth occurred was taken as Minimum Inhibitory Concentration (MIC).

2.7 Minimum bactericidal/fungicidal concentration (MBC/MFC)

The minimum bactericidal/fungicidal concentration of the extracts was determined from the tubes that showed no visible growth in the MIC determination. The micro filter paper discs bearing the organisms were lifted and seeded on fresh 5ml sterile Nutrient Broth (bacteria) and Sabourand Liquid medium (fungi) in labeled bottles. The incubation cycles were repeated. The lowest concentration of the extracts that showed no visible colony growth (turbidity) on the medium was regarded as minimum bactericidal/fungicidal concentration.

3. RESULTS AND DISCUSSION

3.1 Comparative Solvent Extractive Yields

The results from extraction yields of the four solvents used for the two extraction methods is shown in Table 1. The results is presented in the form of percent extractive yield of each solvent from the two methods

Table 3.1: Comparative Extractive Yield of *Beloperone asclepiadea*

SOLVENT	PERCENT (%) EXTRACTIVE YIELD	
	MICROWAVE ASSISTED EXTRACTION	COLD MACERATION
N-hexane	0.64	1.0
Dichloromethane	1.15	3.08
Ethyl acetate	0.74	0.97
Methanol	2.43	3.94

3.2 Phytochemical Screening of Extracts

The results of different phytochemical tests carried on the extracts of different solvents for the presence of steroids,

triterpenes, tannins, flavonoids, saponins, cardiac glycosides, anthraquinones and alkaloids is presented in Table 2.

Table 3.2: Phytochemical Screening of *Beloperone Asclepiadea* Extracts

	PHYTOCHEMICALS	TESTS	N-HEXANE EXTRACT	DICHLORO-METHANE EXTRACT	ETHYL ACETATE EXTRACT	METHANOL EXTRACT
1	STERIODS AND TRITERPENOIDS	a. Salkowski's Test b. Liebermann Buchard Test	+ +	+ +	+ +	+ +
2	TANNINS	a. Ferric Chloride Test b. Bromine Water Test c. Lead Sub-acetate Test	- - -	+ + +	+ + +	+ + +
3	FLAVONOIDS	a. Shinoda Test b. Sodium Hydroxide Test	- -	+ +	- -	+ +
4	SAPONINS	a. Frothing Test b. Haemolysis Test	- -	- -	+ +	+ +
5	CARDIAC GLYCOSIDES	a. Keller-Killiani Test b. Kedde's Test	+ +	+ +	+ +	+ +
6	ANTHRAQUINONES	a. Bontragers b. Modified Bontragers	- -	- -	- -	- -
7	ALKALOIDS	a. Dragendoff's Test b. Wagner's Test c. Meyer's Test	- - -	+ + +	+ + +	+ + +

Key: + = positive (detected), - = negative (detected)

3.3 Sensitivity test for antimicrobial activity

Table 3 shows the results of sensitivity tests by zones of inhibition (ZOI) of the four different extracts and positive standards against the selected bacterial strains and fungal strains. ZOI (in millimeter) is presented in mean values from duplicate petri dishes plus or minus Standard Deviation.

Table 3.3: Zones of inhibition (mm) of extracts of *B. asclepiadea* against test microorganisms

EXTRACT	ORGANISM	100 (mg/mL)	50 (mg/mL)	25 (mg/mL)	12.5 (mg/mL)	CIP 50µg/mL	TBF 50µg/mL
N-hexane	<i>Staphylococcus aureus</i>	20.5 ±0.5	15.0 ±0.0	13.0 ±0.0	11.0 ±0.0	30	
	<i>Escherichia coli</i>	30.0 ±0.0	24.5 ±0.5	20.0 ±0.0	15.0 ±0.0	40	
	<i>Salmonella typhi</i>	18.0 ±0.0	15.0 ±0.0	12.0 ±0.0	0.0 ±0.0	32	
	<i>Bacillus subtilis</i>	20.0 ±0.0	16.5 ±0.5	13.5 ±0.5	11.5 ±0.5	34	
	<i>Streptococcus pyogenes</i>	20.5 ±0.5	17.0 ±0.0	14.0 ±0.0	11.0 ±0.0	30	
	<i>Candida albicans</i>	16.5 ±0.5	14.0 ±0.0	11.5 ±0.5	0.0 ±0.0	-	30
	<i>Aspergillus niger</i>	12.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	-	30
Dichloromethane	<i>Staphylococcus aureus</i>	15.5 ±0.5	13.0 ±0.0	11.0 ±0.0	0.0 ±0.0	30	
	<i>Escherichia coli</i>	15.0 ±0.0	14.0 ±0.0	0.0 ±0.0	0.0 ±0.0	40	
	<i>Salmonella typhi</i>	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	32	
	<i>Bacillus subtilis</i>	12.5 ±0.5	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	34	
	<i>Streptococcus pyogenes</i>	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	30	
	<i>Candida albicans</i>	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	-	30
	<i>Aspergillus niger</i>	12.5 ±0.5	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	-	30
Ethyl acetate	<i>Staphylococcus aureus</i>	20.0 ±0.0	18.0 ±0.0	15.0 ±0.0	13.0 ±0.0	30	
	<i>Escherichia coli</i>	27.5 ±0.5	24.0 ±0.0	18.0 ±0.0	14.0 ±0.0	40	
	<i>Salmonella typhi</i>	30.0 ±0.0	26.0 ±0.0	20.0 ±0.0	17.0 ±0.0	32	
	<i>Bacillus subtilis</i>	16.0 ±0.0	14.0 ±0.0	12.0 ±0.0	0.0 ±0.0	34	
	<i>Streptococcus pyogenes</i>	16.0 ±0.0	14.0 ±0.0	11.5 ±0.5	0.0 ±0.0	30	
	<i>Candida albicans</i>	18.0 ±0.0	16.0 ±0.0	13.0 ±0.0	11.5 ±0.5	-	30
	<i>Aspergillus niger</i>	12.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	-	30
Methanol	<i>Staphylococcus aureus</i>	21.0 ±0.0	17.5 ±0.5	14.0 ±0.0	11.5 ±0.5	30	
	<i>Escherichia coli</i>	30.0 ±0.0	25.0 ±0.0	18.0 ±0.0	15.5 ±0.5	40	
	<i>Salmonella typhi</i>	25.0 ±0.0	21.0 ±0.0	18.0 ±0.0	15.0 ±0.0	32	
	<i>Bacillus subtilis</i>	24.0 ±0.0	20.0 ±0.0	15.5 ±0.5	0.0 ±0.0	34	
	<i>Streptococcus pyogenes</i>	19.5 ±0.5	17.0 ±0.0	14.0 ±0.0	12.0 ±0.0	30	
	<i>Candida albicans</i>	13.5 ±0.5	11.5 ±0.5	0.0 ±0.0	0.0 ±0.0	-	30
	<i>Aspergillus niger</i>	15.0 ±0.0	12.5 ±0.5	11.0 ±0.0	0.0 ±0.0	-	30

Key: CIP=Ciprofloxacin (anti-bacterial); TBF = Terbinafin (anti-fungal)

3.4 Minimum inhibitory concentration (MIC) of extracts

The results of Minimum Inhibitory Concentration (MIC) assays is presented in Table 4.4. The MIC's or the lowest concentrations of the extracts that showed antimicrobial activity against the organisms have been highlighted.

Table 3.4: Minimum Inhibitory Concentration of *B. asclepiadea* Extracts

EXTRACT	ORGANISM	100 Mg/mL	50 Mg/mL	25 Mg/mL	12.5 Mg/mL	6.25 Mg/mL	3.125 Mg/mL	1.5625 Mg/mL	0.78125 Mg/mL	0.3906 Mg/mL	0.1953 Mg/mL
N-hexane	<i>Staphylococcus aureus</i>	-	-	-	-	-	-	-ϕ	+	+	+
	<i>Escherichia coli</i>	-	-	-	-	-ϕ	+	+	+	+	+
	<i>Salmonella typhi</i>	-	-	-	-ϕ	+	+	+	+	+	+
	<i>Bacillus subtilis</i>	-	-	-	-	-ϕ	+	+	+	+	+
	<i>Streptococcus pyogenes</i>	-	-	-	-	-ϕ	+	+	+	+	+
	<i>Candida albicans</i>	-	-	-ϕ	+	+	+	+	+	+	+
	<i>Aspergillus Niger</i>	-	-ϕ	+	+	+	+	+	+	+	+
Dichloromethane	<i>Staphylococcus aureus</i>	-	-	-ϕ	+	+	+	+	+	+	+
	<i>Escherichia coli</i>	-	-	-ϕ	+	+	+	+	+	+	+
	<i>Salmonella typhi</i>	-ϕ	+	+	+	+	+	+	+	+	+
	<i>Bacillus subtilis</i>	-ϕ	+	+	+	+	+	+	+	+	+
	<i>Streptococcus pyogenes</i>	+	+	+	+	+	+	+	+	+	+
	<i>Candida albicans</i>	+	+	+	+	+	+	+	+	+	+
	<i>Aspergillus Niger</i>	-ϕ	+	+	+	+	+	+	+	+	+
Ethyl acetate	<i>Staphylococcus aureus</i>	-	-	-	-ϕ	+	+	+	+	+	+
	<i>Escherichia coli</i>	-	-	-	-	-	-ϕ	+	+	+	+
	<i>Salmonella typhi</i>	-	-	-	-	-ϕ	+	+	+	+	+
	<i>Bacillus subtilis</i>	-	-	-ϕ	+	+	+	+	+	+	+
	<i>Streptococcus pyogenes</i>	-	-	-ϕ	+	+	+	+	+	+	+
	<i>Candida albicans</i>	-	-	-	-ϕ	+	+	+	+	+	+
	<i>Aspergillus Niger</i>	-ϕ	+	+	+	+	+	+	+	+	+
Methanol	<i>Staphylococcus aureus</i>	-	-	-	-ϕ	+	+	+	+	+	+
	<i>Escherichia coli</i>	-	-	-	-	-	-ϕ	+	+	+	+
	<i>Salmonella typhi</i>	-	-	-	-ϕ	+	+	+	+	+	+
	<i>Bacillus subtilis</i>	-	-	-	-ϕ	+	+	+	+	+	+
	<i>Streptococcus pyogenes</i>	-	-	-ϕ	+	+	+	+	+	+	+
	<i>Candida albicans</i>	-ϕ	+	+	+	+	+	+	+	+	+
	<i>Aspergillus Niger</i>	-	-ϕ	+	+	+	+	+	+	+	+

Key: + = Growth, - = No growth, -ϕ = minimum inhibitory concentration

3.5 Minimum Bactericidal/Fungicidal Concentration (MB/MFC) of Extracts

The results for minimum bactericidal/fungicidal concentration are shown below (Table 5) with the lowest concentration that killed organism (MBC/MFC) for the extracts against various microorganisms highlighted.

Table 3.5: Minimum Bactericidal/Fungicidal Concentration *B. asclepiadea* Extract

EXTRACT	ORGANISM	100 Mg/mL	50 Mg/mL	25 Mg/mL	12.5 Mg/mL	6.25 Mg/mL	3.125 Mg/mL	1.5625 Mg/mL	0.78125 Mg/mL	0.3906 Mg/mL	0.1953 Mg/mL
N-hexane	<i>Staphylococcus aureus</i>	-	-	-ϕ	+	+	+	+	+	+	+
	<i>Escherichia coli</i>	-	--ϕ	+	+	+	+	+	+	+	+
	<i>Salmonella typhi</i>	-	--ϕ	+	+	+	+	+	+	+	+
	<i>Bacillus subtilis</i>	-	--ϕ	+	+	+	+	+	+	+	+
	<i>Streptococcus pyogenes</i>	-	--ϕ	+	+	+	+	+	+	+	+
	<i>Candida albicans</i>	--ϕ	+	+	+	+	+	+	+	+	+
	<i>Aspergillus Niger</i>	+	+	+	+	+	+	+	+	+	+
Dichloromethane	<i>Staphylococcus aureus</i>	--ϕ	+	+	+	+	+	+	+	+	+
	<i>Escherichia coli</i>	--ϕ	+	+	+	+	+	+	+	+	+
	<i>Salmonella typhi</i>	+	+	+	+	+	+	+	+	+	+
	<i>Bacillus subtilis</i>	+	+	+	+	+	+	+	+	+	+
	<i>Streptococcus pyogenes</i>	+	+	+	+	+	+	+	+	+	+
	<i>Candida albicans</i>	+	+	+	+	+	+	+	+	+	+

	<i>Aspergillus Niger</i>	+	+	+	+	+	+	+	+	+	+
Ethyl acetate	<i>Staphylococcus aureus</i>	-	--C	+	+	+	+	+	+	+	+
	<i>Escherichia coli</i>	-	-	--C	+	+	+	+	+	+	+
	<i>Salmonella typhi</i>	-	--C	+	+	+	+	+	+	+	+
	<i>Bacillus subtilis</i>	--C	+	+	+	+	+	+	+	+	+
	<i>Streptococcus pyogenes</i>	--C	+	+	+	+	+	+	+	+	+
	<i>Candida albicans</i>	-	--C	+	+	+	+	+	+	+	+
	<i>Aspergillus Niger</i>	+	+	+	+	+	+	+	+	+	+
Methanol	<i>Staphylococcus aureus</i>	-	--C	+	+	+	+	+	+	+	+
	<i>Escherichia coli</i>	-	-	--C	+	+	+	+	+	+	+
	<i>Salmonella typhi</i>	-	--C	+	+	+	+	+	+	+	+
	<i>Bacillus subtilis</i>	-	--C	+	+	+	+	+	+	+	+
	<i>Streptococcus pyogenes</i>	--C	+	+	+	+	+	+	+	+	+
	<i>Candida albicans</i>	+	+	+	+	+	+	+	+	+	+
	<i>Aspergillus Niger</i>	+	+	+	+	+	+	+	+	+	+

Key: + = Growth, - = No growth, -C = minimum bactericidal/fungicidal concentration

4. DISCUSSIONS OF RESULTS

4.1 Comparative Extraction

The results in Table 3.1 shows that cold maceration method, with higher recovery yields, provided better extraction efficiency for *Beloperone asclepiadea* compared to Microwave Assisted Extraction. Extraction efficiency is dependent on factors such as temperature, surface area of the material, extraction time, solvent polarity, etc. However, almost all the parameters were similar for both methods. Cold maceration is a conventional process done at room temperature whose one advantage over the hot process is its less likelihood to cause degradation of thermolabile compounds present in the plant. It may be suggested that the plant material is laden with thermolabile constituents that may not have been tolerated by the hot MAE process. Sankeshwari *et al.* (2018) also obtained a higher yield of extracts from CM results from the extraction from the roots of Licorice (*Glycyrrhiza glabra*) plant. The four solvents have different extractive yields depending mainly on the polarity, with methanol having the highest percentage yield. This is either because there may be plenteous highly polar (as against highly non-polar) phytochemicals in the plant that the earlier solvents could not remove or because methanol can extract both the polar and some of the non-polar compounds. (Dixon & Jeena, 2017). The former reason is supported by the lowest yield recorded by n-hexane in both methods after exhaustive extraction. The greater extractive yield of dichloromethane than ethyl acetate and n-hexane may mean that moderately non-polar constituents are present in higher amounts than moderately polar and strongly non-polar constituents (Barchan *et al.*, 2014). It is perhaps worth pointing out that generally speaking extraction yields are low for both two methods with methanol having 2.43% & 3.94 % (for MAE and CM respectively), ethyl acetate, 0.74% & 0.97%, dichloromethane, 1.15% & 3.08% and hexane with as low as 0.64% & 1.0% (for MAE and CM respectively). Ibrahim *et al.* (2017) had obtained similarly very low yields for extraction from roots and stem bark of *Ceiba pentandra* which gave percentages such as 5.0%, 1.87% and 0.80% for methanol, ethyl acetate and n-hexane respectively. Extremely low yield of crude extracts is,

however, consistent with the fact that phyto-constituents in nature usually occur in very low yields far below the demand for bioactive compounds from them used in modern medicine (Ivor, 2000; Beutler, 2009). This is the factor that promotes their artificial synthesis after isolation and characterization.

4.2 Phytochemical Screening

4.2.2 Phytochemical Screening

Table 3.2 indicates the presence in *B. asclepiadea* of cardiac glycosides, saponins, steroids/triterpenes, flavonoids, alkaloids and tannins while anthraquinones were absent. The methanol extract tested positive for all the classes of phytochemicals found in the plant. The ethyl acetate extracts tested negative for anthraquinones and flavonoids while the n-hexane extract only indicated the presence of cardiac glycosides and steroid/triterpenes. The result appears to be in consonance with phytochemical results observed in some plant species of the Acanthaceae family from which active compounds have been isolated (Renjini Haridas *et al.*, 2017; Sultana & Das, 2019 and Sathiyabalan *et al.*, 2018). The spread of positive phytochemical results across solvents of different polarities may imply the presence of both polar and non-polar active compounds. However there is more tilting towards highly polar and moderately polar bioactive compounds. A number of vital secondary metabolites with polar groups, especially flavonoids and poly phenolic compounds with their high hydroxyl content may be involved. They are precursors for drug synthesis due to their peculiar activities as good scavengers of free radicals group in them (Kumar and Pandey, 2013). The same goes with saponins which are known to possess both antimicrobial and anti-inflammatory activities. The positive test for alkaloids and steroids/triterpenoids shows likely concentrations of these classes of phytochemicals known for their physiological, pharmacological and hormonal activities (Lu *et al.*, 2012; Einbond, 2009). *B. plumbaginifolia*, a related plant, had some isolated compounds with reported antioxidant, anti-inflammatory and antinociceptive properties (Rajasekaran *et al.*, 2012).

4.3 Antimicrobial Activity

From Table 3.3 almost all the extracts showed some antibacterial activity at a concentration of 100mg/mL except that the dichloromethane extracts showed no antifungal activity against *Candida albicans* and no antibacterial activity against *Streptococcus pyogenes* and *Salmonella typhi*. It is also the only extract that showed no activity against any of the microorganisms at a concentration of 12.5 mg/mL. The other extracts had very interesting antimicrobial ZOI's (at 100 mg/mL and at least up to 12.5 mg/mL) against *S. typhi*, *E. coli*, *B. subtilis*, *S. aureus* and *S. pyogenes* compared to the standard drugs at 50µg/mL. Inhibition against *S. typhi* has one of the highest values, 30±0.0 mm (ethyl acetate) and 25.5±0.0 mm (methanol) compared to 32.0 mm for ciprofloxacin. Inhibition against *E. coli* is equally high and even more

widespread, with 30±0.0 mm (methanol and n-hexane), 27.5±0.5 mm (ethyl acetate) and 15.0±0.0 mm (dichloromethane) compared to 40.00 mm for ciprofloxacin. *B. subtilis* had ZOI's 24.0±0.0 mm (methanol), 20.0±0.0 mm (n-hexane), 16.0±0.0 mm (ethyl acetate) and 12.5±0.5 mm (dichloromethane) compared to 34.0 mm for ciprofloxacin. *S. aureus* had ZOI's of 21.0±0.0 mm (methanol), 20.5±0.5 mm (n-hexane), 20.0±0.0 mm (ethyl acetate) and 15.0±0.0 mm (dichloromethane) as against 30.00 mm by ciprofloxacin while ZOI values for *S. pyogenes* are 20.5±0.5 mm, 19.5±0.5 mm and 16.0±0.0 mm (ethyl acetate) as against 30.00 mm activity by the standard drug.

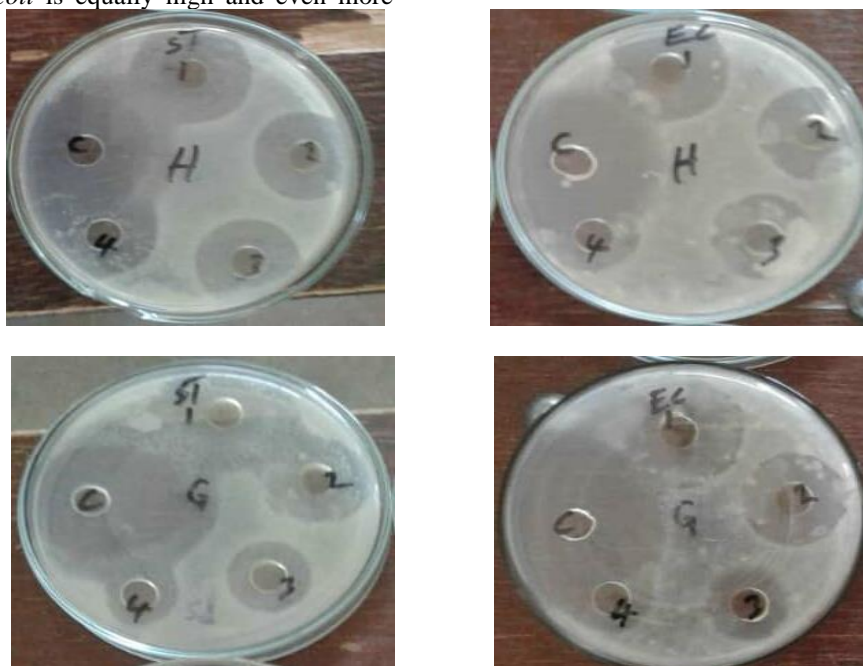


Fig 4.1 Prominent zones of inhibition of *B. asclepiadea* extracts: Ethyl acetate (H) against *S.typhi* (ST) and *E.coli* (EC); Methanol extracts (G) against *S.typhi* (ST) and *E. coli*(EC). 1,2,3 and 4 are concentrations 100, 50, 25 and 12.5 mg mL⁻¹ respectively of plant extract, while 5 is the standard drug.

The strongest antifungal activity against *C. albicans* was observed for ethyl acetate extract (18.0±0.0 mm) followed by n-hexane (16.5±0.5 mm) and methanol extract ((13.5±0.5 mm) whereas against *A. niger* it is (12.5±0.5 mm) for n-hexane and (12.0±0.0 mm) for both ethyl acetate and methanol extracts. The antifungal ZOI for the standard drug, *terbinafin* is 30.0mm at 50 µg/mL.

From the foregoing the presence in the plant extracts of useful phytochemicals with strong activity against *S. typhi*, *E. coli*, *B. subtilis*, *S. aureus* and *S. pyogenes* and *C. albicans* among others is no more a matter of pontification. *Salmonella typhi* is gram negative and a well known microorganism that causes typhoid fever. *Escherichia coli* is gram negative and known to cause urinary tract infection, cholangitis and food poisoning. *Bacillus subtilis* is gram positive and implicated in meningitis, ear and urinary tract infections. *Staphylococcus aureus* is gram positive and responsible for meningitis, osteomyelitis, food poisoning and pneumonias while *Streptococcus pyogenes* is the causative agent for tonsillitis, scarlet fever and

pharyngitis. The fungus *Candida albicans* is known to be one of the causative agents for candidiasis, skin infections, urinary infections and genital yeast infections while *Aspergillus niger* causes aspergillosis and pneumonia (Paul *et al.*, 2010; Otang & Afolayan, 2016). It seems safe to conclude that the plant extracts harbor phytochemicals having strong potency against most or all the listed diseases. Of particular significance from the result in Table 3.4 is the relatively very low MIC's of 1.5625 mg/mL observed for activity against *S. aureus* in the N-hexane extract and 3.125 mg/mL observed for activity against *E. coli* in the ethyl acetate and methanol extracts. The same argument may be stretched to *S. typhi*, *B. subtilis* and *S. pyogenes* with MIC's of 6.25 mg/mL in the ethyl acetate and n-hexane extracts. There may also be great optimism against fungal diseases with the lowest antifungal MIC of 12.5 mg/mL recorded against *Candida albicans* by the ethyl acetate extracts and 50 mg/mL against *A.niger* by the methanol and n-hexane extracts. These depositions are further strengthened by the MBC/MFC results in Table 3.5

which shows the minimum concentrations of the extracts that will cause death of the microbial strains. For instance the lowest MBC values of 25 mg/mL were observed for *S. aureus* (n-hexane extract) and *E. coli* (methanol and ethyl acetate extracts) while 50 mg/mL were recorded for others like *S. typhi*, *B. subtilis*, *S. pyogenes* and *C. albicans* by various solvent extracts.

It is now almost certain from the foregoing that the, at least, six microbial strains that are most likely critical to medicinal potential of *B. asclepiadea* include *S. aureus*, *E. coli*, *S. typhi*, *B. subtilis*, *S. pyogenes* and *C. albicans* in that order. Medicinal potency and pharmacological utility of the plant can be subsequently narrowed down to possible activity against bacterial infections as meningitis, sepsis, osteomyelitis (all linked to *S. aureus*), urinary tract infection, cholagitis, cholecystitis (all linked to *E. coli*), typhoid fever (*S. typhi*), food poisoning, meningitis (*B. subtilis*), tonsillitis, scarlet, fever, pharyngitis (*S. pyogenes*), and fungal infection: candidiasis, skin infections, urinary and genital yeast infections (all linked to *C. albicans*).

Plant species in the Acanthaceae family already had demonstrated substantial antimicrobial activity against several microbes (Asifjavid *et al.*, 2014; Sultana. & Das, 2019). Also plants from the family tend to follow a trend in medicinal applications (Sharma & Kumar, 2016) Many have been reported to cure diseases like pneumonia, urinary tract diseases, meningitis, skin infections, fever, food poisoning, gastrointestinal diseases and some also possess anticancer, anti-diabetic and anti inflammatory properties (Zabta *et al.*, 2017; Rahman *et al.*, 2014).

Furthermore, increased drug resistance has been linked to some of the microbes to which our plant of interest showed substantial potency thus raising new hopes of fresh therapeutic options. For instance, resistance to fluoroquinolone antibiotics which is one of the most widely used drugs for treatment of urinary tract infections (*E. coli*), resistance to first line drugs used to treat sepsis and meningitis (*S. aureus*), resistance to fluconazole a common drug for the treatment of thrush (*C. albicans*) and a rare resistance to amphotericin B and other drugs used to treat Aspergillosis (*A. niger*) were reported in 2016. Also ciprofloxacin is the most common antibiotic used to treat typhoid fever (*S. typhi*) to which cases of microbial resistance have been identified (WHO, 2018; Berkow & Lockhart, 2017). There is again the nagging issue of diseases without cure caused by some of the microorganisms like arthritis which is caused by *Staphylococcus aureus* (Corrado. *et al.*, 2016). The plant *B. asclepiadea* having showed high activity against some of the microbes implicated in those unanswered medical questions may provide a lead to finding a new way around these subsisting medicinal problems. The plant obviously has to be investigated further not only for activity against more bacteria and fungi but more importantly for isolation and characterization of bioactive compounds.

5. CONCLUSIONS AND RECOMMENDATIONS

Cold Maceration Extraction of *B. Asclepiadea* proved to be the better method for the extraction of the plant than

microwave assisted extraction, suggesting that the plant material is laden with thermolabile constituents that may not have been tolerated by the hot MAE process. The comparatively high extractive yield of methanol and dichloromethane compared to n-hexane and ethyl acetate may mean preponderance of highly polar and moderately non polar phyto-constituents as against non-polar and moderately nonpolar ones. This is further confirmed by the positive phytochemical results for cardiac glycosides, saponins, steroids/triterpenes, flavonoids, alkaloids and tannins and their spread across solvents of polarities that favour highly polar and moderately polar bioactive compounds.

The antimicrobial results showed strong activity against both gram positive and gram negative bacteria *S. typhi*, *E. coli*, *B. subtilis*, *S. aureus*, *S. pyogenes* and fungus *C. albicans* with zones of inhibition with competitive values to those of the standard drugs. The relatively very low MIC's of 1.5625 mg/mL observed for activity against *S. aureus*, 3.125 mg/mL for activity against *E. coli* and 6.25 mg/mL for activity against *S. typhi*, *B. subtilis* and *S. pyogenes* almost conclusively prove potency against corresponding bacterial infections as (*S. aureus*) meningitis, sepsis, osteomyelitis, (*E. coli*) urinary tract infection, cholagitis, cholecystitis, (*S. typhi*), typhoid fever, (*B. subtilis*) food poisoning, meningitis, (*S. pyogenes*) tonsillitis, scarlet fever and pharyngitis. There is also great optimism against candidiasis, skin infections, urinary and genital yeast infections with the lowest antifungal MIC of 12.5 mg/mL recorded against *Candida albicans*.

Medicinal potency for the plant may stretch beyond antibacterial and antifungal to cover anti-inflammatory, antioxidant and other activities. Related plants have been reported both to possess antioxidant and anti-inflammatory activities (Sathiyabalan *et al.*, 2018; Shafique & Qureshi, 2015) and to show high activity against selected microbes that appear to follow a trend (Sharma & Kumar, 2016; Asifjavid *et al.*, 2014). Finally high potency of the plant extracts against *S. aureus*, *E. coli*, *S. typhi*, *B. subtilis*, *S. pyogenes* and *C. albicans* raises hope of a fresh inroads around drug resistance to diseases linked to the microbes, and to others with no known cure. The plant is currently being further investigated for activity against more microbes and for isolation and characterization of implicated bioactive compounds.

Recommended areas of further research include *in vivo* pharmacological activity tests and medicinal risk assessment.

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