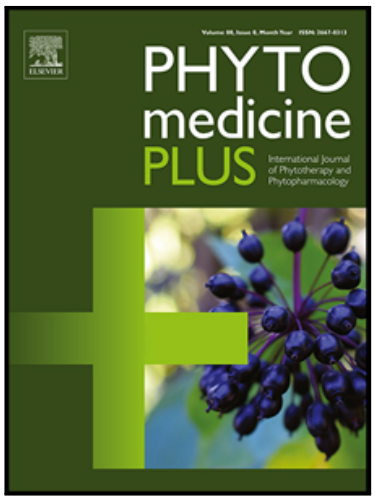
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Evaluation of multifunctional activity of bioactive peptide fractions from the leaves of Nauclea diderrichii (De Wild. and T. Durand) Merrill and Ixora brachypoda DC

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Highlights

- The presence of peptides was confirmed in two plants, Nauclea *diderichii* and *Ixora brachypoda DC* from family Rubiaceae
- Crude and pre-purified peptides were obtained from both plants using solid-phase extraction
- Peptide fractions of both plants displayed good biological activity in different assay methods
- The wide range of activity displayed by the peptide fractions offers a perspective on their potential application in drug development.

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Evaluation of multifunctional activity of bioactive peptide fractions from the leaves of *Nauclea diderrichii* (De Wild. and T. Durand) Merrill and *Ixora brachypoda* DC

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Abstract

Bioactive peptides are known for health-promoting properties due to several pharmacological effects they modulate in physiological systems. Herein, the presence of peptides particularly cyclic peptides, was investigated in two Rubiaceae members; *Nauclea diderichii* (De Wild. and T. Durand) Merrill and *Ixora brachypoda* DC by thin layer chromatography (TLC) chemical detection, and their multifunctional bioactivity evaluated. Crude and pre-purified peptides were obtained from both plants using aqueous and solid-phase extractions, respectively. The peptide fraction of *N. diderrichii* showed good DPPH scavenging activity (IC_{50} = 3.43 µg/mL) compared to the standard Gallic acid (IC_{50} = 1.44 µg/mL). Peptide fractions of both plants displayed inhibitory effects (MIC = 6.25 µg/mL) against bacteria and fungi strains except *Escherichia coli*, relative to standard drugs (MIC = 0.31 µg/mL). Both displayed significant toxicity to brine shrimp nauplii ($LC_{50} \leq 220.8$ µgmL^{-1}) as well as MCF-7

and RD cancer cells ($IC_{50} \leq 34.0$ μgmL^{-1}). The enterovirus species B and C tested were remarkably susceptible to the peptide fractions ($IC_{50} \leq 3.20$ μg/mL).

Keywords: Cyclic peptides, Rubiaceae, Antimicrobial, Antiviral, Antioxidant, Cytotoxicity

Abbreviations

FHI- Forestry Herbarium Ibadan; AMP- antimicrobial peptides; HDP- host defence peptides; DMSO- dimethyl sulfoxide; ATCC- American type culture collection; UCH- University College Hospital, Ibadan; *p*-INT- *p*-iodonitrotetrazollium violet; vCPE- virus-induced cytopathic effect; MNTC- maximum nontoxic concentration; CV- Coxsackievirus; WHO- World Health Organization; MTT- 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium salt; DPPH- 2,2-Diphenyl-1-picrylhydrazyl.

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Introduction

Bioactive peptides are present ubiquitously in nature particularly, multicellular organisms (Wang, et al., 2015). Plant-derived bioactive peptides, also known as antimicrobial peptides (AMP) or host defence peptides (HDP) are endogenous molecules that form part of plant innate immunity against infections and pests (Koehbach and Craik, 2019). Due to their broad-range bioactivities, AMP are being explored for the discovery and development of therapeutic agents for various human disease conditions (Kosikowska and Lesner, 2016; Yili, et al., 2014). Generally but inadequately, peptides are faced with limitation of poor pharmacokinetic properties which threaten their viability as chemotherapeutic agents (Morrison, 2018). Nevertheless, circular peptides provides better pharmaceutical scaffold due to their conformational rigidity, and resistance to thermal, chemical, and enzymatic degradation (Attah, et al., 2016; Gang, et al., 2018; Joo, 2012). Some plant families, including Rubiaceae, have been reported to contain circular peptides and especially, a class of cysteine-rich circular peptides of plant origin known as cyclotides (Hellinger, et al., 2015; Koehbach, et al., 2013). *Nauclea diderrichii* (De Wild. and T.Durand) Merrill and *Ixora brachypoda* DC belong to the coffee family, Rubiaceae.

Ixora brachypoda grows as a small bushy tree or shrub with height about 3-4 feet. It is an ornamental plant native to the Tropical Africa, including Nigeria. In Ethnobotany, the root is used as an analgesic as well as a vermifuge along with the bark; it is also used in some parts of Nigeria to treat diabetes (Ezeigbo and Asuzu, 2010). *Nauclea diderrichii* known as *Opepe* in Yoruba language of Nigeria is an evergreen tropical tree which can be up to 160 feet in height with cylindrical boles which remain clear for about 80 to 100 feet (Agnaniet, et al., 2016). In West Africa, its wood is valuable for construction due to its high resistance to fungi and insects especially, termites (Ogbole, et al., 2016).

The health-promoting properties of plant-derived bioactive peptides, as pharmaceuticals or nutraceuticals, have been associated with their several pharmacological activities evaluated by various *in vitro* and *in vivo* studies (Maestri, et al., 2016). Herein, the presence of circular peptides in the leaves of *Nauclea diderrichii* and *Ixora brachypoda* was determined and the cytotoxic, antimicrobial, antiviral, and antioxidant activities of the peptide extracts were evaluated.

Material and Methods

Preparation of Extracts

Fresh leaves of *Ixora brachypoda* and *Nauclea diderrichii* were collected from the University of Ibadan environs. The plants were identified and authenticated by Mr. Samuel Odewo of Forestry Research Institute of Nigeria, Ibadan. Herbarium specimen for *I. brachypoda* and *N. diderrichii* were deposited at the Forestry Herbarium Ibadan (FHI) with voucher numbers 112263 and 112265, respectively. The leaves of both plants were air-dried for two weeks under shade, pulverized and extracted using equal volumes of methanol: dichloromethane under continuous agitation for 18–24 h at room temperature. Then distilled water was added to the extraction system and vortexed, and left for 24 h. The supernatant aqueous amino acid/peptide-rich extracts (CP) were decanted and concentrated using the rotary evaporator to remove methanol prior to C_{18} flash pre-purification. The aqueous extracts were pre-purified on RP-C_{18} solid-phase with C_{18}-parked Cartridges (Strata Gigatubes C18-E; 5 g, 20 mL, Phenomenex, Germany). Following initial preconditioning, equilibration and sample application, bound peptides were washed with 10% buffer B (90%v/v acetonitrile, 0.08% v/v trifluoroacetic acid) and finally eluted with 50 mL of 80% buffer B to obtain the pre-purified peptide fractions (PF). Cryodesiccation of eluted fractions was achieved with freeze-drying equipment.

Preparation of the modified G-250 dye and ninhydrin reagent

The G-250 dye was prepared according to the modified method described by (Wang, et al., 2017). G-250 (200 mg) was dissolved in 40 mL ethanol. Then, 320 mL of 50% ethanol in distilled water was prepared and 40 mL of orthophosphoric acid was added. The ninhydrin reagent was 0.2 g ninhydrin in 100 mL of ethanol, while pyridine reagent was 5 mL pyridine in 1 mL glacial acetic acid.

TLC Chemical detection

The pre-purified peptide fractions (PF) were chemically detected using Thin Layer Chromatography (TLC). A modified method previously described by (Wenyan, et al., 2008; Jork, et al., 1994). Pre-coated TLC plates (F_{254} MERCK, Germany) were used, and the solvent system used was n-butanol: acetic acid: water (2.5:1:1.5 v/v). The solvent reconstituted aqueous peptide fraction was spotted on the TLC plate and developed in the above-mentioned solvent system. The developed plates were allowed to dry, and sprayed with freshly prepared G-250 modified stain, ninhydrin and pyridine. The sprayed plates were

allowed to dry and viewed under daylight. *Viola tricolor*, a plant previously established to be rich in peptides was used as standard (Hellinger, et al., 2015).

Extract Preparation

For the antioxidant and antimicrobial assays, CP and PF (2.5 mg) each from *N. diderrichii*, and *I. brachypoda* leaves were dissolved in 12.5 mL of 1% dimethyl sulfoxide (DMSO) to make 200 μgm/L stock solution and stored at 4 °C until use. For Brine shrimp lethality assay, stock solutions (1000 μg/mL) were prepared by dissolving CP and PF (2 mg each) in 2 mL DMSO. For antiviral assay, PF (10 mg) of both plants were dissolved in 1 mL DMSO to obtain a stock concentration of 10 mg/mL.

DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging activity

DPPH solution was prepared by dissolving 6 mg of DPPH in 150 mL of methanol to give a 0.004% w/v solution. Equal volumes (3 mL) of the DPPH solution and various concentrations of fractions (PF and CP) and Gallic acid (standard) were added to each designated test tube. All test tubes were then incubated in a dark chamber for 30 minutes after which their absorbance were measured at 517 nm using UV VTS spectrophotometer (Spectrumlab 752s). Equal volumes (3 mL) of methanol and the prepared DPPH solution served as negative control. The 50% inhibitory concentrations (IC_{50}) of the PF and CP were statistically analysed and compared to that of Gallic acid.

Microbial cultures

Gram-negative strain, *Escherichia coli* (ATCC 35218), and Gram-positive strain, *Staphylococcus aureus* (ATCC 29213) and strains of fungi, *Aspergillus niger* and *Candida albicans* were maintained on Nutrient agar, and Sabouraud dextrose agar (SDA), respectively. A single colony of each organism was inoculated into 5 mL Tryptic Soy Broth (TSB) and Sabouraud dextrose broth (SDB) for preparation of bacterial and fungi culture, respectively. All microbes were sub-cultured from the original culture and incubated overnight at 37 °C. The bacteria were obtained from the Pharmaceutical Microbiology Department, University of Ibadan, Ibadan, Nigeria, and the fungi were obtained as clinical isolates from the University College Hospital (UCH), Ibadan. The standard drug, gentamycin and ketoconazole were used as the positive control.

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Antibacterial assay

Broth microdilution:

The antimicrobial assay was done using the broth micro-dilution method and 96 well plates were used. The samples were dissolved in DMSO to obtain a stock solution of 1000 µg/mL. This was then diluted serially in the micro plate wells to obtain a concentration range of 200 - 6.25 µg/mL. Tryptic soya broth was the medium used and the drugs used as the reference were gentamycin and ketoconazole (10 µg/mL) for the anti-bacterial and anti-fungal assays, respectively. The reference drugs were also diluted to obtain concentration range of 10 - 0.3125 µg/mL concentrations which were used in the assays.

Antibacterial and Antifungal assay:

The *p*-iodonitrotetrazollium violet (*p*-INT) colorimetric redox assays were adopted for this study, each of the micro plate wells were inoculated with 40 µL of the microorganisms, incubated at 37 °C for 24 hours for bacteria and 25 °C for 48 hours for fungi. After incubation, 40 µL of 0.2 mg/mL *p*-INT was added to each well and incubated for another 30 minutes. Then spectrophotometer reading was taken at a wavelength of 630 nm using (ELISA reader, CLX800-BioTek Instruments) to obtain the optical density, and the IC_{50} was determined. P-iodonitrotetrazolium Violet is a tetrazolium dye used as an electron acceptor for the colorimetric assay of various dehydrogenases. *P*-iodonitrotetrazolium violet dye is often used to visualize dehydrogenase activity histochemically that on reduction produces a red formazan dye that can be used for quantitative analysis.

The percentage microbial growth inhibition was calculated using the formula:

% inhibition = (A-B)/A x 100

Where: A= optical density of untreated well, B= optical density of wells with plant extract.

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Minimum inhibitory concentration (MIC) determination

The Minimum Inhibitory Concentrations were determined visually in the broth dilution as the lowest concentrations of the extracts at which no bacteria/fungi growth was visible (or greatly reduced in comparison to the controlled growth) or colour changed from violet to red formazan in the case of the *p*-INT dye broth assay.

Cytotoxicity screening

Brine shrimp (*Artemia salina*) lethality assay (BSLA)

The assay was carried out according to the method of (Alara, et al.). After hatching, nauplii were collected by dropping pipette. The crude and peptide fractions were dissolved in DMSO to obtain a stock solution of 1000 µg/mL, which were serially diluted two-fold into six concentrations. The diluted test solutions of the CP and PF were added to designated test tubes in different concentrations. Then, ten (10) active brine shrimp (nauplii) were transferred into each of these vials using Pasteur pipette. Triplicates of each of the dose levels were prepared, using seawater as negative control and cyclophosphamide positive control. Number of survivors and deaths were recorded after 24 h.

Cells and Viruses Culture

Rhabdomyosarcoma (RD) and Human breast adenocarcinoma (MCF-7) cells lines of human origin obtained from WHO National Polio Laboratory Department of Virology, University of Ibadan, Nigeria was used for both antiviral studies. Cells were grown in Eagle's minimum essential medium (MEM) supplemented with 10% foetal bovine serum (FBS), 100 units. /mL of penicillin, 100 µg/mL of streptomycin, 2 mM L-glutamine, 0.07% NaHCO3, 1% non-essential amino acids and vitamin solution. Three enterovirus species C including two serotypes of coxsackievirus A (CV-A13 and CV-A20) and a numbered Enterovirus C serotype (EV-C99), and two enterovirus species B enterovirus members including coxsackievirus B3 (CV-B3) and Echovirus 20 (E20) were obtained from stool isolates (Faleye, et al., 2017) by the Enterovirus research group, Department of Virology, University of Ibadan, Nigeria. The test medium used for cytotoxic assays and antiviral assays contained only 2% FBS. Virus titre was determined by virus-induced cytopathic effect (vCPE) in cell culture and were expressed as 50% tissue culture infective concentration ($TCID_{50}$) per mL. All viruses were stored at −70°C until use.

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MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium) cell viability assay

The effects of the peptide fractions on viability of RD and MCF-7 cells were determined by MTT cytotoxicity assay (Mosmann, 1983), a colorimetric assay that reliably measures cell viability. Briefly, seeded monolayer of cells in a 96-well microtitre plate was treated with six ten-fold serial dilutions (1000 to 0.01 µg/mL) of stock solutions (10,000 µg/mL) of peptide fractions in maintenance medium (2% MEM) for 72 h. Plates were then observed for maximum non-toxic concentrations (MNTC) on the cells under an inverted microscope (OLYMPUS CKX31). Afterward, old medium was removed and prepared MTT reagent (25 µL) in phosphate buffer saline (PBS) (2 mg/mL) was added to each well, including controls and plate returned to the incubator for 2 h. DMSO (75 µL) was added to solubilize the formazan crystals formed. Optical density values were obtained by spectrophotometry (Multiscan 347, MTX lab) at 490 nm. Data obtained was used to determine 50% cytotoxic concentration (CC_{50}). Standard drug was vincristine sulphate.

Tissue culture infective dose ($TCID_{50}$)

Cells were seeded into a T25 cell culture flask (Corning®) and incubated at 37 ºC for 24 hours to form a confluent monolayer. Virus suspension (100 µL) was inoculated into the T25 flask of cultured cells, and incubated for about 72 hours to increase the quantity of virion stocks. A 96-well microtitre plate of monolayer cells was inoculated with virus suspension (100 µL) into the eight replicate wells of each column 1-10, with varying (ten-fold serial dilutions) concentration per column; cell control was Column 11 and 12. Plate was incubated at 37°C, and daily CPE scoring was done for about 7 days when cell control wells started dying off. The $TCID_{50}$ values were determined using Spearman-Karber's method and 100 $TCID_{50}$ was used for the antiviral assay. The species B and species C enteroviruses were inoculated on RD and MCF-7 cell lines, respectively. Species B and C enteroviruses are classified as human enteroviruses which are associated with clinical manifestations ranging from aseptic meningitis to acute flaccid paralysis particularly, in children between 0-5 years of age.

Antiviral Assay

Neutralization method as previously described(Lin, et al., 2012) was employed to evaluate the vCPE inhibition effects of the peptide fractions on the viruses. Briefly, six two-fold serial dilutions made from the MNTC of each fraction was added to confluent cell monolayers in a 96-well plate, and allowed to adsorb for about 1 h at 37°C, after which 100 $TCID_{50}$ virus

suspension was added. Plates were incubated at 37°C for 72 h. Virus control wells were infected with the same concentration of virus but untreated with fractions, while cell control wells contained only maintenance medium (uninfected and untreated). Plates was observed preliminarily under the microscope for vCPE. Thereafter, MTT colorimetric measure was employed as described earlier. The concentration that reduced 50% of CPE with respect to the virus control was defined as the 50% inhibitory concentration (IC_{50}). No standard drug was used since there is no approved chemotherapy for enterovirus treatment.

Statistical analysis

CC_{50}, IC_{50} and Selective index

The 50% inhibitory concentrations (IC_{50}) and the 50% cytotoxic concentrations (CC_{50}) for the extracts were calculated from concentration-effect curves after non-linear regression analysis using GraphPad prism5 software. Selective index was defined for antiviral evaluation as index of safety margin (CC_{50} over IC_{50}). Experiments were performed in triplicates, and results presented as mean ± standard deviation.

Results

The presence of free amino acids, linear and circular peptides were detected in the peptide fractions of both plants on spraying spotted TLC plates with ninhydrin (violet-pink), pyridine (yellowish) and modified G-250 stain (bright blue), respectively (Figure 1).

The peptide fraction of *N. diderrichii* relatively showed the highest (Table 1) DPPH radical scavenging ability among fractions. Overall, peptide fractions of both plants displayed enhanced radical scavenging effect than their crudes though not as the standard gallic acid.

Generally, both fractions (CP and PF) of both plants had equal good inhibitory effect (MIC= 6.25 μg/mL) on tested microorganisms (bacteria and fungi) though lower than the standard drugs (Gentamycin and Ketoconazole) except Gram-negative *E. coli* (Table 2). However, only the peptide fraction of *I. brachypoda* was found to relatively inhibit Gram-negative *E. coli*. (MIC= 6.25 μg/mL).

Fractions (CP ad PF) from both plants were moderately lethal (LC_{50} < 500 μg/mL) to brine shrimp nauplii relative to standard drug cyclophosphamide (LC_{50} <63.8 μg/mL). Peptide fractions showed enhanced lethality against the nauplii (Table 3) though *N. diderrichii* PF was more. The peptide fractions of both plants displayed moderate cytotoxicity on MCF-7

and RD cancer cells (CC_{50} 30-36 µg/mL range) when compared to the standard drug vincristine sulphate (CC_{50} <0.27 µg/mL) (Table 4).

The peptide fractions of both plants displayed very good antiviral activity against the tested enterovirus species B and C members with IC_{50} values ≤ 3.2 µg/mL (Table 5). The peptide fractions notably displayed good selectivity on Coxsackievirus A20 (SI ≥98) compared to low selectivity (SI <20) observed with CV-A13, and CV-B3.

Discussion

Plant-derived bioactive peptides also known as AMPs are receiving increasing attention in peptide drug discovery and development due to their wide-range biological activity, diversity, occurrence, abundance and low propensity towards drug resistance (Koehbach and Craik, 2019; Mitra, et al., 2019). The detection of linear and circular peptides in *Nauclea diderrichii* and *Ixora brachypoda* leaves by TLC chemical method further confirms their widespread presence in this plant family. Rubiaceae family is included in the angiosperm families containing circular peptides (cyclotides) (Koehbach, et al., 2013). It is noteworthy of mention here that the first isolated cyclotide (Kalata B1) was from aqueous preparation (tea) of the leaf of *Oldenlandia affinis* (Rubiaceae) (Gran, 1973).

Generation of free radicals have been implicated in chronic health problems and during various immune responses to infections. Thus, the marked DPPH radical scavenging activity displayed by the peptide fraction of *N. diderrichii* could be implied synergistic in its traditional anti-infective potentials, and perhaps the antimicrobial and antiviral activities. Peptides are known to be very potent at reducing intracellular ROS (reactive oxygen species) and preventing cell death caused by the oxidant and may be very beneficial in the treatment of aging and diseases associated with oxidative stress {Yan, 2014 #273;Cai, 2018 #563}.

The general issue with managing infections (microbial, viral or parasitic) by chemotherapy has been the constant and fast development of resistant strains to conventional anti-infective drugs. There are several reports on the antimicrobial and antiviral activities of plant-derived AMPs (Ogbole, et al., 2020; Koehbach and Craik, 2019) and several mechanisms of action have been suggested (Yeaman and Yount, 2003). Among the seven species of *Nauclea* genus in Africa, *N. diderrichii* has been widely reported for anti-infective remedies in traditional medicine. Also, it is used as chewing stick for oral and dental care (Haudecoeur, et al., 2018), as anti-helminthic in Democratic Republic of Congo (D. R. C.) and to treat jaundice and

hepatitis in Gabon and D. R. C. (Mbuta, et al., 2012; Betti, et al., 2013), respectively. The broad-spectrum antimicrobial and antiviral activities of the peptide extract of *N. diderrichii* leaf further validate the anti-infective properties of this plant. The lack of difference in the MIC values of CP and PF of plant samples (Table 2) might suggest no improved activity in the PF. However, it suggests that activity observed in CP was retained in PF (fraction from CP), and that PF could be the a major constituent responsible for antimicrobial activity in CP. Antimicrobial peptides are commonly considered as immunity primitive mechanisms and have been extensively studied in a wide variety of organisms including plants, with their primary biological activities recognised as antifungal, antibacterial, and insecticidal (Zhang, et al., 2020; Tonk, et al., 2016).

The broad spectrum of activities of AMP and the low incidence of resistance to these molecules suggest a potential benefit in cancer treatment (Guzmán-Rodríguez, et al., 2015). Possible cytotoxic peptides can be obtained from the peptide fractions of both plants as demonstrated by their lethality on brine shrimp nauplii (LC_{50} <500 µg/mL), and moderate cytotoxicity on the MCF-7 and RD cancer cell lines (CC_{50} ≤34 µg/mL). However, this finding is discrete from the previously reported non-toxicity (LC_{50} >1000 µg/mL) of the methanol extracts of *N. diderrichii* (Ogbole, et al., 2016), thus emphasizing the cytotoxic potential of the peptide constituents of *N. diderrichii.* The varying extraction process which informs difference in the nature of phytochemical constituents of the methanol and the peptide extracts from *N. diderrichii*, could be responsible for the toxicity discrepancy on brine shrimp.

Conclusively, peptide fractions of *N. diderrichii* and *I. brachypoda* leaves displayed good inhibitory effects against tested strains of bacteria, fungi, and virus, and toxicity against brine shrimp and tested cancer cell lines. Also, good DPPH radical scavenging activity was displayed. So far known, this work represents the first report of bioactivities of peptide fractions from *N. diderrichii* and *I. brachypoda* leaves. There is need to further isolate and characterize the peptides responsible for the indicated biological activities.

Author statement/contribution

Omonike Ogbole: Conceptualization, Methodology, Supervision, Validation, Writing - review & editing. **Abraham Nkumah:** Formal analysis, Investigation, Writing - original draft. **Toluwanimi E. Akinleye:** Formal analysis, Investigation, Writing - review & editing. **Fredrick E. Olisaedu:** Investigation. **Alfred F. Attah:** Methodology, review & editing

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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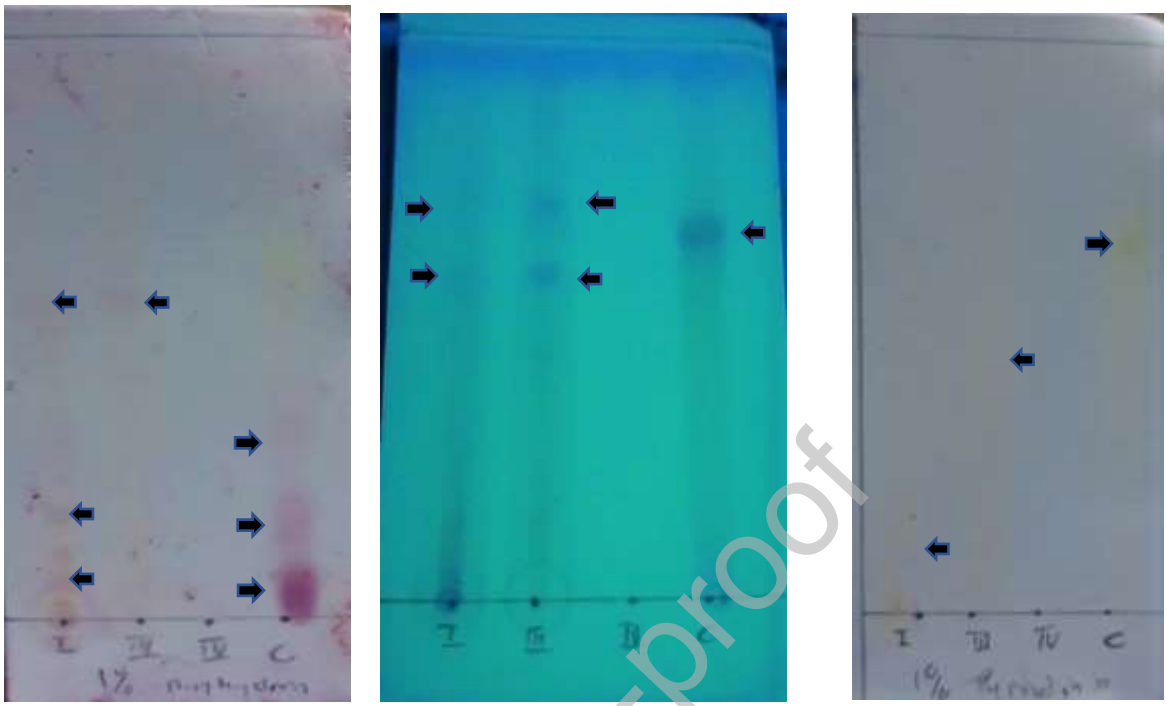
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Journal Pre-proof



A. Ninhydrin **B. Modified G250** **C. Pyridine**

Key: I = *Ixora brachypoda* PF, II = *Nauclea diderrichii* PF, C = Standard (*Viola tricolor*)

Figure 1: Chromatogram showing separated spots detected by different spray reagent.

Journal Pre-proof

Table 1: DPPH antioxidant assay of crude and pre-purified peptide fractions of plant samples

Sample	IC_{50} (µg/mL)
I. brachypoda CP	49.93±0.01
I. brachypoda PF	20.09±0.01
N. diderrichii CP	41.91±0.02
N. diderrichii PF	3.43±0.01
Gallic acid	1.44±0.00

PF- Peptide fractions; CP- crude peptide.

Journal Pre-proof

Table 2: Minimum inhibitory concentration (MIC) of crude and pre-purified peptide fractions of plant samples

Sample	MIC value (µg/mL)			
	E. coli	***S. aureus***	***A. niger***	***C. albicans***
N. diderrichii CP	50	6.25	6.25	6.25
N. diderrichii PF	50	6.25	6.25	6.25
I. brachypoda CP	12.5	6.25	6.25	6.25
I. brachypoda PF	6.25	6.25	6.25	6.25
Gentamycin	<0.31	<0.31	-	-
Ketoconazole	-	-	0.31	5.00

PF- Peptide fractions; CP- crude peptide.

Journal Pre-proof

Table 3: Brine shrimp lethality of crude and pre-purified peptide fractions of plant samples

Sample	LC_{50} (µg/mL)
N. diderrichii PF	159.5±0.70
N. diderrichii CP	469.7±0.31
I. brachypoda PF	220.82±0.32
I. brachypoda CP	236.00±0.01
Cyclophosphamide	63.82±0.45

PF- Peptide fractions; CP- crude peptide.

Journal Pre-proof

Table 4: Cytotoxic activity on peptide fractions on plant samples on cancer cell lines

Sample	CC_{50} (µg/mL)	
	MCF-7	**RD**
I. brachypoda PF	33.99±1.4	35.60±0.2
N. diderrichii PF	30.68±0.7	32.51±0.9
Vincristine sulphate (Standard)	0.27±0.05	0.07±0.05

PF- Peptide fractions; MCF-7- Breast adenocarcinoma; RD- Rhabdomyosarcoma.

Journal Pre-proof

Table 5: Antiviral activity of pre-purified peptide fractions (PF) of plant samples on enteroviruses

Sample	Species B				Species C					
	IC_{50} (μg/mL)		SI		IC_{50} (μg/mL)			SI		
	CV-B3	E20	CV-B3	E20	CV-A13	CV-A20	EV-C99	CV-A13	CV-A20	EV-C99
I. brachypoda PF	3.20± 0.6	1.79± 0.2	11.1± 0.6	19.9± 0.2	1.93± 0.1	<0.3 1	1.04± 0.2	17.7± 0.1	>108 .8	32.6± 0.2
N. diderrichii PF	2.31± 0.4	0.96± 0.1	14.1± 0.4	33.9± 0.1	1.99± 0.2	<0.3 1	1.16± 0.1	15.4± 0.2	>98. 2	26.5± 0.1

PF- Peptide fractions; SI- selective index; CC_{50}- 50% cytotoxic concentration; IC_{50}- 50% inhibitory concentration; CV-A13- Coxsackievirus A13; CV-A20- Coxsackievirus A20; EV-C99- Enterovirus C99; CV-B3- Coxsackievirus B3; E20; Echovirus 20.

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