Isolation and Spectroscopic Identification of Some Constituents of Bioactive Fractions of Aerial Parts of *Mirabilis Jalapa*

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Abstract: *The plant Mirabilis jalapa is renowned for its medicinal properties. This study focuses on the bioguided isolation of constituents present in aerial parts of this plant. Subfractions of pulverized aerial parts of M. jalapa were screened for activity against selected enteropathogenic bacteria. The MIC and MBC were also determined for the subfractions. Three compounds were isolated from the most active methanol subfraction subjected to several chromatography purification steps and their structures elucidated by NMR and MS (EI and ESI) spectroscopic techniques. Susceptibility to extract activity was observed for B. cereus, E. coli, S. typhi and S. dysentereae, with 5mg/ml being the least recorded MBC against S. typhi and S. dysenterae. The compounds trolline (**1**), a pyrrolo[2,1-a] isoquinoline alkaloid and the flavonoids isorhamnetin (**2**) and its rhamnoside derivative isorhamnetin-glucosyl-rhamnoside (**3**) were identified as constituents of most active methanol subfraction. The partial purification of medicinal plants may enhance their functionality and minimize risks to body homeostasis*

Keywords: *Mirabilis jalapa, anti bacterial, diarrhea, spectroscopy*

I. Introduction

For centuries, plants have been major sources of substances known to possess different pharmacologic effects in humans and animals. Plant biodiversity around the world not only provide cheap and ready source of new chemical entities that may serve as lead candidates in drug discovery but play functional roles in the general well being of the body. Though other sources of natural products like fungi, actinomycetes and marine organisms are equally being explored for novel carbon scaffolds.

The plant *Mirabilis jalapa* is famous for its medicinal properties known to different cultures of the world. In Brazilian folk medicine for example, the leaf extract is used in treatment of inflammation (Walker et al., 2008). It is also used as a purgative and in treatment of dysentery (Encanacion *et al.*, 1998) and diarrhea (Holdsworth, 1992 Comeford, 1996). The root of this plant is known to the Chinese for its anti-diabetic properties (Zhou *et al.,* 2012b). Phytochemical investigation reveals the presence of β-sitosterol, ursolic acid, amino acids, β-stigmasterol (Singh and Mittal*,* 2012), phytol (Siddique *et al.*, 1994) in the aerial parts while rotenoids (Xu *et al.,* 2010) and trigonelline (Zhou *et al.,* 2012a) alkaloid have been characterized from the roots of *M. jalapa*.

This study investigates some constituents of most active subfraction of aerial parts of *M. jalapa* against selected entero pathogenic bacteria.

II. Materials and methods

Extraction

Soxhlet apparatus was used to extract 900g of pulverized leaves of *M. jalapa*. Hexane, ethylacetate, butanol and methanol were sequentially used for extraction. The extracts were concentrated with a rotavapour and allowed to dry to constant weights which were then preserved at 4^oC under airtight conditions to prevent contamination.

Antimicrobial screening

The well diffusion method was used to assay for anti-bacterial properties of the subfractions. Briefly, 20mg/ml stock solution of each subfraction was prepared and serially diluted to obtain concentrations of 10mg/ml, 5mg/ml and 2.5mg/ml. A cork borer was used to make wells in freshly prepared nutrient agar in petridishes. Each plate was carefully labeled and organisms were streaked on the surface of agar plates and subsequently incubated at 37^oC for 24 hours. Clinical isolates of the organisms *S. typhi, S. paratyphi, E. coli, S. feacalis, V. cholerea* and *S. dysenterea* were screened for growth susceptibility to the fractions. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were also determined using broth dilution method.

Isolation of constituents of bioactive fraction

The following steps were followed in isolation of compounds from most active fraction: 29g of the methanol fraction was partitioned with hydrated butanol. The butanol fraction (9g) after concentration, was

separated on silica column (mesh size 60-120) eluted with 10%MeOH:DCM from which fractions F50-F80 were pooled together. The pooled fractions F50-F80 was further separated on flash column (40%Acetone:Hex) and subsequently on MCI gel (F33-F45) to obtain F23 (60%H_2O:MeOH), F35 (70%MeOH:H_2O) and F39(75%MeOH:H_2O). Final purification steps were performed on HPLC (recycle HPLC, Japan Analytical Industries with reverse phase ODS H-80 column, R.I and UV detectors; flow rate 2.5ml/min-3.0ml/min) to obtain compound **1** (60%H2O:MeOH, 10mg), compound **2** (45%H2O:MeOH, 7mg) and compound **3** (45%H2O:MeOH, 7mg) respectively.

Spectroscopy

The structures of purified compounds were determined by various spectroscopic analyses. Proton (^{1}H) and Broadband ^{13}C NMR (Avance AV-600 Cryo-probe NMR) were analysed for each sample. Further 2D techniques (COSY, HMBC, HSQC) and DEPT (DEPT 90 and 135) experiments were conducted for complete structural elucidation. Electron Ionization (EI MAT 312 centroid type display) and Electrospray ionization (QSTAR Elite ESI-TOF Mass Spectrometers) mass spectroscopy techniques were done to determine molecular weights at low and high resolutions. Functional groups of samples were determined by FTIR analyses (FTIR FTS-65, Biorad).

III.   Results and Discussions

Figure 1 presents the zones of inhibition (in millimeters) of extract subfractions activities on selected enteropathogenic bacteria. The diameter across the area where no growth was observed was measured. The methanol subfraction had highest zones of inhibition recorded for susceptible organisms compared to other subfractions; with *S.*

dysentereae being the most susceptible for all fractions tested (32mm). *S. feacalis* and *V. cholera* were both resistant to all extracts at 20mg/ml.
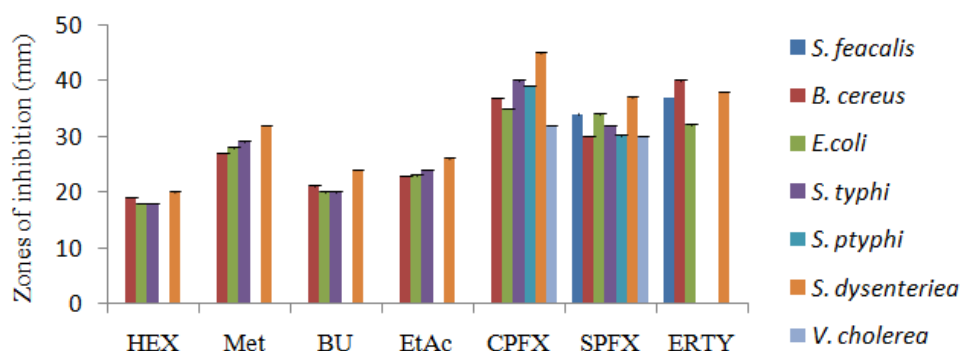

Figure 1:   **Zones of inhibition of extract subfractions of *M. jalapa* leaf on test organisms**

Key: Zones of inhibition are presented as mean±standard deviation of triplicate determinations; Hex=hexane subfraction; Met=methanol subfraction; BU=butanol subfraction; EtAc=ethylacetate subfraction CPFX=ciprofloxacin; SPFX=sparfloxacin ; ERTY=erythromycin

The control drug ciprofloxacin was most active at 5mg/ml concentration but showed no inhibitory effects on *S. feacalis*. Similarly, *S. typhi, S. paratyphi* and *V. cholera* were observed to be resistant to erythromycin while sparfloxacin inhibited growth of all organisms tested.

The MIC values as presented in Table 1 showed all susceptible organisms had MIC of 2.5mg/ml for methanol subfraction. The ethylacetate subfraction also showed bacteriostatic inhibition at 2.5mg/ml against *B. cereus E. coli* and *S. dysentereae*. The least active subfraction however, was hexane fraction where 10mg/ml was required to inhibit growth of *B. cereus, E. coli* and *S. typhi*. The minimum bactericidal concentration (MBC) presented in Table 4.2 showed 10mg/ml of ethylacetate and methanol subfractions completely killed all susceptible organisms. The methanol subfraction appeared to be most active against *S. typhi* and *S. dysentereae* which were completely killed at 5mg/ml.

Table 1: **Minimum Inhibitory Concentration (MIC) of the subfractions of *M. jalapa* leaf extract on test organisms**

Organism	MIC (mg/ml)			
	HEX	Met	BU	EtAc
B. cereus	10.00	2.50	5.00	2.50
E.coli	10.00	2.50	5.00	2.50
S. typhi	10.00	2.50	5.00	5.00
S. dysenteriea	5.00	2.50	5.00	2.50

Key: Hex=hexane subfraction; Met=methanol subfraction; BU=butanol subfraction; EtAc=ethylacetate subfraction. Sample concentrations are serial dilutions of 20mg/ml stock solution.

Table 2: Minimum Bactericidal Concentration (MBC) of the subfractions of *M. jalapa* leaf extract on test organisms

Organism	MBC (mg/ml)			
	HEX	Met	BU	EtAc
B. cereus	20.00	10.00	20.00	10.00
E.coli	20.00	10.00	20.00	10.00
S. typhi	20.00	5.00	20.00	10.00
S. dysenterea	20.00	5.00	10.00	10.00

Key: Hex=hexane subfraction; Met=methanol subfraction; BU=butanol subfraction; EtAc=ethylacetate subfraction.

Extractives of the aerial parts of the plant *Mirabilis jalapa* have been shown to have in-vitro growth inhibitory effects on diarrhea related enteropathogenic bacteria (Eneji *et al.,* 2011). Results presented in Tables 1 and 2 suggest polar substances maybe responsible for the observed antibacterial activities of the plant. Similar findings was reported by Zachariah *et al.,* (2012) supporting the traditional claim of *Mirabilis jalapa* aerial parts as an antibacterial, where polar methanol fraction was also demonstrated to possess components responsible for the observed activities. Gujjaiah, (2012) reported petroleum ether and chloroform extracts to be active against selected bacterial and fungal organisms. The organisms used in the reported research were however less implicated in diarrhoeal and other enteric infectious diseases.

Spectroscopic data for compound 1
   ^{1}H NMR (600MHz, MeOD) δ 6.55-6.53 (s, 2H), 4.72 (dd *J*= 18.0, 7.8, 1H, 4.08 (ddd, *J*= 12.9, 6.0, 2.8, 1H), 3.04 (td, *J*= 12.0, 4.3, 1H), 2.76-2.70 (m, 1H), 2.66-2.60 (m, 2H), 2.56 (dd, *J*= 17.9, 7.9, 1H), 2.41-2.36 (m, 1H), 1.80-1.73 (m, 1H); **^{13}C NMR** (151MHz, MeOD DEPT) C 175.9, 145.6, 145.5, 129.8, 125.5; CH 116.2, 112.4, 58.3; CH$_2$ 38.6, 32.7, 28.8, 28.8; **HREIMS** M* 219.0882 Molecular formula $C_{12}H_{13}O_3N$; Ring plus double bond (RDB) analysis =7; **FTIR**(3425, 2925, 1649, 1527, 1452cm^{-1})

Spectroscopic data for compound 2
   ^{1}H NMR (600 MHz, MeOD) δ 7.92 (d, *J*=1.6, 1H); 7.59-7.57 (dd, *J*=8.4, 2.0, 1H), 6.91, (d, *J*=8.4, 1H), 6.37 (s, 1H). 6.18 (s, 1H), 5.38 (d, *J*=7.2, 1H), 3.94 (s, 3H), 3.74-3.53 (dd, *J*=12.0, 2.0, 1H), 3.57-3.53 (d, *J*=, 1H), 3.46-3.41 (m, 2H), 3.24 (d, *J*=3.20, 1H); **^{13}C NMR** (151 MHz, MeOD DEPT) δ C: 179.29, 167.16, 163.05, 158.57, 158.51, 150.81, 148.42, 135.36, 123.10, 105.47; CH: 123.79, 116.08, 114.31, 103.66, 100.30, 94.97, 78.55, 78.08, 75.91, 71.46; CH$_2$: 62.51; CH$_3$: 56.76; **ToF ESI-MS** (positive mode): Molecular ion peak (M+H) m/z 479.1 with intense isotopic peaks appearing at m/z 480 and m/z 481; Molecular formula: $C_{22}H_{22}O_{12}$; RDB:12

Spectroscopic data for compound 3
   ^{1}H NMR (600 MHz, MeOD) δ 7.93 (d, *J*=1.5,1H); 7.64-7.62 (dd, *J*=8.5,2.0, 1H); 6.93-6.90 (dd, *J*=8.5,3.5, 1H); 6.41 (d, *J*=1.5, 1H); 6.21 (d, *J*=2.0, 1H); 5.22 (d, *J*=7.5, 1H); 4.51 (s, 1H); 3.94 (s, 3H); 3.82 (d, *J*=10.5, 2H); 3.61 (d, *J*=8.0, 2H); 3.49-3.45 (dd, *J*=9.5, 3.0, 2H); 3.43 (m, 4H), 1.10 (d, *J*=6.5, 3H); ^{13}C (151MHz, MeOD DEPT) δ C 179.28, 166.77, 162.89, 158.89, 158.55, 150.84, 148.35, 135.38, 123.01, 105.41; CH 124.00, 116.14, 114.50, 104.36, 102.51, 100.24, 95.12, 78.11, 77.31, 75.87, 73.80, 72.25, 72.05, 71.58, 69.81; CH$_2$ 68.53; CH$_3$: 17.90, 56.79; **ESI-MS**: (ToF Positive mode) MH$^+$ m/z 625.2; Exact mass: 624.1690; Molecular formula: $C_{28}H_{32}O_{16}$; RDB: 13; **FTIR** (3411.8, 1622 , 1425.3, 1114.8 cm^{-1})

Carbon and Proton Assignments for Compound 1
   NMR ^{13}C and DEPT analyses identify presence of 12 carbons: four methylenes, three methines and five quartenary carbons. The chemical shifts in the aromatic region (δ 6.55-6.53ppm) appearing as a tall singlet

and integrating for 2H (H1 and H2) are attached to aromatic methine carbons 112ppm (C10) and 116ppm (C7) (DEPT 90). The tall singlet is probably due to existence of both protons in similar chemical environment and neither in ortho or meta positions on the aromatic ring. The third aliphatic methine carbon with $^C\delta$ 58.30ppm (C10b) has attached proton with $^H\delta$ 4.72 (H3) appearing as a triplet. The HMBC shows the 2-3 bond correlations in the carbon-carbon connectivity as described in the proposed skeleton.
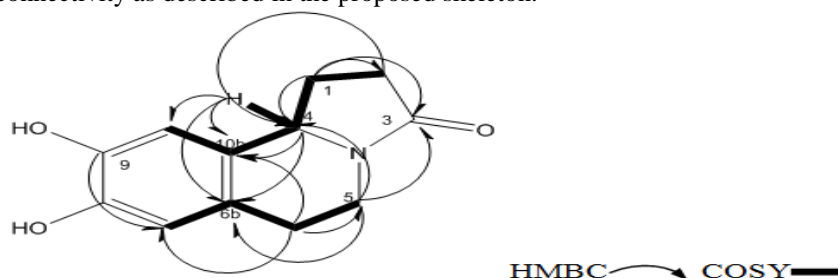

The skeleton presented above is consistent with the observed COSY correlations with characteristic *J* couplings (12-18Hz) especially for the germinal protons. The HR-EIMS elemental analysis, molecular mass of 219 and FTIR pattern observed for the compound is evidence for presence of odd number nitrogen, presented here as a tertiary amide functional group, whose carbonyl appeared at 175ppm.

The IUPAC name for the compound is 8,9-dihydroxy-1,5,6,10b-tetrahydropyrrolo[2,1-a]isoquinolin-3(2H)-one (generated using the ACD i-lab software) commonly known as trolline. The isomer of this compound was previously isolated from *Portulaca oleracea* and reported as oleracein E by Yang *et al.,* (2007). There are no previous reports however, (to the best of our knowledge) documenting the isolation of this compound from the aerial parts of *Mirabilis jalapa*.

**Carbon and Proton Assignments for Compound 2**

The spectroscopic data of compound 2 as presented shows a methoxy carbon observed at δ 56.76ppm (proton shifts of 3.94ppm integrating for 3H). A methylene group at δ 62.51ppm and methine carbons δ 71.46-78.55ppm which correlates with proton chemical shift for oxygenated protons in the region 3-4ppm are characteristic features of a sugar moiety present in the overall structure of the compound. The aglycone part of this compound is aromatic since $^\delta$H 6.18-7.92ppm were observed. Inspection on the proton and HSQC data show the anomeric proton appearing at $^H\delta$ 5.38ppm, a doublet and integrating for 1H is attached to carbon C-1″ ($^C\delta$ 103.66ppm). The CH_2-OH ($^H\delta$ 3.46-3.41ppm) of the sugar moiety appears upfield of the anomeric proton and attached to C-6″ ($^C\delta$ 62.51ppm), seen as a methylene pair in HSQC. The assignment is consistent with DEPT 135 spectrum indicating this carbon as a methylene (negative peak). HMBC data indicate protons H1, H2, H3 show 2-3 bond correlation with carbons C-1′ (123ppm)**,** C-4′ (148ppm), and C-2′ (158ppm) while the methoxy protons correlate with C-3′ (150ppm) suggesting strongly these proton spin systems belong to the same aromatic ring. The anomeric proton shows HMBC with C-3 ($^C\delta$ 135ppm) which most probably is point of attachment to the aglycone moiety of the compound. The ring system proposed describes a flavonoid type skeleton:
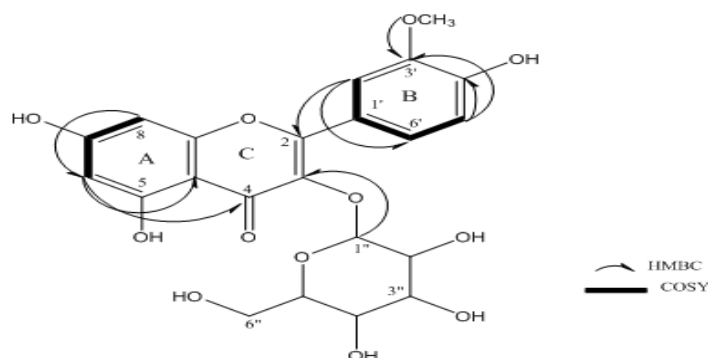
COSY correlations for the aromatic protons (as shown above) are observed for H1/H2 (weak) and H2/H3 (strong). A weak COSY signal is also observed for H4/H5. The IUPAC name generated using the ACD-ilab online software is 5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-3-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one, commonly known as Isorhamnetin. The compound isorhamnetin isolated from various plants have been reported notably *Oenanthe javanica* (water dropwort) (Kim *et al.,* 2013).

Carbon and Proton Assignments for Compound 3

The spectral data for compound 3 shows similar assignments to compound 2 except for the presence of an additional rhamnose sugar molecule (with characteristics methyl ^{13}C peak appearing at 17.90ppm) linked as a disaccharide. The chemical shifts ranging from 71.58-78.11ppm are characteristic of oxygenated protons typical of sugar molecules. Similar pattern for oxygenated protons is observed in proton NMR in the region between 3-4ppm. Appearance of protons in the aromatic region on the ^1H NMR ppm scale strongly indicates a ring system with sugar molecules. Two anomeric protons ($^H\delta$ 5.22 and 4.51ppm) are observed to be attached to carbons with $^C\delta$ 102.51 and 104.36ppm (in HSQC) respectively. While proton with $^H\delta$ 5.22ppm shows HMBC with carbon $^C\delta$ 135.38 as seen in isorhamnetin, the proton with $^H\delta$ 4.51ppm shows HMBC with C $^C\delta$ 68.53 and 71.58ppm respectively:
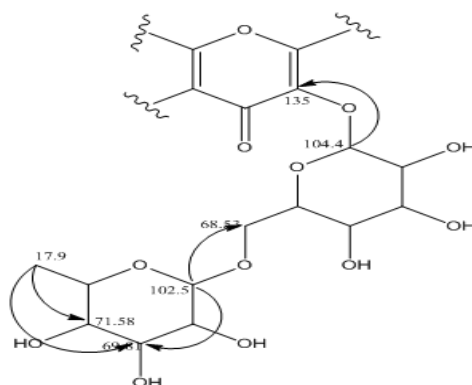
The methyl group carbon $^C\delta$ 17.90ppm (DEPT 135) shows HMBC with $^C\delta$ 69.81 and 71.58 ppm which are carbons assigned to sugar moiety of the compound. This relationship is clearly seen in NOESY, which shows the methyl group correlating through space interactions with the sugar carbons suggesting these carbons exist in the same spin system. The FTIR spectrum for compound 3 shows strong absorbance in the region 3411.8cm^{-1} for hydroxyl group (-OH), 1622cm^{-1} for carbonyl (-C=O) and 1114.8cm^{-1} characteristic of –CO ester linkage. The IUPAC name for compound **3** is 5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-4-oxo-4H-chromen-3-yl6-O-(6-deoxyhexopyranosyl)hexopyranoside (isohermnetin 3-O-glucosyl rhamnoside).

Compounds containing the pyrrolo[2,1-a] isoquinoline skeleton as in compound **1** are known to have biological activity of interest. Oleracein E an isomer of trolline for example has been demonstrated to have potent DPPH free radical scavenging activity and inhibitory effects on hydrogen peroxide induced lipid peroxidation in rat brain homogenates (Yang *et. al.,* 2009). Synthetic pyrrolo[2, 1-a] isoquinoline based compounds have also been reported to have hypotensive and antitumor activities (Mikhailovskii and Shklyaev, 1997). Similarly, findings reported by Wang *et al.,* (2004) demonstrate the antibacterial activity of trolline against *Staphylococcus aureus*, *Staphylcoccus pneumonia*, and *Klesiella pneumonia*.

The antimicrobial properties of various classes of flavonoids have been well reported. Studies on the structure-activity relationship of this class of compounds supports the importance of location of hydroxyl groups on the ring structure of flavonoids; importantly, the existence of hydroxyl groups at positions 5, 7 2' and 4' (Cushnie and Lamb, 2005; Kumar and Pandy, 2013). Combination of several flavonoids may also enhance bacterial growth susceptibility (Amira *et al.,* 2002). Flavonoid rich methanol fraction of *Calotropis procera* containing 3-O-rutinosides of quercetin, kaempferol, isorhamnetin, and flavonoid 5-hydroxy-3,7-dimethoxyflavone-4′-O-β-glucopyranoside was observed to have increased antimicrobial effects (Nenaah, 2013). Partial purification and screening of bioactive fractions may reduce the risks associated with use of whole plant extracts and probably increase the functionality of plant active components in promoting body homeostasis.

IV.   Conclusion

Extractives of the aerial parts of *Mirabilis jalapa* contains bioactive polar compounds including the isoquinoline and flavonoid class of compounds with antimicrobial properties. This study leans support to the use of this plant in the treatment of dysentery and other diarrhoeal related infectious diseases.

Acknowledgement

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