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Full Length Research Paper

In vitro assessment of bioactive components of *Mirabilis jalapa* ethanolic extract on clinical isolates of Salmonella *typhi* and *Bacillus cereus*

Eneji, S.M.*, Inuwa, H.M., Ibrahim, S., Ibrahim, A.B. and Abdulfattah, A.

Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria.

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We investigated the effects of bioactive components of ethanolic leaf extract of *Mirabilis jalapa* on the disease causing enteric pathogens by testing their activity against *Salmonella typhi* and *Bacillus cereus* at an initial stock solution of 20 mg/ml of the crude extract. Following serial dilutions of stock solution, and the determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the crude extract, bioautography technique was used to identify bioactive fractions present in the crude extract. The observed fraction active against both organisms was separated and the MIC and MBC determined. Zones of inhibition for the crude extract at 20 ug/L were 34.33 mm±1.70 and 51.33 mm±1.88 for *S. typhi* and *B. cereus,* respectively while the zones of inhibition of separated bioactive fraction at 3mg/ml were 40.33 mm±1.33 and 40.67 mm±1.70 respectively. The MIC and MBC of the bioactive fraction showed increased in efficacy against the organisms as compared to that of the crude extract. Fourier transform infrared spectroscopy (FTIR) and gas chromatography mass spectrometry (GC-MS) analyses of the bioactive fraction were carried out on the analyte under investigation to identify the constituents of the separated bioactive fraction. The results suggest that the antibacterial activity of the plant extract may be attributed to the alkaloids present.

Key words: Bioassay, bioautography, enteric pathogens, analyte, alkaloids.

INTRODUCTION

Treating infectious diseases using medicinal plants is a common practice in many rural African communities (Ogbe et al., 2009). Among other things, such practices are being further encouraged in recent times due to emergence and possible spread of clinically confirmed drug resistant microorganisms. Indeed several researches in medicinal plants have produced promising results in finding alternatives to failing antibiotics and the discovery of lead compounds in drug design (Newman et al., 2003; Chibale, 2005). The medicinal value of these plants lies in some chemical substances that produce definite physiological action on the human body (Oladunmoye, 2007) and in the treatment of complex health disorders for which the targets are not known (Kayser and Quax, 2007). Thus, it is likely that complex mixtures of secondary metabolites present in plant extracts may modulate several target sites concomitantly. The plant *Mirabilis jalapa* belongs to the family *Nyctaginaceae*, producing beautiful flowers that usually opens around 4 O'clock in the afternoon hence its common name (Nair et al., 2005). It is a perennial herb that grows from a tuberous root to a height of about 50 to 100cm. It is used in traditional herbal practice in the treatment of piles, abscess boils and ulcers (Nath et al., 2010). It is known to contain trigonelline; a purgative alkaloid in addition to oxymethyl anthraquinones, beta-sitosterol galactose and arabinose. This study investigates the potentials of *Mirabilis jalapa* ethanolic leaf extract in its ability to inhibit the growth of selected enteric disease causing microorganisms and to identify bioactive fractions present in the crude leaf extract.

MATERIALS AND METHODS

Plant materials

Fresh leaves of *M. Jalapa* were obtained from the National

*Corresponding author. E-mail: alkarim.alkakani@gmail.com. Tel: +2348096911124

Veterinary Research Institute (NVRI) Vom Plateau State, Nigeria. The plant was identified in the herbarium section of the Biological Science Department, Ahmadu Bello University, Zaria, with deposited voucher number 2441. The leaves were washed, air dried and ground into powder. 350 g of the ground leaves was refluxed using 96% ethanol in a Soxhlet apparatus. The extract was concentrated gently in a water bath at 60°C and stored in a desiccator until analysis.

Phytochemical screening

The ethanolic extract was screened for its phytochemical constituents as described by Trease and Evans (1984).

Bioassay

The ethanolic crude extract (CE) was first screened for antibacterial activity using the well diffusion method. A cork borer was used to bore holes (6 mm) in depth in Petri-dishes containing nutrient agar. Known concentrations of the extract were subsequently added. Agar overlay and direct contact bioautography technique was used to assay for bioactive fractions (BF) present in the crude ethanolic extract. The solvent system ethylacetate: chloroform: methanol: water in the ratio 15:8:4:1 was used to develop the silica gel coated thin layer chromatography (TLC) plates and then inoculated with clinical isolates of *S. typhi* and *B. cereus* obtained from the Microbiology Unit of the Ahmadu Bello University Teaching Hospital, Shika, Zaria. The zones of inhibition were measured in mm and the MIC/MBC of both crude extract and bioactive fractions were also determined using the nutrient broth serial dilution method. Concentration of initial stock solution of CE was 20 mg/ml.

Identification of bioactive fraction

Replica preparative TLC plates were developed using same solvent system as earlier described. Bands that showed activity against all organisms tested were separated out, re-dissolved using solvent of extraction and centrifuged at 700 x *g*. The supernatant was decanted and allowed to evaporate to dryness. 3 mg/ml solution of BF was prepared for sensitivity testing while a portion of the residue was subjected to FTIR analysis (Shimadzu, Japan) and GCMS analysis using a QP 2010 PLUS Shimadzu, (Japan) equipped with an Elite – 5 capillary column (30 mm x 0.25 mm I.D x 0.25 µm film thickness). The carrier gas was helium with a flow rate of 1.61ml/min. initial column oven temperature was maintained at 60°C for 15 min and gradually increased to an injection temperature of 250°C.

Statistical Analysis

Results of zones of inhibition were presented as mean ± standard deviation of triplicate determinations and analyzed using the student's t-test to compare the means.

RESULTS

Table 1 presents the phytoconstituents of *M. jalapa* ethanolic leaf extract. The extract had high amounts of saponins, steroids, alkaloids and tannins. Anthraquinones tested negative to the phytochemical screening.

The zones of inhibition for both the crude and separated bioactive fractions are shown in Figure 1. Zones of inhibition of the crude extract ranged from 34.33±1.70 to 51.33±1.88 at 20 mg/ml extract concentration, with a significant difference in the zones observed for both organisms. Antibacterial screening of the bioactive fraction at 3 mg/ml concentration inhibited growth of the organisms within the range of 30±1.63 to 33±0.82 zones of inhibition with no significant difference between the two.

The MIC and MBC of the crude extract and bioactive fraction is presented in Table 2. Increased efficacy was observed for the bioactive fraction which had 1 mg/ml and 2 mg/ml MIC and MBC values respectively against both organisms.

Figure 3 presents the TLC plates used for agar overlay bioautography. Replica plates were developed using same solvent system and the R_f values were calculated using the formula:

$$R_f = \frac{\textit{Distance travelled by compound}}{\textit{Distance travelled by solvent front}}$$

The R_f values were used to locate bands that inhibited bacterial growth shown in Figures 4A and 4B.

The FTIR analysis of separated fractions (Figure 2) showed a weak to medium broad spectrum with peak at 3431.48cm^{-1} typical of N-H stretching and/or O-H stretch in phenolic compounds. Characteristic N-H bending motion (1627.97cm^{-1}) was also observed in the functional group region. Result of the GCMS analysis of BF is presented in Table 3. The mass spectra of the analyte were matched with those found in the NIST data base software of the equipment.

DISCUSSION

Phytochemical analysis (Table 1) revealed *M. jalapa* ethanolic leaf extract to contain alkaloids, tannins, flavonoids and saponins. Antimicrobial activities of the ethanolic extract have been attributed to the presence of these phytoconstituents (Oladunmoye, 2007). Susceptibility to the effects of the crude extract was higher in *B. cereus* with 51.33±1.88 zone of inhibition (Figure 1). Various leaf extracts of *M. jalapa* have been shown to be significantly effective against gram positive and gram negative bacteria (Muthumani et al., 2009). The result in this work is in agreement with the findings of Devi et al. (2010). Increased susceptibility of *B. cereus* may be attributed to the fact that gram positive bacteria consist of a single layer outer cell wall (Kaladhar and Nandikolla, 2010).

Results of the MIC further suggest *B. cereus* to be more sensitive to the effect of the crude extract as compared to *S. typhi*. However, the MIC of bioactive fraction required to inhibit growth of both organisms was 1 mg/ml. This suggests that not all components present

Table 1. Phytochemical constituents of ethanolic extract of leaves of *M. jalapa.*

Phytochemical	Crude ethanolic extract
Saponins	++
Tannins	++
Anthraquinones	-
Steroids	++
Flavonoids	+
Alkaloids	++

++ = Highly present; + = moderately present; - = absent.

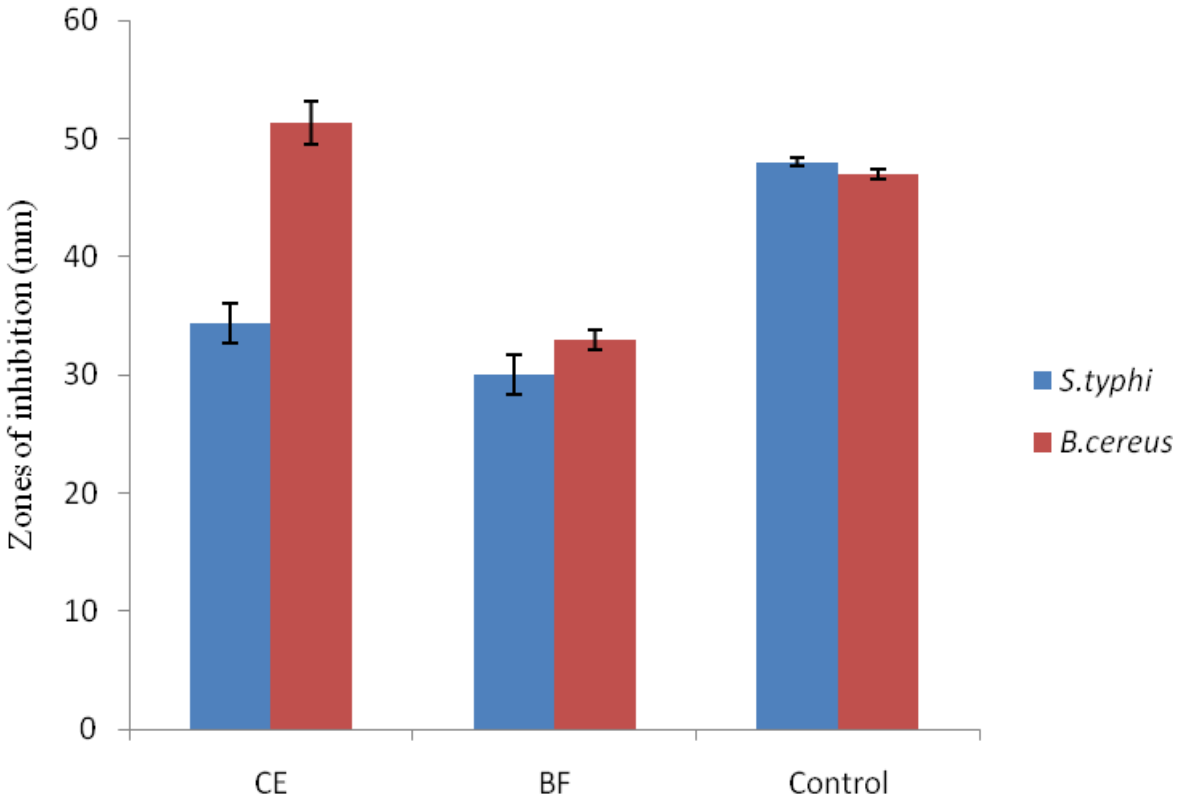

Figure 1. Zones of inhibition of CE and BF of *M. jalapa* leaves. The control is the broad spectrum antibiotic chloramphenicol. Concentrations of CE and control were 20mg/ml while BF was 3mg/ml.

in the extract mixture are directly responsible for the observed activity. Separation of the active components significantly lowered the concentrations needed to inhibit bacterial growth hence increasing the efficacy of the extract. Antimicrobial screening of plants and bioassay guided fractionation has resulted in the characterization of active principles in the search for lead drug candidates (Mattson and Chang, 2006).

The techniques used in the analysis of the bioactive fractions of ethanolic crude extracts of *M. jalapa* suggest the active components are likely to be piperidine and/or isoquinoline based alkaloids. Pivatto et al. (2005) reported the various antimicrobial properties of alkaloid compounds. Other studies have also reported the antibacterial and anti-inflammatory properties of *M. jalapa* alcoholic extracts which may be attributed to individual actions of phytoconstituents like alkaloids and steroids present in it (Kaladhar and Nandikolla, 2010; Nath et al., 2010).

Conclusion

This study demonstrates the potentials of *M. jalapa* alcoholic extract as possible source of active compounds against pathogenic enteric organisms. Further isolation

Table 2. MIC/MBC values of CE and BF of *M. jalapa* leaves.

Organism	MIC (mg/ml)		MBC (mg/ml)	
	CE	BF	CE	BF
S. typhi	5.00	1.00	10.00	2.00
B. cereus	2.50	1.00	5.00	2.00

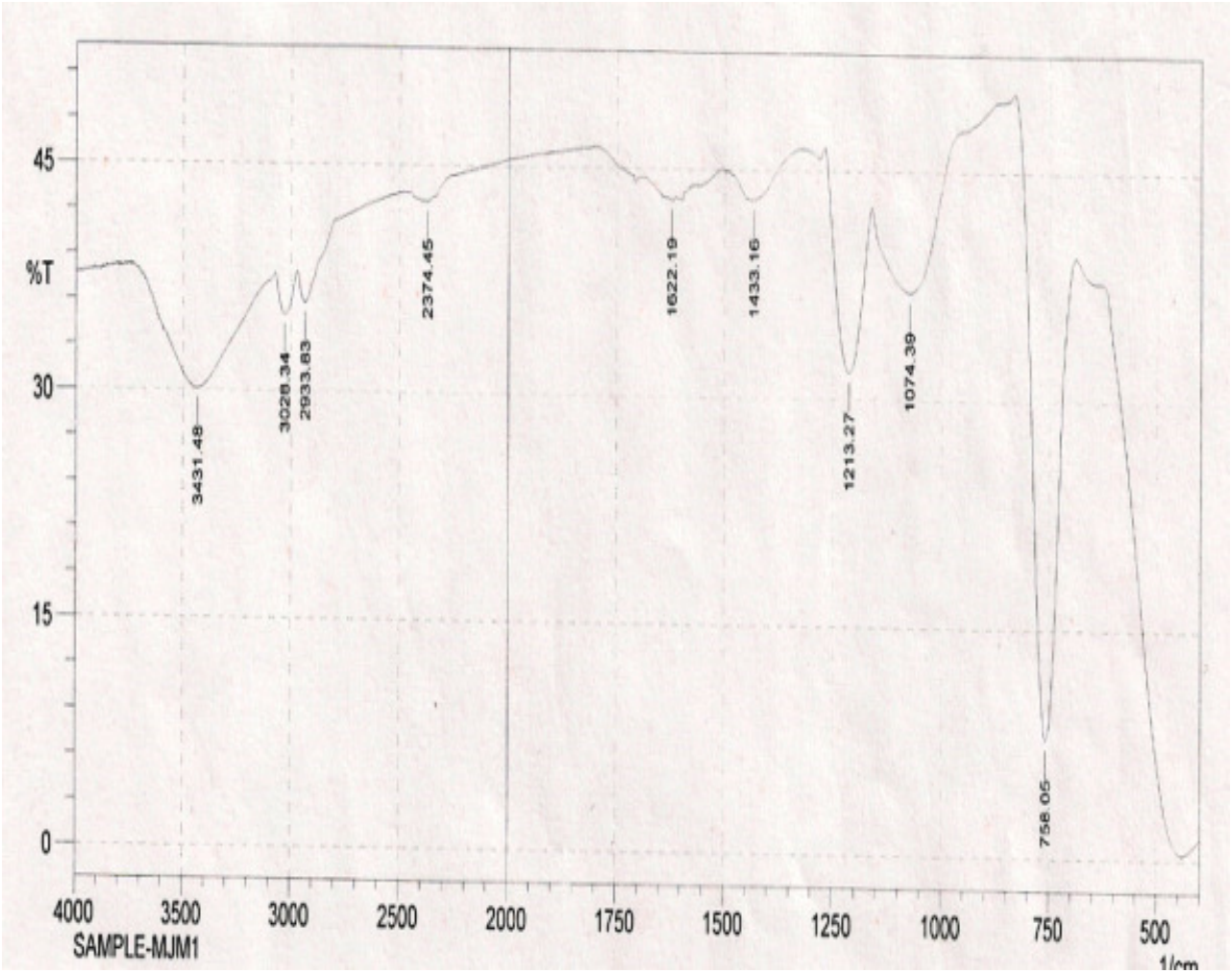

Figure 2. FTIR analysis of the bioactive fractions of ethanolic extract of *M. Jalapa* leaves.

Table 3. Identified compounds of GCMS analysis of BF.

Compound name	Formula	Molecular weight	Retention time (min)	Similarity index (%)
4-phenyl-tetrahydro-pyran-4-nyl-piperidinyl methanone	$C_{17}H_{23}NO_2$	273	41.63	61
Threonine	$C_4H_9NO_3$	119	5.450	70
Isoquinoline-1-(3-benzyloxy 5-hydroxy benzyl)-N-formyl-1,2,3,4	$C_{25}H_{25}NO_4$	403	12.192	64
4-Benzyloxy-3-methoxy-2-nitro benzoic acid	$C_{15}H_{13}NO_6$	303	14.025	81
Oleic acid	$C_{19}H_{36}O_2$	296	19.483	88
2,6,10 trimethyl dodecane	$C_{15}H_{32}$	212	24.233	89
3,8 dimethyl decane	$C_{21}H_{44}$	296	29.975	85

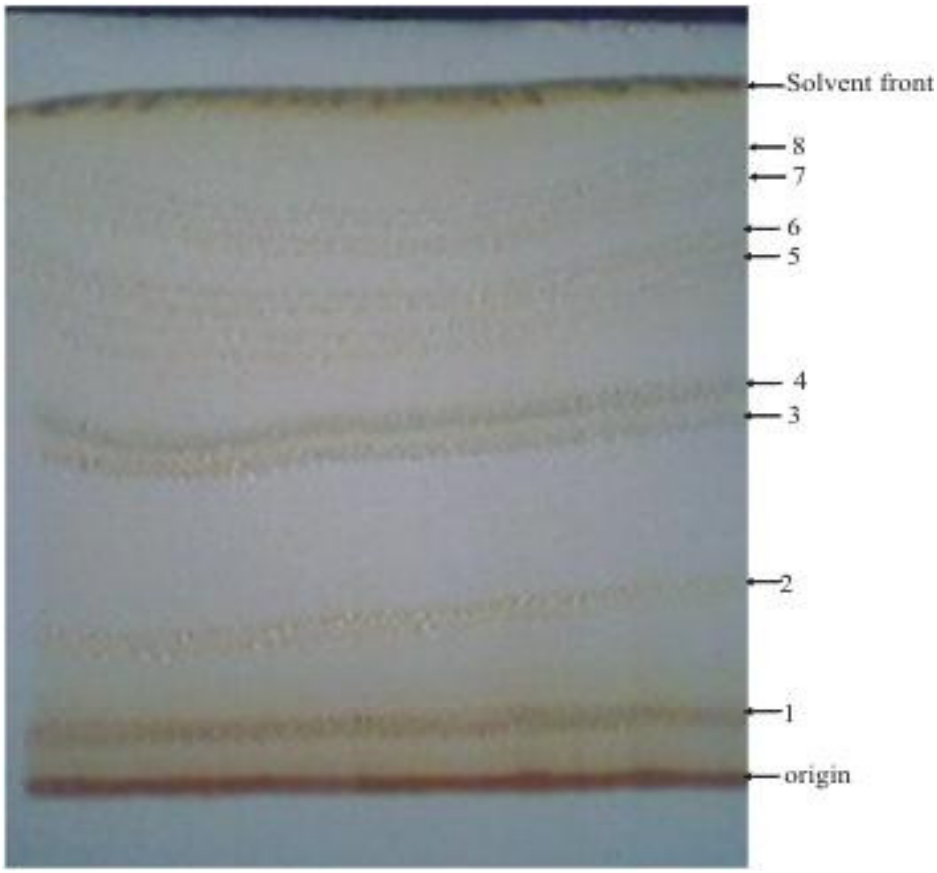

Figure 3. TLC bands of ethanolic extract of *M. jalapa* leaves developed using ethylacetate: chloroform: methanol: water solvent system. The solvent front was 17 cm from the origin.

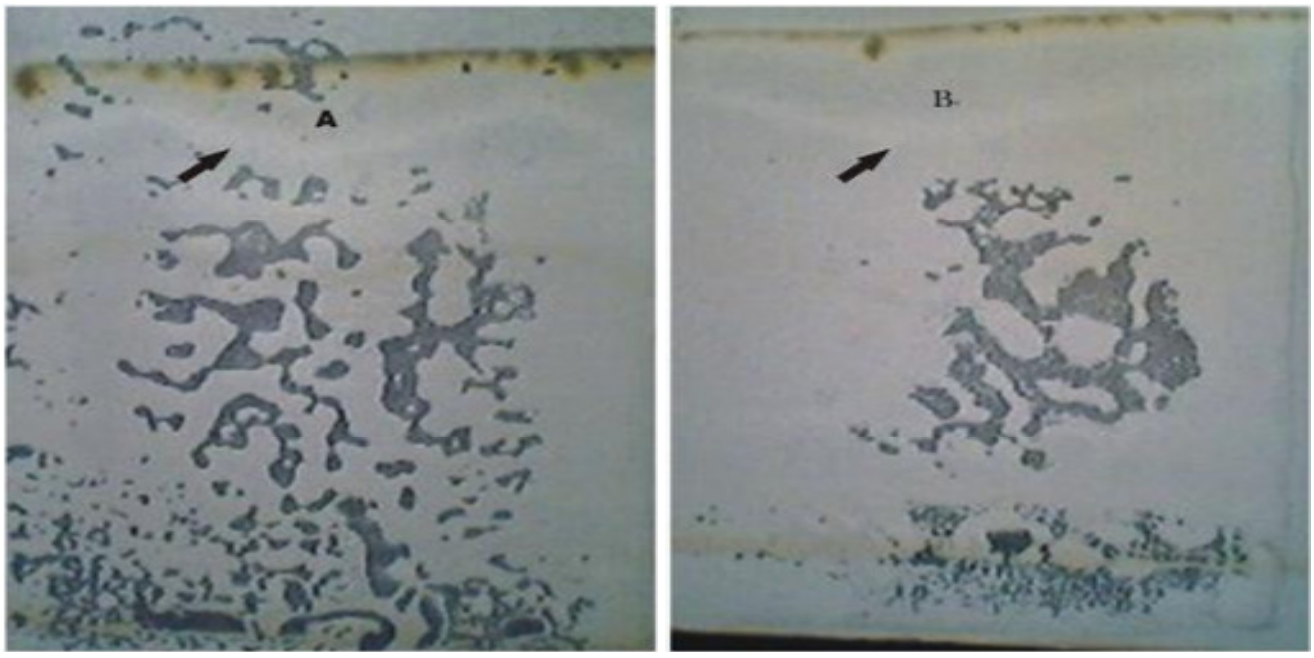

Figure 4. Bioautography for the bioassay of active components present in ethanolic crude extract of *M. jalapa*. Plate 2(A) was inoculated with *S. typhi* and 2(B) with *B. cereus.* The arrows show regions of growth inhibition with R_f values of 0.64.

and characterization of these active components in their pure form may serve as suitable candidates in the design of antibacterial drugs that are of plant origin.

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