Antimicrobial evaluation and phytochemical analysis of leaf extracts of *Mirabilis jalapa* against some human pathogenic bacteria

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Abstract: The aim of this study was to screen for antimicrobial activity and phytochemical constituents of the aqueous and organic solvent extracts of *Mirabilis jalapa* (4'O clock plant) leaf. Samples of *M. jalapa* were collected randomly from the University of Ibadan botanical garden, Ibadan, Oyo State, Nigeria between July and October, 2009 and air dried. Pure cultures of *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi* and *Proteus mirabilis* were obtained from the Medical Microbiology Laboratory, University College Hospital, Ibadan, Oyo State, Nigeria. The cultures were maintained on MacConkey agar slants for a period of 48 hours in a refrigerator before they were subcultured into freshly prepared MacConkey agar slants for nutrient replenishment. The aqueous and organic solvent extracts of *Mirabilis jalapa* (4'O clock plant) leaf were screened for antimicrobial activity and phytochemical constituents using standard techniques. From the results, ethanol extract showed highest antimicrobial effect against some of the tested pathogenic bacteria, followed by Methanol extract. Aqueous and Petroleum ether extracts showed no significant antimicrobial activity. Comparison of the inhibitory effect of the leaf extracts against some broad spectrum antibiotics revealed that Ciprofloxacin had the highest efficacy against the susceptible gram negative bacteria strains used. *Proteus mirabilis* was highly susceptible to the alcohol extracts of *Mirabilis jalapa* compared to the result of its antibiotic sensitivity testing which showed no observable antimicrobial activity. In the case of *Escherichia coli,* there was no noticeable antimicrobial activity with both the aqueous and organic solvent extracts, as well as with the broad spectrum antibiotics used. *Staphylococcus aureus* also showed similar results as *Escherichia coli.* Qualitative analysis of phytochemicals carried out on the leaf extracts revealed the presence of alkaloids, saponins, tannins, and flavonoids, while quantitative analysis showed that alkaloids were present in all the extracts, and in the highest quantity, followed by flavonoids, then tannins. Quantity of saponins was the lowest. Ethanol extract recorded the highest number of phytochemicals identified, followed by the methanol extract, then aqueous extracts. Petroleum ether extracts recorded the lowest value. The results obtained from the *In vitro* antimicrobial assay of the leaf extracts of *Mirabilis jalapa* lend scientific credence for the use of the plant against bacterial infections. This results confirm the therapeutic potency of *Mirabilis jalapa* for use in folklore medicine. The activities observed could be due to the presence of some of the secondary metabolites like alkaloids, saponins, tannins, and flavonoids which have known antimicrobial activity.

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1.0. Introduction

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Medicinal plants are of great importance to the health of individuals and communities. In fact, herbal medicines are known to serve the health needs of about 80% of the world's population; especially for millions of people in the vast rural areas of developing countries rely on traditional or herbal medicines for their primary health care and plants have long formed the basis of sophisticated traditional medicine systems and purportedly provide excellent leads for new drug developments (Pravi, 2006; WHO, 2008; Akinjogunla *et al*., 2009, 2011a).

Herbal medicine is the oldest form of healthcare known to mankind and over 50% of all modern clinical drugs are of natural products origin and natural products play important roles in drug development in the pharmaceutical industry (Preethi *et al.,* 2010).

Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids which have been found to have *in vitro* antimicrobial properties (Edeoga *et al*., 2005). The medicinal value of these plants lies in some chemical substances that provide definite physiological action on the human body. The relatively lower incidence of

adverse reactions to plant preparations compared to modern conventional pharmaceuticals, coupled with their reduced cost consequently encouraged both the consuming public and national health care institutions to consider plant medicines as alternatives to synthetic drugs (Nair *et al*., 2004). Undoubtedly, medicinal plants are relevant in both developing countries and developed nations of the world as sources of drugs or herbal extracts for various chemotherapeutic purposes (Alanis *et al*., 2005).

The rediscovery of the connection between plants and health is responsible for the launching of a new generation of multi-component botanical drugs, dietary supplements and plantproduced recombinant proteins (Akinjogunla *et al.,* 2011a,b).

Mirabilis jalapa (4'O clock plant) is a perennial herb that reaches a height of 50 – 100 cm from a tuberous root. Some cultivated hybrid can grow up to a metre in height. It is a popular ornamental plant grown worldwide for the beauty of its flowers which can be white, red, pink, purple or multicolored and their sweet fragrance (Diggs *et al.,* 1999). *Mirabilis jalapa* (4'O clock plant) has been used in traditional medicine (Nair *et al.,* 2004). This may be due to the presence in it of some biomolecusles which are of pharmacological importance (Edeoga *et al.,* 2005). These biomolecules include terpenoids, arabinose and true alkaloids (Nair *et al.,* 2004).

The aim of this study is to screen for antimicrobial activity and phytochemical constituents of the aqueous and organic solvent extracts of *Mirabilis jalapa* (4'O clock plant) leaf.

2.0. Materials and Methods

2.1. Collection of plant materials

Samples of *Mirabilis jalapa* (4'O clock plant) were collected randomly from the University of Ibadan botanical garden, Ibadan, Oyo State, Nigeria and air dried. The period of collection was between July and October, 2009. The taxonomy of the plant was done and authenticated at the University's botanical garden.

2.2. Collection of test organisms

Pure cultures of bacterial isolates to be used for the in-vitro antimicrobial assay were obtained from the Medical Microbiology Laboratory, University College Hospital, Ibadan, Oyo State, Nigeria. The cultures were maintained on MacConkey agar slants for a period of 48 hours in a refrigerator before they were subcultured into freshly prepared MacConkey agar slants for nutrient replenishment. The organisms collected were *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi* and *Proteus mirabilis.*

2.3. Extraction of *Mirabilis jalapa* (4'O clock plant) leaf

The leaf of the plant was extracted with three different organic solvents, namely; methanol, ethanol, petroleum ether, and an aqueous solvent.

2.4. Solvent extraction of *Mirabilis jalapa* (4'O clock plant) leaf

Seventy grams (70g) of the leaf powder was added to 350ml of each of the organic solvents - 70% methanol, 70% ethanol and petroleum ether until super saturation for a period of 72 hours at room temperature (Oladunmoye, 2007). The leaf extract obtained was protected from sunlight and stirred several times with a sterile glass rod. The resultant suspension was then filtered using muslin cloth. The filtrates were then evaporated under reduced pressure and concentrated in-vacuo using a rotary evaporator at 85°C. The concentrated extracts are then stored in labeled sterile screw cap bottles at 4°C in the refrigerator, until when required for use.

2.5. Aqueous extraction of *Mirabilis jalapa* (4'O clock plant) leaf

Seventy grams (70g) of the leaf powder was weighed out and soaked in 350 ml of distilled water in 500ml conical flask which was thereafter stoppered with a rubber cork and left for 24 hours. After this period, it is filtered using a sterile Whattman no. 1 filter paper into a clean conical flask. It is then subjected to water-bath evaporation where the liquid was evaporated at its boiling temperature of 100°C. The remaining extract obtained was stored in a refrigerator at 4˚C until when required for use (Akueshi *et al.,* 2002).

2.6. pH determination of leaf extract of *Mirabilis jalapa* (4'O clock plant)

The pH of the leaf extract was carried out in order to determine the degree of acidity or alkalinity of the extract. The electrometric method was used which adopted the use of an Exstik^{TM} pH waterproof meter. Three consecutive readings were taken at intervals following a stable value on the equipment.

2.7. *In vitro* antimicrobial assay

The technique used in the *in vitro* antimicrobial assay was the agar well diffusion method by Perez *et al.,* (1990). This technique was used to evaluate the antimicrobial activity of the leaf extracts against the bacterial isolates collected. An antibiotic sensitivity testing for comparative efficacy was also carried out using the disk diffusion test (Bauer *et al.,* 1966).

2.7.1. Agar well diffusion

From fresh broth culture, 0.2 µl of each of the pathogenic isolates were streaked on nutrient agar plate that has a diameter of 90mm, using a sterile inoculating wire loop. Afterwards, six wells at adjacent distance of about 25mm between wells, and towards the periphery of the plates, were punched with a sterile cork borer of diameter 6mm on the agar plate. However, 0.3 ml of each of the leaf extracts (methanol, ethanol, petroleum ether, and aqueous) at varying concentrations (100mg/ml, 200mg/ml, 300mg/ml, 400mg/ml and 500mg/ml) were aseptically dispersed into five of the wells using a 10ml sterile syringe. Distilled water (0.3ml) was introduced into the 6th well to serve as control. The plates were allowed to stand on the laboratory bench for about 40 minutes at room temperature in order to allow for pre-diffusion of the extract in the agar wells (Esimone *et al.,* 1998). Four replicates were made. The plates were afterwards incubated at 37°C for 24 hours. After this period, the plates were observed for zones of inhibition around the well. The observed zones of inhibition were measured using a divider and transparent ruler, and were recorded in millimeters (mm). The degree of antimicrobial activity was evaluated using the values obtained from the readings of the zone of inhibition on each of the agar plates.

2.7.2. Antibiotic sensitivity test

Antibiotic susceptibility testing was done with the use of antibiotic discs (gram negative and gram positive) by the disk diffusion method (Bauer *et al.,* 1966), using broad spectrum antibiotics. Before each antibiotic disc was placed on each of the media surface, the pathogenic bacterial isolates from MacConkey agar slants were streaked on each of the nutrient agar plates after which the antibiotic discs were aseptically placed on each of the agar plate using a sterile forceps. The agar plates were then incubated at 37°C for 24 hours. Afterwards, the plates were examined for zones of inhibition. The zones of inhibition around each antibiotic disc were measured in millimeters using a transparent ruler. Antibiotic susceptibility was evaluated from the readings obtained from the diameter of the zone of inhibition. The antibiotic susceptibility patterns from each of the zones of inhibition observed on the agar plates is referred to as antibiogram.

3.0. Results Analysis

The results obtained from the *in vitro* antimicrobial assay are shown in Table 1. The diameter of the zone of inhibition of each of the four extracts of the leaf of *Mirabilis jalapa* on the agar plate containing clinical isolates were measured in millimeters as shown in Tables 1. The results showed that two of the leaf extracts (methanol and ethanol) had significant antimicrobial activity against three of the strains tested for (*Pseudomonas aeruginosa, Proteus mirabilis* and *Salmonella typhi*). The minimum inhibitory concentration (MIC) of the extracts ranged between 300mg/ml and 500mg/ml. MIC values of 300mg/ml were the same for the alcohol (methanol and ethanol) extracts used against the clinical isolates tested for. *Pseudomonas aeruginosa* and *Salmonella typhi* had the lowest MIC values (300mg/ml) for the alcohol extracts; *Pseudomonas aeruginosa* for the ethanol extract and *Salmonella typhi* for methanol extract. The remaining bacterial strains (*Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus*) for both aqueous and petroleum ether extracts showed no observable minimum inhibitory concentration (MIC).

Table 2 showed antibiotic sensitivity profiles of clinical isolates using standard antibiotics in comparison with the experimental methanolic and ethanolic extracts. Effect of the leaf extracts on clinical isolates compared with that in the antibiograms after antibiotic sensitivity testing is shown in Table 2. It showed the antibiotics used had activities on *Salmonella typhi* and *P. aeruginosa*. It also showed that the antibiotics used had no activity on *E. coli, Proteus mirabilis* and *Staphylococcus aureus*. Only two antibiotics had activities on *K. pneumoniae*. In the same vein, methanolic and ethanolic extracts had activities on the clinical isolates except for *S. aureus*, *E. coli* and *K. pneumoniae* as shown in Table 2. However, the activities of the methanolic and ethanolic extracts on the clinical isolates are comparable with that of standard antibiotics (Table 2).

Table 3 shows the pH of the four leaf extracts. It showed that all the leaf extracts were acidic with aqueous extract having the highest pH value (pH 6.4). This was closely followed by petroleum ether (pH 6.2), methanol (pH 5.3) and ethanol (pH 5.1).

Table 4 shows the qualitative phytochemical characteristics of the leaf extracts of *Mirabilis jalapa*. It showed that alkaloids, saponins, and flavonoids were present in all the leaf extracts. Trepenoids was only detected in aqueous and methanol extracts. Steroids and cardiac glycosides were detected in ethanol extracts only. Phlobatannins and anthraquinones were not detected in any of the leaf extracts as shown in Table 4.

Table 5 shows the quantitative determination of common bioactive constituents in the four extracts of *Mirabilis jalapa* leaf in milligram. The quantitative values for saponins, tannins, flavonoids and alkaloids ranged from 2.50 to 15.00mg for aqueous extract,

7.50 to 22.50mg for ethanol extract, 7.50 to 20.00mg for methanol extract, and 3.75 to 12.50mg for petroleum ether extract respectively as shown in Table 5.

4.0. Discussion

The antimicrobial activity of the four extracts of *Mirabilis jalapa* leaf against the six clinical isolates presented in Table 1 indicates the assessment of the potency of the leaf extracts from the observation for inhibition zones that occurred on the culture plates. Among all the extracts used in this study, the ethanol extract was found to be the most active against the tested bacterial strains, followed by methanol extract. This is similar to the findings of Obi and Onuoha (2000), who reported that alcohol was the best organic solvent for the extraction of most plant bioactive principles of medical importance. Significant antimicrobial activity was not observed in aqueous and petroleum ether extracts. The observed variation in susceptibility pattern of the tested clinical isolates used in this study may be related to the genetic diversity among the organisms. Odoemena and Essien (1995) have reported that genetic properties of tested organisms play a major role in their resistance to effects of the extracts.

Pseudomonas aeruginosa was found to be the most susceptible to the ethanol extract with an inhibition zone diameter ranging between 2mm and 11mm at concentrations between 300mg/ml and 500mg/ml followed by *Proteus mirabilis* with an inhibition zone diameter ranging between 1mm and 3mm at concentrations between 400mg/ml and 500mg/ml then *Salmonella typhi* with an inhibition zone diameter of 3mm at 500mg/ml. The remaining three bacterial strains were resistant to the ethanol extract. The methanol extract showed maximum antimicrobial activity against *Proteus mirabilis* at concentrations between 400mg/ml and 500mg/ml (inhibition zone diameter ranging between 4mm and 6mm), followed by *Salmonella typhi* with an inhibition zone diameter ranging between 2mm and 5mm (of concentrations ranging between 300mg/ml and 500mg/ml). *Pseudomonas aeruginosa* showed the least susceptibility with inhibition zone diameter ranging between 2mm and 3mm.

From all these results, it can be inferred that the activity of the extract is concentration-dependent. This is in an agreement to an earlier report that an increase in the concentration of an antimicrobial agent might result in an increase in its effectiveness (Aspen, 2000). There was no observable susceptibility in *Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus*. All the controls used in the experiment did not yield any zone of inhibition. The low inhibitory concentration observed for the alcohol extracts of *Mirabilis jalapa* leaf may be of great significance in the health delivery system, since it could be used as an alternative treatment to orthodox antibiotics in the treatment of the pathogens used especially as they frequently develop resistance to antibiotics (Singleton, 1999) and this could go a long way in reducing the cost of obtaining good health care.

The alcohol extracts showed greater antimicrobial activity than the corresponding aqueous and petroleum ether extracts. These findings is interesting in that the traditional method of treating a bacterial infection was by administering a concoction of the plant parts or body of the plant in water whereas according to this results, preparing an extract with an organic solvent is shown to provide a better antimicrobial activity which is in accordance with the results obtained by Nair *et al.* (2005). As a result, reports that traditional healers and herbalists use aqueous solution to extract biologically active compounds due to its easy availability (Shale *et al.,* 1999) has been contradicted by the findings of these experiments with alcoholic extracts recording a greater antimicrobial activity. It has been reported by Atawodi *et al.* (2003) that the popularity of an herbal recipe in traditional medicine practice may not necessarily be an indication of its effectiveness.

Aqueous and Petroleum ether extract have been shown to be ineffective against the human pathogenic bacteria used. The absence of zone of inhibition in these extracts made a confirmation that even if antimicrobial constituents are present in the extracts; they are not potent enough to inhibit microbial growth and survival. Oladunmoye (2007) showed that *Mirabilis jalapa* possesses antimicrobial activity against some human pathogenic bacteria. These antimicrobial activities explain many uses of plant in ethno-medicine (Aiyelaagbe *et al.,* 2007).

From the findings of this study, gram negative bacteria (GNB) used are more susceptible to the plant extract than gram positive bacteria (GPB) which in a way contradicts previous reports that plant extracts are more active against GPB than GNB (Kamatou and Viljoen, 2005; Delmare and Moschen-Pistorello, 2007; Tepe and Daferera, 2005). However, results from this study also revealed that alcohol extracts contain certain bioactive constituent with significant antibacterial property which enables the extract to overcome the barrier in gram negative cell wall (Scalbert, 1991). The antimicrobial properties of *Mirabilis jalapa* probably explain its traditional use for treating bacterial disease. Freiburghans *et al.* (1996) indicated that different solvent extracts of some plants may exhibit different pharmacological properties. Also, the susceptibility of the pathogenic bacteria to the alcohol extracts (methanol and

ethanol) or its resistance to the extract (especially aqueous and petroleum ether) at varying concentrations might be ascribed to the differences in the morphology of the cell structure and chemical composition between these organisms as well as variation in permeability and osmotic potential (Hailu *et al.,* 2005).

It is a general belief that GPB are more susceptible than GNB due to differences in the cell wall structure. GNB are considered to be more resistant due to their outer membrane acting as a barrier to many environmental conditions including antibiotics (Tortora *et al.,* 2001). Of all the microorganisms used, *Pseudomonas aeruginosa* recorded the highest susceptibility with the ethanol extracts. This indicates that the extract could be a good first line treatment against infection caused by it.

From the antibiotic sensitivity testing, comparative efficacy with gram positive and gram negative antibiogram against the six clinical isolates were highly encouraging. Gram negative antibiogram showed significantly higher antimicrobial activity against *Pseudomonas aeruginosa, Klebsiella pneumonia* and *Salmonella typhi* around ciprofloxacin and norfloxacin compared to the organic solvent and aqueous extract. These results may be attributed to the methods of production, purification, concentration as well as quality control and assurance of the antibiotics. The presence of likely unidentifiable impurities in the leaf extracts might be responsible for the lower values of zone of inhibition exhibited against the tested microorganisms (Sayed *et al.,* 1987).

Proteus mirabilis frequently associated with urinary tract infection, bacteraemia, pneumonia and focal lesions in debilitated patients or those receiving intravenous infusions (Brooks *et al.,* 2007) is highly susceptible to the alcohol extracts of *Mirabilis jalapa* compared to gram negative antibiogram which showed no observable antimicrobial activity. The absence of zone of inhibition in *Proteus mirabilis* to the gram negative antibiogram could be streamlined to the findings of Thomas (1972), who reported that *Proteus mirabilis* have been found to be more resistant to many of the commonly used antibiotics and is liable to cause super-infection during antibiotic therapy.

Norfloxacin and ciprofloxacin recorded significant antimicrobial activity against *Klebsiella pneumoniae* compared to the organic solvent extract and aqueous extract which showed no observable antimicrobial activity. In the case of *Esherichia coli,* there was no noticeable antimicrobial activity with both the aqueous and organic solvent extracts, as well as with broad spectrum antibiotics. *Staphylococcus aureus* also showed similar results as *Escherichia coli.* However, the increasing problems of multi-drug resistant (MDR) bacteria is of great concern to both the clinicians and pharmaceutical industries and this has made it significant to search for newer drugs that are highly effective, affordable, acceptable and available (Akinjogunla *et al*.. 2010, 2011a).

The findings of this study revealed that the alcohol extracts of *Mirabilis jalapa* leaf is within the acidic range of the pH scale. This acidity may constitute a barrier to the survival of microorganisms because most of them are capable of growth at a neutral pH value and it may be attributable to the acidic nature of tannin (Swaminathan and Kochlar, 1989). A decrease towards the acidic range of the leaf extracts may have contributed to its inhibition to the growth and survival of the clinical isolates used. This agrees with the findings of Akueshi *et al.* (2000) that acidity inhibits growth of microorganisms. The aqueous and petroleum extracts were mildly acidic with aqueous ether extract having a pH of 6.4 and petroleum ether extract having a pH of 6.2. These values are closer to the neutral pH value (7.0) and it may be a reason for their ineffective antimicrobial potency.

The four extracts of the *Mirabilis jalapa* leaf were analyzed for phytochemical constituents. Results from the qualitative phytochemical analysis revealed that the leaf contains some bioactive components. Four of the bioactive components detected were present in all the extracts; they include alkaloids, saponins, tannins and flavonoids. Terpenoids were present only in both methanol and aqueous extract. Steroids and cardiac glycosides were present only in the ethanol extract. This is similar to what was reported by Akinjogunla *et al.* (2011a) in a phytochemical analysis of the aqueous leaf extracts of *Ocimum gratissimum* and *Vernonia amygdalina* which revealed the presence of phyto-constituents as observed in our study. Also in this study, two of the phytochemical constituents were absent in all the extracts and these include anthraquinones and phlobatannins. This is a deviation from what was reported by Akinjogunla *et al.* (2011a), who reported the presence of anthraquinones and phlobatannins in their study.

Table 3: pH of Leaf extracts of *Mirabilis jalapa*

Leaf Extract	pH
Aqueous	6.4
Methanol	5.3
Ethanol	5.1
Petroleum Ether	6.2

Table 1: *In Vitro* Antimicrobial Activities of four graded solvent extracts of *Mirabilis Jalapa* leaf on clinical isolates

Test organisms	Concentration (mg/ml) and zone of inhibition (mm)																			
	100mg/ml				200 mg/ml				300 mg/ml				400 mg/ml				500 mg/ml			
	AE	ME	EE	PE	AE	ME	EE	PE	AE	ME	EE	PE	AE	ME	EE	PE	AE	ME	EE	PE
Escherichia coli	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pseudomonas aeruginosa	0	0	0	0	0	0	0	0	0	0	2	0	0	2	6	0	0	3	11	0
Proteus mirabilis	0	0	0	0	0	0	0	0	0	0	0	0	0	4	1	0	0	6	3	0
Klebsiella pneumonia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Staphylococcus aureus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Salmonella typhi	0	0	0	0	0	0	0	0	0	2	0	0	0	3	0	0	0	0	0	0

Key: **AE:** aqueous extract, **ME:** methanol extract, **EE:** ethanol extract, **PE:** petroleum extract, **0:** No inhibition

Table 2: Antibiotic Sensitivity Profiles of Clinical Isolates using standard antibiotics in comparison with the experimental methanolic and ethanolic extracts

Test organisms	Zone of Inhibition (mm)													
Gram Negative Bacteria (GNB)	**N**	**AG**	**NB**	**T**	**GN**	**CIP**	**C**	**AM**	**NA**	**CF**	**400mg/ml (EE)**	**400mg/ml(ME)**	**500mg/ml(EE)**	**500mg/ml(ME)**
Escherichia coli	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. aeruginosa	0	3	11	5	7	17	6	4	5	4	6	2	11	3
Proteus mirabilis	0	0	0	0	0	0	0	0	0	0	1	4	3	6
K. pneumoniae	0	0	3	0	0	8	0	0	0	0	0	0	0	0
Salmonella typhi	3	6	10	6	9	16	11	7	6	5	0	3	3	5
Gram Positive Bacteria (GPB)	**D**	**CX**	**NB**	**GN**	**E**	**CIP**	**CD**	**SXT**	**AX**	**AC**	**400mg/ml(EE)**	**400mg/ml(ME)**	**500mg/ml(EE)**	**500mg/ml(ME)**
S. aureus	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Keys: **N** = Nitrofurantoin, **AG** = Augmentin, **NB** = Norfloxacin, **T** = Tetracycline, **GN** = Gentamicin, **CIP** = Ciprofloxacin, **C** = Chloramphenicol, **AM** = Ampicillin, **NA** = Nalidixic acid, **CF** = Cefuroxime, **D** = Drovid, **CX** = Cephalexin, **E** = Erythromycin, **CD** = Clindamycin, **T** = Tetracycline, **AX** = Amoxil, **AC** = Ampiclox, **ME**= Methanolic extract, **EE=** Ethanolic extract, **0** = No inhibition.

Table 4: Qualitative phytochemical screening of the four extracts of *Mirabilis jalapa* leaf

Bioactive Constituents	Aqueous Extract	Ethanol Extract	Methanol Extract	Petroleum Ether Extract
Alkaloids	+	+	+	+
Saponins	+	+	+	+
Tannins	+	+	+	+
Phlobatannins	-	-	-	-
Flavonoids	+	+	+	+
Terpenoids	+	-	+	-
Steroids	-	+	-	-
Cardiac Glycosides	-	+	-	-
Anthraquinones	-	-	-	-

Key: + = detected (present), **-** = not detected (absent)

Table 5: Quantitative phytochemical determination of common bioactive constituents in the four extracts of *Mirabilis jalapa* leaf

Bioactive Constituent	Aqueous Extract (mg)	Ethanol Extract (mg)	Methanol Extract (mg)	Petroleum Ether Extract (mg)
Saponins	2.50	7.50	7.50	3.75
Tannins	5.00	7.50	8.75	5.00
Flavonoids	10.00	20.00	15.00	10.00
Alkaloids	15.00	22.50	20.00	12.50

Plants are a rich reservoir of antimicrobials. It is observed that a single plant is known to contain several bioactive principles of biological significance (Cowan, 1999). In this study, the quantitative phytochemical analysis revealed that alkaloids had the highest quantity in all the four leaf extracts, followed by flavonoids, then tannins and lastly, saponins. Alkaloids have been known to have pronounced physiological effect particularly on the nervous system (Sofowora, 2008). The higher quantity of flavonoids determined in the four extracts were similar to the findings of Egunjobi (1969). Also, it is observed from the results that ethanol extract recorded the highest amount of the bioactive components analyzed, followed by methanol extract, then aqueous extract. Bioactive constituents were quantitatively least in petroleum extract. The common bioactive agents found in the alcohol extracts are alkaloids, saponins, tannins and flavonoids. The bioactive components contained in the plant are connected with its antimicrobial properties (Adegoke *et al.,* 2009; Kunle and Egharevba, 2009, Egharevba et al., 2010).

The mechanism of action of the bioactive constituents of *Mirabilis jalapa* may be difficult to speculate, however many antibacterial agents may exhibit their action through inhibition of nucleic acids, proteins and membrane phospholipid biosynthesis (Franklin *et al.,* 1987). It is probable that the bioactive components in the alcohol extracts of *Mirabilis jalapa* act via some of the above. All these are attributable to two reasons; Firstly, the presence and nature of biologically active components whose activity can be enhanced with the alcohol. Secondly, the strong extracting capacity of the organic solvent has produced greater number of bioactive constituents responsible for antimicrobial activity (Okigbo and Omodamiro, 2005).

Antibiotics provide the main basis for the therapy of bacterial infections. However, the genetic variability of bacteria enables them to rapidly evade the action of antibiotics by developing antibiotic resistance (Kumaraswamy *et al.,* 2008). In recent years, development of multidrug resistance in pathogenic bacteria and parasites has created major clinical problems in the treatment of infectious diseases (Davies, 1994). This and other problems, such as toxicity of certain antimicrobial drugs in the host tissue (Idose *et al.,* 1968) have triggered interest in search of new antimicrobial substances/drugs of plant origin. However, knowing the phytochemical constituents of the plant can help one to speculate on the medicinal value of the leaf. These constituents are said to be responsible for antimicrobial activities in plants (Edeoga *et al.,* 2005). The observed antimicrobial activities of the extract may be traced to these bioconstituents. This confirms the ability of the alcohol extract to inhibit *Pseudomonas aeruginosa, Salmonella typhi* and *Proteus mirabilis*.

5.0. Conclusion

Considering the rich diversity of *Mirabilis jalapa* in bioactive constituents, screening of the various natural organic compounds and their identification to reveal the active principles by isolation and characterization of their antimicrobial constituents, must be considered as a fruitful approach in the search of new herbal drugs for folkloric usage. The antimicrobial activities can be advanced if the active components are purified and adequate dosage determined for proper administration. This may go a long way in preventing the administration of inappropriate concentrations, a common practice among many folklore medical practitioners.

The results from the *in vitro* antimicrobial assay confirm the therapeutic potency of *Mirabilis jalapa* for use in folklore medicine. The activities observed could be due to the presence of some of the secondary metabolites like alkaloids, saponins, tannins, and flavonoids which have known antimicrobial activity. In line with the assertions of Kumaraswamy *et al.* (2008), these findings of this study would form a good basis for selection of the plant for further phytochemical and pharmacological investigation, suggesting antibacterial properties that can be used as antimicrobial agents in new pharmaceuticals for the therapy of infectious diseases caused by bacterial pathogens.

However, in line with Stephen *et al.* (2009), further studies should be carried out in order to isolate, characterize and purify the bioactive constituents of the plant with a view to determining its spectrum of activity as well as adding it to already established antimicrobial agents especially those that are active against resistant strains of bacteria.

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