Antimicrobial Activity of Aqueous Methanol Extracted
Rigla (*Portulaca oleracea* Linn)

Mohammed Abdel Wahab Mohammed Gismalla
B.Pharm.Omdurman Islamic University, Oct 2004

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Department of Pharmaceutics
Faculty of Pharmacy

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Mohammed Abdel Wahab Mohammed Gismalla

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Antimicrobial Activity of Aqueous Methanol Extracted Rigla (*Portulaca oleracea* Linn)

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Declaration

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree of the university or other institute, except where due acknowledgment has been made in the text.

Name: Mohammed Abdel Wahab Mohammed Gismalla
Signature: .............................................
DEDICATION

This work is dedicated to

To my father, to

My lovely mother, to

My brothers and sisters, to

My sweet wife and kids,

To my family,

To

My teachers and friends

To all those who believe in the richness of learning
I am deeply thankful to God almighty upon completion of this work successfully. My thanks extended to my supervision committee Dr. Hassabelrasoul Elfdil Hassan and Prof. El-hadi Mohamed M Ahmed, for their valuable advices, insightful criticisms, and patient encouragement.

Also I would like to express my gratitude to the Faculty of Pharmacy, University of Gezira for giving me the opportunity, time and place to conduct this work . I am grateful to many persons who shared their memories and experiences, especially Dr. Sahar Fadalla Aljak, Dr. Waleed Elsidig and Dr. Muhammed Alhassan . I must acknowledge as well the members of the microbiology Laboratory, Faculty of Pharmacy, University of Gezira, especially Abdullah Mustafa for his kind assistance throughout this work. My thanks to my wife Dr. Hiba Albasheer, and to all my family members and colleagues for their motivation and unconditional support.
Antimicrobial Activity of Aqueous Methanol Extracted
Rigla (Portulaca oleracea Linn)

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Abstract
Many of the available antimicrobials especially antibacterials are no longer effective because of emerging resistance, caused by many factors. Scientists and pharmaceutical industries considered medicinal plants as a good choice for research for new antimicrobials. Portulaca oleracea Linn (Portulacaceae) common (Purslane, Rigla) exhibits a wide range of pharmacological effects including antiseptic and antibacterial.

The objectives of this study is to investigate the antimicrobial activities of methanol (70%) extract of fresh Portulaca Oleracea Linn (whole plant) against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae and fungal Candida albicans clinical isolates. The study made use of the Gel Diffusion Sensitivity Method, and their MICs were determined by using Broth Two Fold Dilution Method. Results obtained showed that, the aqueous methanol (70%) extract of Portulaca oleracea Linn possessed a significant antibacterial activity against S.aureus with MIC 10 mg/ml, the plant extract was found to inhibit the growth of P. aeruginosa with MIC 25 mg/ml, while against P. mirabilis and K. pneumoniae the plant extract produced an inhibitory effect at MIC of 50 mg/ml. At concentration up to 100 mg/ml the plant extract inhibited E. coli and failed to inhibit the growth of C. albicans. It could be concluded and recommended that Portulaca Oleracea Linn is a rich vegetable source of antibacterial bioactive molecules.
النشاط المضاد للميكروبات مستخلص الميثانول المائي
من نبات الرجلة (أو البقلة الحمقاء)

محمد عبدالوهاب محمد قسم الله
درجة ماجستير الصيدلة في الأحياء الدقيقة الصيدلانية
كلية الصيدلة جامعة الجزيرة

ملخص الدراسة
لم تعد العديد من مضادات الميكروبات المتوفرة حالياً (خصوصا مضادات البكتريا) ذات فعالية دوائية مفيدة و ذلك بسبب المقاومة الدوائية الناشئة نتيجة العديد من العوامل المطلوبة والموضوعة. أدى ذلك إلى اتجاه العديد من العلماء والأبحاثين في مجال الصناعات الدوائية للاهتمام بالنباتات الطبية كخيار مثالي مبتكر والبحث في استخلاص مضادات جديدة للميكروبات ذات أصول نباتية. نبات الرجلة (أو البقلة الحمقاء) عرف كنبات له بعض التأثيرات الدوائية بما في ذلك الأثر المطهر والمضاد للجراثيم. هدفت هذه الدراسة إلى التقصي عن النشاط المضاد للميكروبات من مستخلص الميثانول المائي (70%) من نبات الرجلة (البقلة الحمقاء) الطازج ضد عدد من العزلات السريرية وهي المكورات العنقودية الذهبية، الإشريكية القولونية، الزائفة الزنجارية، المتقلبة الرائعة، الكلبسيلة الرئوية والثقافات الفطرية من المبيضات البيض. وذلك باستخدام طريقة الإنتشار على قرص الجل، وتحديد أقل تركيز ثبتت باستعمال طريقة التخفيف المضاعف في المرق السائل. أظهرت النتائج أن مستخلص الميثانول المائي (70%) من نبات الرجلة (البقلة الحمقاء) يمتلك نشاطاً ملحوظاً كمضاد للبكتيريا موضع الدراسة. حيث أثبتت الدراسة فعالية مستخلص الميثانول المائي للبقلة الحمقاء على المكورات العنقودية الذهبية مع تركيز ثبتتتي أدنى بحوالي 10 ملغ/مل. كما أثبتت فعالية على الزائفة الزنجارية مع تركيز ثباتي أدنى بحوالي 50 ملغ/مل. وعند تركيز 100 ملغ/مل أثبت المستخلص الميثانول فعالية ضد الإشريكية القولونية وفشل في تثبيط الثقافات الفطرية من المبيضات البيض. خلصت الدراسة إلى أن نبات الرجلة (البقلة الحمقاء) غني بمركبات مضادة للميكروبات. وأوصت الدراسة إجراء بحوث أخرى متعمقة للعديد الطبية من النبات.
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1. Introduction

1.1. Background

Antimicrobials have always been considered as one of the wonder discoveries of the 20th century. This is true, but the real wonder is the rise of antibiotic resistance in hospitals, communities and the environment concomitant with their use. Since the discovery of antimicrobials they play a major role in the treatment of infectious diseases. Many of the available antimicrobials especially antibacterials are no longer effective because of emerging resistance, which is caused by many factors such as inappropriate antimicrobial description by prescribers (rarely including sensitivity testing), misuse and self-prescription by consumers, counterfeit medicines, inappropriate storage of drugs, lack of patient knowledge in dealing with antimicrobials, in addition, lack of continuous medical professional training for the prescribers as well as pressure from the pharmaceutical industries. All these factors and many others are some of the important determinants influencing resistance (Xiang et al., 2005).

Bacterial and fungal resistance is an increasing threat to the successful treatment of nosocomial infections. As bacterial resistance continues to evolve, some pathogens that were once considered routine to be treated have become resistant to almost all antimicrobial agents. Many strains of pneumococci, staphylococci, enterococci, and tuberculosis are currently resistant to most or all antimicrobials which were once effective. Multi-drug resistant Klebsiella and Pseudomonas aeruginosa are prevalent. This problem is particularly critical in developing countries where more expensive second-line antibiotics may not be available or affordable, also beside the well known pathogens, resistance has been appeared in opportunistic microorganisms (Brink, 2005).

Antibiotic resistance occurs when bacteria change in some way that reduces or eliminates the effectiveness of drugs, chemicals or other agents designed to cure or prevent the infection, thus the bacteria survive and continue to multiply causing more harm (Bisht et al., 2009).

Antimicrobial resistance results in increased illness, deaths and health-care costs, which is highlights the need for novel antimicrobial agents and opening a new era of naturally occurring antimicrobials.
Scientists and pharmaceutical industries considered medicinal plants are a good choice for research for new antimicrobials, because these natural resources have ordinary fewer side effects, costless and contains effective antimicrobials components (Londonkar et al., 2012).

The use of plants as medicines predates written human history, it is estimated that about 60 to 70% of people living in developing countries still rely on plant medicine, and 80% of the world’s population presently uses herbal medicine for some aspects of primary health care according to WHO survey at 2012 (Agyare et al., 2015).

Portulaca oleracea Linn (Portulacaceae) common known as Purslane and Rigla in Arabic (Fig 1) has spread throughout the world as an edible and nutritious plant. It has been used in folk medicine in many countries as a diuretic, febrifuge, antiseptic, antispasmodic and vermifuge. It exhibits a wide range of pharmacological effects including antibacterial, analgesic, anti-inflammatory, skeletal muscle relaxant, wound-healing activities, animal bites and bleeding (Xiang, et al., 2005; Agyare et al., 2015).

1.2. Rationale

Antimicrobial resistance (AMR) is an increasingly serious threat to global public health, it develops when a microorganism (bacteria, fungus, virus or parasite) no longer responds to a drug to which it was originally sensitive. This means that standard treatments no longer work, infections are harder or impossible to control, the risk of the spread of infection to others is increased, illness and hospital stays are prolonged. All these will added economic and social costs and the risk of death is greater in some cases twice that of patients who have infections caused by non-resistant bacteria. Considering that the need for newer antimicrobials is challengeable, and for that, this study seeks to investigate the antimicrobial activities of methanol (70%) extract of fresh "Rigla" Portulaca Oleracea Linn (whole plant) against some bacterial and fungal cultures, especially the plant is well known as safe, edible and nutritious food.
1.3. Objectives of the study

1.3.1. General objective

- *In Vitro* assessment of antimicrobial activity of fresh "Rigla" *Portulaca oleracea* Linn methanol (70%) extract.

1.3.2. Specific objectives

- To determine the yield of 24 hours of methanol (70%) extract of fresh *Portulaca oleracea* Linn and it's total percentage.
- To investigate the antimicrobial activity of methanol (70%) extract of *Portulaca oleracea* Linn against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae* and *Candida albicans* clinical isolates, by determination of the zones of inhibition using well diffusion assay method.
- To determine the Minimum Inhibitory Concentration (MIC) of *Portulaca oleracea* Linn extract to each microorganism exhibits activity, using broth two fold dilution method.
2. Literature Review

2.1. Botanical view

2.1.1. Common Names of *Portulaca oleracea* Linn

*Portulaca oleracea* Linn. (*Portulacaceae*) commonly known as Purslane (in USA and Australia), Baqlatul humqa, Khurfa, Baqlatul mubarika and Rigla (in Arabic), Pigweed (in England), Pourpier (in France), Ma-Chi-Xian (in China) Papasan (in Yoruba), Babajibji (in Hausa), (Ntioke, Ntilimoke, Ntiike or Idiri in Igbo), Rudravanti (in Hindi), Dahna (in Oriya) and Nuner (in Kashmiri). The name Portulaca is thought to be derived from the Latin ‘porto’ to carry ans ‘lac’ meaning milk, since the plant contains a milky juice. And has been reported officially in the French, Mexican, Spanish, and Venezuelan Pharmacopoeias (Zhou *et al*., 2015; Okafor *et al*., 2014; Masoodi *et al*., 2011; Sultana *et al*., 2013).

2.1.2. Scientific Classification of *Portulaca oleracea* Linn

Kingdom – *Plantae*

Subkingdom – *Tracheobionta*

Superdivision – *Spermatophyte*

Division – *Magnoliophyta*

Class – *Magnoliopsida*

Subclass – *Caryophyllidae*

Order – Caryophyllales

Family – *Portulacaceae*

Genus - *Portulacae L.*

Species - *Portulacea Oleracea* Linn (Okafor *et al*., 2014).
2.1.3. Description and characteristics features of the plant

2.1.3.1. Macroscopic characteristics

The plant has a round, smooth, procumbent or prostrate, and succulent stem, growing about six inches high, with small, oblong, wedge-shaped, dark-green leaves alternate clustered at stem joints and ends, thick and stalked, destitute of the bristle in their axils which others of the genus have. The flowers are small, yellow, solitary or clustered, stalkless placed (0.24 inches). The reddish stems originate from a central rooting point, radiating out like spokes of a wheel. The stems vary in length, commonly up to 12 inches. Leaves are stalkless (sessile), oval, smooth, succulent, and shiny, and vary from 0.5 to 2 inches in length, the leaves, although generally arranged opposite, very short petiolated, stipular appendages minute or absent, taste sour without any smell, petiole short about 1 to 1.5 mm long and 0.5 mm thick with greenish upper surface and reddish lower. Seeds are borne in a small pod with a top that comes off like the lid on a cookie jar. The seeds of an individual plant have been known to produce both green and golden leaved plants. Seeds are reddish brown to black, oval, and tiny (about 0.02 to 0.03 inch in diameter). Common Purslane is a prolific seeder, a single plant may produce 240,000 seeds, which may germinate even after 5 - 40 years. (Okafor et al., 2014; Masoodi et al., 2011).

Figure 1: Portulaca oleracea Linn (Portulacaceae) plant.
2.1.3.2. Microscopic characteristics

In transverse section, the whole mesophyll consists of almost solely of aqueous tissue; the vascular bundles are surrounded by a sheath of green palisade cells. The eragstic substance occurs in the form of prismatic and rosettes of calcium oxalate crystals of different sizes. Transverse section of petiole reveals that the lower surface is comparatively very much bulged, while the upper one is slightly depressed. The uniseriate epidermis is made up of tangentially elongated tubular parenchymatous cells. The anticlinal wall of lower epidermal cells is curved and cells contain some dark pigment too. Ground tissue comprised of 4-6 layers of thin walled, rounded parenchymatous cells having distinct intercellular spaces. The vascular bundle about 2-4 in number are collateral, closed, placed more or less centrally and arranged in an arch which opens towards adaxial side. Vesicles having helical and scaliferous thickenings show simple perforations, fibers often grow intrusively (Okafor et al., 2014; Masoodi et al., 2011).

2.2. Distribution and History

The plant has an extensive distribution, assumed to be mostly anthropogenic, throughout the Old World extending from North Africa and Southern Europe through the Middle East, China, Japan, Ascension Island, India Subcontinent to Malaysia and Australia. The species status and how it reached the New World is currently unknown. In general, it is considered an exotic weed, however, suggesting that it reached North America in the pre-Columbian era. Scientists suggested that the plant was already eaten by native Americans, who spread its seeds. It is naturalized elsewhere and in some regions is considered an introduced weed. It is a weedy summer annual species that is abundant throughout the world, invading vegetable gardens, bare areas, low-maintenance lawns, ornamental plantings, and agricultural areas. It is particularly well adapted to the warm, moist conditions. In general Purslane is found all over the world, in the temperate countries (Okafor et al., 2014; Masoodi et al., 2011).
2.3. Phytochemical constituents

Some of the reported biologically active compounds in Purslane include alkaloids, coumarins, flavonoids, cardiac glycosides, anthraquinone glycosides, alanine, catechol, saponins, tannins and organic acids like oxalic acid, cinnamic acids, caffeic acid, malic acids and citric acids. Furthermore, the occurrence of glutathione, glutamic acid and aspartic acid. Moreover, the whole plant contains large amounts of 1-norepinephrine (0.25% in fresh herb), soluble carbohydrates, fructose, vitamins, A, B1, B2, B6 and it is rich in ascorbic acid. The seeds contain 17.4% of a fixed oil containing β-sitosterol. The leaves contain 0.42% mucilage, which is composed of an acidic and a neutral fraction. The acidic fraction consists of galacturonic acid residues joined by α-(1→4) linkages. The neutral fraction is composed of 41% of arabinose and 43% of galactose residues, besides traces of rhamnose residue. The fatty acid content in *P. oleracea* ranged from 1.5 to 2.5 mg/g of fresh mass in leaves, 0.6 to 0.9 mg/g in stems and 80 to 170 mg/g in seeds. The β-carotene content ranged from 22 to 30 mg/g fresh mass in leaves. Longer-chain omega-3 fatty acids were not detected. α-Linolenic acid accounted for around 40% and 60% of the total fatty acid content in leaves and seeds, respectively. It is the uniqueness of Purslane as the "richest vegetable source" of omega-3 fatty acids and protein compared to other vegetables has been concluded. A water-soluble anionic low molecular weight polysaccharide (gum) with surface, interfacial, and emulsification properties was extracted from leaves of *P. oleracea* and named *P. oleracea* gum which probably considered as a new good food emulsifier (Sultana *et al.*, 2013).

2.4. Benefits of Purslane

2.4.1 Folkloric benefits

Purslane in ancient times was looked upon as one of the anti-magic herbs, and strewn around a bed was said to afford protection against evil spirits and it was believed to protect that person from having nightmares. If carried was supposed to attract love and luck. It was carried by soldiers to protect them in battle. It is under the dominion of the moon and is supposed to work on the psychic senses and taken regularly helps develop clairvoyant faculties. In Ghana it is an emblem of peace and is mixed with oil to act as a palliative against evil spirits. It has use in religious ceremonies and in purification after
sickness. It is a children's charm for good luck. In Yoruba folklore all the plants of the forest owed money except papas and who paid his debts. In Lesotho the plant is a protection against illness and lightening. It is used as a charm by the Suto (Okafor et al., 2014).

2.4.2 General Benefits

The young leaves are a very acceptable addition to salads, their mucilaginous quality also making them a good substitute for okra as a thickener in soups. Older leaves are used as a potherb. The seed can be ground into a powder and mixed with cereals for use in gruels, bread, pancakes. The plant is antibacterial, antiscorbutic, depurative, diuretic and febrifuge, and the fresh juice is used in the treatment of strangury, coughs and sore. The leaves are poulticed and applied to burns, both the leaves and the plant juice are particularly effective in the treatment of skin diseases and insect stings. A tea made from the leaves is used in the treatment of stomach aches and headaches. The leaf juice is applied to earaches, it is also said to alleviate caterpillar stings. The leaves can be harvested at any time before the plant flowers, they are used fresh or dried. This remedy is not given to pregnant women or to patients with digestive problems. The seeds are tonic and vermifuge. They are prescribed for dyspepsia and opacities of the cornea. To complete the range of its applications, one could mention its use as an insecticide, in which case its juice is poured on to anthills, and also its ornamental use in Roman and medieval gardens. Another authority declared that the distilled water took away pains in the teeth, the seeds, bruised and boiled in wine, were given to children as a vermifuge. In Africa, the whole plant is considered antiphlogistic (takes the heat out) and bactericide in bacillary dysentery, diarrhoea, hemorrhoids, enterorrhagia. It has been used in prescriptions as an antidiabetic. Externally it is used as a cataplasm of fresh leaves for maturing of abscesses. The seeds are also calming and will help slake a thirst. An infusion is used as antihelmintics for children to expel roundworms, in high doses as an emetic and also as a cooling drink, with a mild diuretic effect. In Nigeria the plant is used as a diuretic, the bruised leaves are used in external application for erysipelas, treatment of burns and are applied topically to swellings. In Benin area, the plant along with other ingredients is taken as an aid to the development of the foetus (Okafor et al., 2014).
2.5. Antimicrobial activities of "Rigla" Portulaceae Oleracea Linn

The methanolic extract of Portulaca oleracea leaves showed pronounced activity against Pseudomonas syringae pv, Bacillus subtilis, Vibrio cholerae and Yersinia pseudotuberculosis, however, none of the water extracts showed any antibacterial activity against these microorganisms (Ercisli et al., 2008).

The hydroalcoholic extract of Portulaca oleracea Linn leaves and seeds had antibacterial effects on selected drug resistant bacterial strains of Streptococcus pyogenes, S. pneumoniae, S. saprophyticus, Hafnia alvei, Acinetobacter baumannii, Enterococcus faecalis, Proteus mirabilis, Serratia marcescens, and Staphylococcus aureus, it was tested by using agar well diffusion method. The highest MIC for the Portulaca oleracea leaves was 200 ppm and three strains were inhibited at this concentration, while the highest MIC for the Portulaca oleracea seeds was 100 ppm. The lowest MIC concentration for Portulaca oleracea seeds and leaves was 50 ppm and eight strains were inhibited at this level. The leaves and seeds extract of Portulaca oleracea has a remarkable antibacterial effect and it can be a good alternative when we are faced with drug resistant bacteria (Mousavi et al., 2015).

Antibacterial activities of aqueous and ethanolic extracts of Portulaca oleracea L could inhibit the four pathogenic bacteria responsible of cow mastitis (Escherichia coli, staphylococcus aureus, Streptococcus agalactiae and Streptococcus dysgalactiae) at different levels. For Portulaca oleracea L, ethanolic extracts had higher antibacterial activities than aqueous extracts except for against Escherichia coli in which the maximum inhibition zone was 22.7 mm (Peng, 2014).

Whole plant of P. oleracea extracted in ethanol was found inhibitory to Bacillus subtilis and those extracted in chloroform, ethanol and hexane to Rhizobium leguminosarum, the species failed to prove antagonistic to E. coli (Banerjee et al., 2003).

A pectic polysaccharide isolated from the aerial part of this P. oleracea displays anti herpes property against simplex virus type 2 which is due to the inhibition of virus penetration and not virus adsorption (Dong et al., 2010).

A 70% methyl alcohol extract of Portulaca oleracea shows antibacterial activity against the Gram negative stains Escherichia coli, Pseudomonas aeruginosa, and Neisseria gonorrhea with inhibition zones of 14, 15, and 15 mm, respectively, and the Gram-
positive strains: Staphylococcus aureus, Bacillus subtilis, and Streptococcus faecalis with inhibition zones of 13, 14, and 15 mm, respectively, as well as antifungal activity against Candida albicans with inhibition zone of 12 mm (Elkhayat et al., 2008).

Antimicrobial effect of P. oleracea ethyl acetate extract on food borne pathogens was found to have highest anti microbial activity against Staphylococcus aureus and Shigella dysentrica in comparison to petroleum ether, chloroform and methanol extracts. The ethyl acetate extract of P. oleracea showed strong antimicrobial activity against Staphylococcus aureus at 4000 ppm concentration, this concentration also retarded S. dysentrica (Bae, 2004).

Aqueous and ethanolic extracts of root and leaves of P. oleracea were screened for antimicrobial activity against two gram-positive bacteria (Bacillus subtilis, Staphylococcus aureus), one gram-negative bacterium (Pseudomonas aeruginosa) and a mould Aspergillus niger by agar diffusion method. The highest antibacterial and anti fungal activity was observed at the concentration of 750 μg/ml. Ethanolic root extract was more potent to inhibit growth of Pseudomonas aeruginosa, while aqueous extract was comparatively more potent for other three microbes (Dhole et al., 2011).

The antibacterial activity of Portulaca oleracea L were evaluated on Multi Drug Resistant (MDR) strains of Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis, Salmonella spp, Enterococcus faecalis, Citrobacter freundii, Acinetobacter baumannii, Streptococcus pneumoniae, Enterococcus faecium and Enterobacter cloaceae. Antibacterial activity of five different solvent extracts (Methanol, acetone, ethanol, petroleum ether and n-Hexane) were prepared by using Soxhlet extractor. In Vitro antibacterial activity was performed by agar well diffusion method, and the maximum antibacterial activity of leaves of Portulaca oleracea L found in methanolic extract, while, the highest zone of inhibition of methanolic leaves extract was found against E.coli (26 mm) followed by S. aureus (24 mm), S. pneumoniae (24 mm), K. pneumoniae (22 mm), S. typhi (22 mm) whereas in ethanolic extract maximum zone of inhibition was found against S. pneumoniae (22 mm), E. coli (20 mm), S. aureus (18mm), C. freundii (18 mm) and K. pneumoniae (18 mm). The lowest MIC value was found in methanolic extract was 0.79 mg/ml against S. aureus, E.coli and S. pneumoniae alternatively the lowest MIC value of ethanolic extract
was 1.56 mg/ml found against *S. aureus*, *E.coli*, *S. typhi*, *E. faecalis*, *A. baumannii* and *S. pneumoniae* (Wasnik *et al.*, 2016).

By using disc diffusion assay, the mean diameters of the inhibition zones of apigenin (which is a flavonoid isolated from aerial part of *P. oleracea L*) against five pathogenic bacterial strains *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Salmonella typhimurium*, *Proteus mirabilis*, *Enterobacter aerogenes*, among all bacterial strains tested, *Salmonella typhimurium* (17.36 ± 0.18) and *Proteus mirabilis* (19.12 ± 0.01) have shown significant (*P* < 0.05) zone of inhibition, where remaining bacterial strains have shown less significant (*P* < 0.05) zone of inhibition when compared with control values (14.56 ± 0.21) and (11.68 ± 0.13), respectively. The experimental data demonstrated that the apigenin of *Portulaca oleracea* L, has displayed the antibacterial activity with MIC value > 4 mg/ml against all pathogenic bacterial strains. The MIC values did not change after 48 hours and also did not correlate well with the diameter of inhibition zones from the disc diffusion assay (Nayaka, 2014).

In investigating antibacterial activity of total flavonoids extracted from aerial part of *Portulaca oleracea* L, five standard pathogenic bacterial strains were used like *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Proteus mirabilis*, *Klebsiella pneumonia* and *Enterobacter aerogenes*, among all the bacterial strains *Salmonella typhimurium* (14.33±0.2886) and *Proteus mirabilis* (17.16±0.0281) had shown maximum zone of inhibition for total flavonoids and remaining bacterial strains have shown moderate zone of inhibition when compared with control (20.66±0.2881). In case of bio-assay method *Salmonella typhimurium* was shown more sensitive by low turbidity of OD value (0.187) indicating most significant result. The Minimum inhibitory Concentration (MIC) of the total flavonoids isolated from *Portulaca oleracea* L was tested at the concentration ranging from undiluted sample to 10mg/ml. the minimum inhibition concentration (MIC) for the total flavonoids for all tested bacterial strains was >10mg/ml. Experimental results supports that these flavonoids have antibacterial properties which helps in the developing antibacterial agents in the form of drugs for the therapy of infectious diseases caused by these bacterial pathogens (Londonkar *et al.*, 2012).
Two active ingredients, namely linoleic and oleic acids, were identified from *Portulaca oleracea* found with synergistic antibacterial activity when combined with erythromycin against MRSA RN4220/pUL5054 and possibly act by inhibiting the efflux pumps of the bacteria cells (Chan *et al*., 2015).

The antifungal activity of *P. oleracea* extracts against hyphal growth of various fungi was evaluated in real time using an automatic single-cell bioassay system. The antifungal activity of each fraction of *P. oleracea* was evaluated based on the dynamic hyphal growth response curves of test fungi Aspergillus and Trichophyton and the yeast Candida, a crude sample obtained by ethyl acetate extract showed a specific and marked activity against dermatophytes of the genera Trichophyton (Oh *et al*., 2000). Fungitoxicity of aqueous and organic solvent (e.g. hexane, ethanol and chloroform) extracts were tested against *Aspergillus niger*, *Rhizopus artocarpi* and Fusarium sp, by agar cup assay and filter disc methods, hexane and aqueous extracts showed antifungal activity against Fusarium sp, while ethanol and chloroform extracts of the same herb inhibited the growth of *Rhizopus artocarpi* (Banerjee *et al*., 2002).
3. Materials and Methods

3.1. Description of the Study
The study was experimental study, searching for new antimicrobial activity of natural product. The whole work has been done in the pharmacognosy, pharmaceutics and microbiology laboratories of the faculty of pharmacy, University of Gezira, during February 2017.

3.2. Collection and processing of *Portulaca oleracea* Linn Plant material

3.2.1. Collection of *Portulaca oleracea*
The fresh plant was purchased in at morning, from Wad Madani Central Market for vegetables and fruits, then verified and authenticated by the department of Pharmacognosy, faculty of pharmacy, University of Gezira. The whole plant was included in the study (aerial part and root).

3.2.2. Processing of *Portulaca oleracea*
First the plant was washed with tap water then distilled water, weighted, cut by stainless steel cutter into small pieces and homogenized with electric blender while fresh.

3.3. Methods

3.3.1. Process of extraction method
500 gm of processed Rigla plant *Portulaca oleracea* Linn (Portulacaceae) was extracted by cold maceration with 1500 ml of (70%) methanol with intermittent shaking for 24 hours. Then it was filtered by using multiple layers of sterile cotton under suction pressure maded by Bokhner fennel. The filtration process was done twice for the extract. The solution obtained was evaporated to dryness under vacuum using rotatory evaporator at 60 °C, then, freeze dried ( AMSCO/FINN-AQUA. LYOVAC GT 2®, Germany ) to calculate the yield percentage and kept in a refrigerator until use.
3.3.2 Preparation of extract solutions of different concentrations

Solutions of *Portulacae Oleracea* Linn extract were prepared in concentrations of 200, 100, 20, 10 and 1 mg/ml by using a sterile distilled water (w/v dilution), and kept in closed, well labeled containers for antimicrobial testing (well diffusion and MIC testing).

3.3.3. Antimicrobial susceptibility testing

3.3.3.1. Microorganisms isolates

Well identified bacterial and fungal cultures were used, they include one gram positive bacteria (*Staphylococcus aureus*), four gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae*) and one fungi (*Candida albicans*). Cultures were obtained from the Laboratory of microbiology, Faculty of Pharmacy, University of Gezira.

3.3.3.2. Preparation and selection of culture Media

3.3.3.3. Nutrient Broth preparation

Seven grams of Nutrient broth medium was suspended in 250 ml distilled water, then sterilized by autoclave at 121°C for 15 minutes, then aseptically transferred into sterile tubes of 2.5 ml and 5ml volumes and left for 24 hours to confirm that it is free from contamination (Cheesbrough, 2006).

3.3.3.4. Nutrient Broth seeding

From cultures obtained each microorganism aseptically seeded in separated tubes to refresh and activate the microorganisms. The seeded tubes were incubated at 37 °C for 24 hours in order to be used in sensitivity testing of *Portulaca oleracea* Linn aqueous methanol extract.

3.3.3.5. Medium used in Sensitivity testing

Mueller-Hinton's agar was recommended medium for bacteria because it produces rapid growth of most of pathogens, as it contains no inhibitors, can be used with many compounds, and it gives a sharp end point, then carefully poured into 90 mm diameter
sterile Petri dishes to depth of 4 mm, so as to establish a uniform depth of the medium. Sabouraud agar also recommended for *Candida albicans* (Cheesbrough, 2006).

### 3.3.3.6. Mueller-Hinton's Agar preparation

Nineteen grams of the medium was suspended in 500 ml of distilled water, then heated to dissolve completely, distributed in sterile tubes each contain 20 ml, then sterilized by autoclave for 15 minutes at 121°C (Cheesbrough, 2006).

### 3.3.3.7. Preparation of Sabouraud agar

Sixty five grams of the medium was suspended in 1000 ml of distilled water, then heated until boiled to dissolve completely, distributed in sterile tubes each contain 20 ml, then sterilized by autoclave for 15 minutes at 121°C.

### 3.3.3.8. Agar well diffusion method

#### 3.3.3.8.1. Description of Agar well diffusion method

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants extracts. The agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface, then, a hole with a diameter of 6 to 8 mm is punched aseptically, and a volume up to 200 μl of the antimicrobial agent or extract solution at desired concentration is introduced in to the well, then, agar plates were incubated under suitable conditions depending upon the tested microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested, also in inoculation phase microorganism can be added to the melted agar at 45 °C then mixed well to ensure proper distribution of microorganism in plates (Mounyr *et al.*, 2016).

#### 3.3.3.8.2. Mueller-Hinton's Agar inoculation

Tubes which contain 20 ml of sterile melted Mueller-Hinton agar were transferred in to the water bath and stabilized at 45 °C then seeded with 100 μl of microorganism (equivalent to 0.5 McFarland's scale) which was prepared from a 24 hours broth culture of activated bacteria aseptically, three tubes were used for each microorganism, and the seeded agar then was poured aseptically into sterile petri dishes and allowed to set.
3.3.3.8.3. Sabouraud agar inoculation

Tubes which contain 20 ml of melted Sabouraud agar were transferred to the water bath and stabilized at 45 °C, then seeded with 100 μl of *Candida albicans* (equivalent to 0.5 McFarland's scale), which was prepared from a 24 hours nutrient broth culture of activated fungi aseptically, the seeded Sabouraud agar then was poured aseptically into sterile petri dishes and allowed to set.

3.3.3.8.4. Wells formation and addition of extract

In each petri dish, 4 wells (8 mm wide) were performed by using sterile inverted tubes, of equidistance distribution in the plate, labeled appropriately. In each petri dish three wells were filled with 200 μl of one concentration of methanol extract of *P. oleracea*, (concentrations used were 1, 10 and 100 mg/ml respectively). The fourth well was filled with 100 μl of ceftriaxone 100 mg/mL (Hikma pharmaceutical, Jordan) used as a positive control for bacteria, and 100 μl of Nystatin 100,000 IU/ml (Delta Pharma, Egypt) as a positive control for *Candida albicans* in the fourth well of Sabouraud agar, then plates were allowed to stand on the bench for two hours to ensure adequate diffusion of the extract and the referenced drug, finally the plates were incubated at 37 °C for 24 hours for bacteria and 36 hours for fungi.

3.3.3.8.5. Measuring zones of inhibition

After 24 hours of incubation at 37 °C, antimicrobial activity was expressed as the diameter of the zones of inhibition using calibrated ruler (mm) produced by the extract around the wells. All tests were carried out in triplicate and the mean of zones of inhibition and Standard Error of Mean (SEM) were calculated to each microorganism.

3.3.3.9. Minimum inhibitory concentration (MIC)

3.3.3.9.1. Definition of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration of the extract at which the microorganism does not demonstrate any visible growth, the microorganism growth was indicated by turbidity (Mousavi *et al.*, 2015). In other words MIC is the lowest concentration of antimicrobial agent that completely inhibits growth of the organism in tubes as detected by the unaided eye (Balouiri *et al.*, 2016).
3.3.3.9.2. Two-fold Broth serial dilution method for MIC determination
Two-fold Broth macro-dilution is one of the most basic antimicrobial susceptibility testing method, also used to determine MIC approximately. The procedure involves preparing two-fold dilutions of the antimicrobial agent in a liquid growth medium dispensed in tubes containing a minimum volume of 2.5 ml, then, each tube is inoculated with a microbial inoculum prepared in the same medium after dilution of standardized microbial suspension adjusted to 0.5 McFarland's scale (Balouiri et al., 2016).

3.3.3.9.3. Procedure for MIC determination
Minimum inhibitory concentration was carried out by two-fold macro-dilution method. Six tubes of 2.5 ml of seeded nutrient broth were used to each microorganism. Four of them were used for serial dilution for Portulacae Oleracea Linn extract which started at its inhibitory concentration in well diffusion assay. One tube was left as negative control (containing only microorganism) to determine the microbial turbidity. The last tube contains 0.5 ml of ceftizoxime 100 mg/mL (Hikma pharmaceutical, Jordan) as a positive control for bacteria to determine the clearance of the media.
4. Results and Discussion

The yield of 500 gm of *Portulacae Oleracea* Linn extracted by 70% methanol (by cold maceration) was 8 grams (1.6%).

The results of the qualitative agar well diffusion method and MIC results were recorded and detailed in Table 1 and Fig 2, after calculation the means ± SEM.

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**Table 1. Results of antimicrobial activity of methanol (70%) extract of *Portulaca oleracea* L and MIC values to each tested microorganisms.**

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Clear Zones of inhibition in mm Mean ± SEM (Standard Error of Mean)</th>
<th>MIC in mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P O meth 70% extract concentrations</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 mg/ml 10 mg/ml 100 mg/ml</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ceftizoxime Nystatin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 mg/ml 100 mg/ml 100.000 IU/ml</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>---- ---- 12.33 ± 0.16 26 ± 0.47</td>
<td>100</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>--- --- 23.33 ± 0.55 55 ± 0.47</td>
<td>25</td>
</tr>
<tr>
<td><em>P. mirabilis</em></td>
<td>--- --- 19.3 ±0.65 64.33±0.54</td>
<td>50</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>--- --- 21 ± 0.47 34.65±0.27</td>
<td>50</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>--- 21 ± 0.27 23 ± 0.94 43 ± 0.75</td>
<td>10</td>
</tr>
<tr>
<td><em>C. Albicans</em></td>
<td>--- --- --- 30 ± 0.54</td>
<td></td>
</tr>
</tbody>
</table>
Determination of antibiotic sensitivity against microbial cultures used in this study demonstrated that, the aqueous methanol extract of Portulaca oleracea Linn (Portulacaceae) showed activity at concentration of 100 mg/ml against all bacterial cultures tested, while against Staphylococcus aureus showed a considerable activity at 10 mg/ml concentration, the plant extract was found to possess no activity against Candida albicans up to 100 mg/ml concentration (Table 1; Fig 2).

In general as recorded earlier (Oh et al., 1998; Banerjee et al., 2003; Elkhayat et al., 2008; Bae, 2004; Dhole et al., 2011; Wasnik et al. 2016; Nayaka, 2014; Londonkar et al., 2012; Peng, 2014; Mousavi et al., 2015; Ercisli et al.,2008; Chan,et al., 2015). The plant extract showed a significant antibacterial activity against both gram positive and gram negative bacterial strains with varying responses depending on the method of extraction or the type of solvent used and/or different assay methods.

Moreover, the quantitative Minimum Inhibitory Concentration (MIC) of the aqueous methanol extract of Portuleca oleracea L was tested by two-fold broth macro-dilution method, whereas, the plant extract scored MIC of 10, 25, 50, 50 and 100 mg/ml for Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella...
pneumoniae and Escherichia coli respectively (Fig 3-7). The antifungal test against Candida albicans of the investigated plant extract showed negative response, although it is well noted from previous screening of Portulaca oleracea Linn to exert a potent antifungal activity against number of fungal strains including Candida (Banerjee et al., 2002; Banerjee et al., 2003; Dhole et al., 2011; Elkhayat et al., 2008), this may be attributed to differences in procedures used to produce the plant extract or in performing the antimicrobial assay methods.

As shown in (Table 1) the highest inhibition (21 mm) of 10 mg/ml concentration of the extract was reported versus Staphylococcus aureus. This concentration inhibits on growth of other organisms in this study. S. aureus is a major human pathogen responsible for many diseases due to their toxin production and they can breach in mucosal barriers and enter underlying tissues. Diseases can caused by S. aureus include, local lesions of skin (such as, S tyes, Furuncles, Carbuncles, Wound infections, Impetigo), deep abscess (e.g. breast abscess), systemic infections (e.g. Osteomyelitis, septic arthritis and septicemia), Staphylococcal food poisoning, Toxic shock syndrome and Staphylococcal Scalded Skin Syndrome (SSSS) (Greenwood, 2012), and it is one of the most resistant bacteria to some antibacterial drugs such as methicillin. For that, the extract contains new compounds which inhibit the growth of S. aureus significantly considered one of the outcomes of the study, that should further be investigated especially versus MRSA.

The inhibitory effect of Portulaca oleracea extract is mainly related to the total flavonoids content including apigenin ( Londonkar et al., 2012; Nayaka 2014), fatty acids of different types including linoleic and oleic acids also found to be active components identified in plant extract (Chan, et al., 2015).

Furthermore, such phytoconstituents especially flavonoids which physiologically active constituents have been isolated from the plant and used to treat and offer some protection against human diseases and being potent antioxidant found in the investigated edible vegetable food of Portulaca oleracea ( Londonkar et al., 2012).
Figure 3. Results of MIC of *S. aureus* by two-fold broth dilution method.

Figure 4. Results of MIC of *P. aeruginosa* by two-fold broth dilution method.
Figure 5. Results of MIC of *E. coli* by two-fold broth dilution method.

Figure 6. Results of MIC of *P. mirabilis* by two-fold broth dilution.
Figure 7. Results of MIC of *K. pneumoniae* by two-fold broth dilution method.
5. Conclusion and Recommendations

5.1 Conclusion

Based on the findings of this study, the following conclusions were drawn:

- The aqueous methanol extract of *Portulaca oleracea* Linn (*Portulacaceae*) has promising antibacterial activity against bacterial cultures used (*Staphylococcus aureus*), (*Escherichia coli, pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae*).
- The extract was found to be effective against both Gram's positive and Gram's negative bacteria, especially *S.aureus* and *P.aeruginosa* which exerts MIC 10 mg/ml and 25 mg/ml respectively.

5.2 Recommendations

Based on the findings of this study the following can be recommended:

- The findings of the study reflect potential antibacterial capabilities of this plant.
- Further researches are highly recommended to investigate the bioactive molecules of this medicinal plant and to investigate their prospective clinical outcomes in the treatment of microbial infections.
- The Extract can be investigated in other bacterial strains regarding their ethnopharmacological studies
- More specific methods can be used for detection of MIC e.g. Micro-dilution method.
- The extract showed activity on *S. aureus* which is significant result, also its activity against MRSA strains could be investigated.
- Different extraction methods and extraction solvents could be used.
References


