

EFFECTS OF SOME TRADITIONAL PLANTS EXTRACTS ON BACTERIA ISOLATED FROM BURNS, WOUNDS & SKIN DISEASES

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ABSTRACT

Treating ailments is one important aspect of alternative medicine by used herbal medicines/drugs; plant extracts may be used for the preservation of processed foods as well as pharmaceutical and natural therapies for the treatment of infectious diseases in humans. From various skin bacterial infections, we selected 10 isolates For each of the *Staphylococcus aureus*, *streptococcus* and *Pseudomonas aeruginosa* in ultimately the total becomes 30 isolates Equally, that associated the common infections in hospitalized patients & the out patients. This study tested in vitro activity of flowers of *Calendula officinalis*, the roots of *Cichorium Intybus* and leaves *Portulaca Oleracea*. The antibacterial assay results showed for these plants were

effective against all 30 clinical bacterial isolates tested. The extract of *Cichorium Intybus* was the most active against all Gram positive & negative bacteria From the other two extracts. Based on our results, it is concluded that plant extracts have great potential as antimicrobial compounds against microorganisms and they can be used in the treatment of infectious diseases caused by resistant microorganisms.

KEYWORDS: Garden Marigold, Purslane, Chicory, wound, burns & skin diseases.

INTRODUCTION

Staphylococcus aureus, *streptococcus pyogenes* & *Pseudomonas aeruginosa* have the ability to causes skin diseases.^[1,2,3] The skin has a complex flora; infections can result when there is a breakdown in the integrity of the skin or when the immune defense is compromised. The microorganisms from the burned wound invade the unaffected tissue and local sepsis develops; if they invade the lymphatic and vascular system, systemic sepsis develops.^[2]

Wound infection is one of the most common diseases in developing countries because of poor hygienic conditions. Wounds are the physical injuries that result in an opening or breaking of the skin and appropriate method for healing of wounds is essential.^[4] Microorganisms colonizing the burn, wound originate from the patients endogenous skin and gastrointestinal and respiratory flora.^[5]

Microbial, immunological and even other factors such as Local infection, hypoxia, trauma, foreign bodies and systemic problems such as diabetes mellitus, may be responsible for causing connective tissue loss. Inflammatory mediators are constantly produced by wounds that show no sign of healing and the risk of death is involved when multiple organ failure. 6 million people prove to be the sufferers especially from chronic wounds.^[6,7] Some skin diseases caused by microorganisms are listed in Table 1 and some photographs are shown in Fig. 1.

Table 1- Some skin diseases caused by microorganisms.

Diseases	Organisms	Entry site
Impetigo	<i>S. pyogenes, S. aureus</i>	Skin around the nose and Mouth.
Carbuncle	<i>S. aureus</i>	Hair follicles.
Wounds	<i>S. aureus, S.Pyogenes, P. aeruginosa</i>	Skin.
Cellulitis	<i>Streptococcus pyogenes</i>	Small breaks in the epidermis.
Hot tub” Folliculitis	<i>P. aeruginosa</i>	Hair follicles.
Burn infections	<i>Pseudomonasaeruginos, staph aureus</i>	from the hands of health care workers, the environment or GI tracts of patients with burns .

Major injury due to burns has been demonstrated to increase susceptibility to infectious complications and related multiple organ failure.^[2] The burn wound surface is a protein rich environment consisting of a vascular necrotic tissue that provides a favorable rich for microbial colonization and proliferation.^[5]

The continuous use of antibiotics microorganism have become resistant, addition to this problem, antibiotics are sometimes associated with adverse effects on host which include hypersensitivity, immunosuppressant and allergic reactions.^[8]

A large number of plants/plant extracts/decoctions or pastes are equally used by tribal's and folklore traditions in India for treatment of cuts, wounds and burns.^[9] These plants are considered a source of treatment in rural areas, The people in these areas believed that these supporting natural repairing process of skin. Plants are also variable for use in blood

coagulation and cleaning of skin/wounds^[10] in the table 2, We mentioned the traditional uses of these plants.

Table 2- The Traditional Uses Of The Plants.

Common Name	Scientific Name	Traditional Uses
Garden Marigold	<i>Calendula Officinalis</i>	A herbal tea & in salads fresh or dried and used to color cheese or as a replacement for saffron.
Purslane	<i>Portulaca Oleracea</i>	Eaten as a leaf vegetable may be used fresh as a salad, or cooked as spinach .
Chicory	<i>Cichorium Intybus</i>	Used as a drink coffee substitute gives to coffee a bitterish taste , a dark color & a caffeine-free .

Fig. 1 Some skin diseases caused by microorganisms.



Impetigo



Wounds



Carbuncle



Boils



Cellulitis



Hot tub" Folliculitis



Burn infections

Several in vitro studies have looked at the effect of plant extracts on *staphylococcus aureus*, *streptococcus pyogenes* & *Pseudomonas aeruginosa*. In our study has effect these plants (Garden Marigold, Purslane & Chicory) on *staphylococcus aureus*, *streptococcus pyogenes* & *Pseudomonas aeruginosa* Isolated from several skin disorders in vitro but more work needs to be done in determining the active ingredients of these plants as well as performing studies on a larger number of patients.

The objectives of the present study was to explore the utility of plants among poor people (people that depend Therapeutically on traditional medicine) for various use especially for Boil, Burn and for Wounds in nearby of the rural areas.

METHODOLOGY

Collection of the plant materials

The selected part of air dried plants (*Portulaca Oleracea*, *Cichorium Intybus*, *Calendula Officinalis*) were purchased in September from commercial sources at Baghdad city-Iraq. Every part of plant Sample (leaves, roots and flower) was ground into uniform powder using grinder machine.

Preparation of plants Extracts

10 grams of this powder was extracted using 100 ml of boiling distilled water for 10 min. Then the extract was soaking for 2 day, filtered from the leaves by filtered using Whatman filter paper No1.^[11] The filtrate was then centrifuged at the highest speed (3000 rpm for 5 min).^[12] This process was repeated three times.^[13]

Patients and specimen collection

Were obtained 30 diagnosed isolates from patients suffered from burn infection, wound and other skin diseases from hospitals laboratories in Baghdad during October – December 2015.

All bacterial isolates were re-diagnosed & identified in microbiology laboratories of biology department /science collage / Al-Mustansiriyah university according to their colony morphology, microscopic gram stain investigation and biochemical tests.

Bacterial isolates

The isolates number employed in this study were 30; consisted 10 isolates each of the *Staphylococcus aureus* & *streptococcus pyogenes* (Gram Positive) and 10 isolates of the *Pseudomonas aeruginosa* (Gram negative). The microorganisms were obtained from various

sources of skin disorders such as burn, wound, boil. The general features for clinical isolates of bacteria Table-3.

Table - 3 General features for clinical isolates of bacteria

Bacteria species	Gram nature & Morphology	Location the pathogenic Bacteria as microflora in body	Symptoms and signs of skin infection
<i>staphylococcus aureus</i>	is a Gram-positive, cocci as grape-like clusters, facultative anaerobic.	live as a commensal organism on the skin, nose, mouth and throat.	From small benign boils to more severe of furuncles or carbuncles, impetigo and skin abscesses and worldwide responsible for skin and soft tissue infections.
<i>streptococcus pyogenes</i>	gram-positive coccoid-shaped grows in chains. is an aerobic, non-motile, non-sporing.	Pharynx, mouth, rectum and skin	Redness, pain, swelling, raised temperature, fever. large bleeding ulcers, pus; formation of golden-colored crusts.
<i>Pseudomonas aeruginosa</i>	is a gram-negative, motile rod, yellow-green colonies resulting from two pigments. aerobic (and at times facultative anaerobic.)	colonization can occur upper respiratory tract, lower GIT	wound infections may secrete a blue-green colored fluid and have a fruity smell. Burn wound infections usually with bleeding, swelling and a blue-green drainage.

Assay of Antibacterial activity by using Agar well diffusion method

Petri plates containing sterile Mueller Hinton agar 20ml, after solidification, Few colonies (2-4) from overnight culture were transferred to 2ml of normal saline in order to prepare the bacterial suspension and were adjusted to (0.5 McFarland standard turbidity) equal to 1.5×10^8 CFU/ml. were swabbed of each Bacterium isolate and spreaded with sterile swabs. thus were left at room temperature for 15 minutes allowing the absorption of the inoculums into the agar . Wells or cups of 5 mm size were made with sterile cork borer into agar plates containing the bacterial inoculums. Using the micropipette 50 μ l volume of the extract was added into a well of inoculated plates. Sterilized distilled water was used as a negative control which was introduced into a well instead of extract. Then, the plates incubated at 37 C, for overnight. The diameter of the zones of inhibition was measured with scale.^[14]

RESULTS AND DISCUSSION

Three types of bacterial species were isolated from the hospital; Thirty isolates of skin diseases, burn infections and wound infections were obtained from patients in the Baghdad. Results in Table 4 showed that *Calendula Officinalis*, *Portulaca Oleracea* & *Cichorium Intybus* have antibacterial agents. The *Cichorium Intybus* revealed highest inhibition zone

high effects on positive & negative isolates of all specie (*staphylococcus aureus*, *streptococcus pyogenes*, *Pseudomonas aeruginosa*). The other hand, the inhibition zones effect of *Calendula Officinalis* was higher to on all gram negative isolates of *Pseudomonas aeruginosa* from positive isolates.

While the effects *Portulaca Oleracea* was high inhibition zone against all gram positive isolates of the *staphylococcus aureus* & *streptococcus pyogenes*.

The several active components in plants like saponin, alkaloids and flavonoids & triterpenes; fatty acids and sterols; amino acids and amino glycosides are essentially effective in case of both skin infections as well as healing of wounds.^[6] Concentrated natural antimicrobials in plants such as phenolic compounds can be effect in degree of inhibition; at low concentration of phenols affect enzyme activity, while at high concentrations they cause protein denaturation.^[15]

The different groups of compounds table 5, show antimicrobial effect and serves as plant defense mechanisms against pathogenic microorganisms.^[16]

Table- 4 Effects The Plants and Bacterial Isolates.

NO. Isolates	Tested isolate	Antibacterial activity Zone of inhibition(mm)		
		<i>Calendula Officinalis</i>	<i>Cichorium Intybus</i>	<i>Portulaca Oleracea</i>
1	<i>Staphylococcus aureus1</i>	10	16	16
2	<i>Staphylococcus aureus2</i>	11	18	14
3	<i>Staphylococcus aureus3</i>	10	15	14
4	<i>Staphylococcus aureus4</i>	12	-	-
5	<i>Staphylococcus aureus5</i>	9	-	-
6	<i>Staphylococcus aureus6</i>	-	15	15
7	<i>Staphylococcus aureus7</i>	-	16	18
8	<i>Staphylococcus aureus8</i>	11	-	-
9	<i>Staphylococcus aureus9</i>	10	17	-
10	<i>Staphylococcus aureus10</i>	9	15	16
11	<i>Streptococcus pyogenes1</i>	13	17	15
12	<i>Streptococcus pyogenes2</i>	12	14	16
13	<i>Streptococcus pyogenes3</i>	11	17	18
14	<i>Streptococcus pyogenes4</i>	11	-	16
15	<i>Streptococcus pyogenes5</i>	10	-	-
16	<i>Streptococcus pyogenes6</i>	11	13	-
17	<i>Streptococcus pyogenes7</i>	13	16	15
18	<i>Streptococcus pyogenes8</i>	-	-	16
19	<i>Streptococcus pyogenes9</i>	-	15	-

20	<i>Streptococcus pyogenes 10</i>	11	-	15
21	<i>Pseudomonas aeruginosa 1</i>	15	19	10
22	<i>Pseudomonas aeruginosa 2</i>	17	-	8
23	<i>Pseudomonas aeruginosa 3</i>	19	18	8
24	<i>Pseudomonas aeruginosa 4</i>	15	17	11
25	<i>Pseudomonas aeruginosa 5</i>	-	16	-
26	<i>Pseudomonas aeruginosa 6</i>	-	19	7
27	<i>Pseudomonas aeruginosa 7</i>	18	16	9
28	<i>Pseudomonas aeruginosa 8</i>	19	-	8
29	<i>Pseudomonas aeruginosa 9</i>	16	15	10
30	<i>Pseudomonas aeruginosa 10</i>	16	16	-

Table 5. Mode of action of phyto chemicals

Class	Mechanism
Phenolics	Membrane disruption, substrate deprivation
Phenolic acids	Bind to adhesins, complex with cell wall, inactivate enzymes
Terpenoids, essential oils	Membrane disruption
Alkaloids	Intercalate into cell wall
Tannins	Bind to proteins, enzyme inhibition, substrate deprivation
Flavonoids	Bind to adhesions, complex with cell wall, Inactivate enzymes
Coumarins	Interaction with eukaryotic DNA
Lectins and polypeptides	Form disulfide bridges

Start with the first plant compounds of *C. officinalis* includes Some of the important bioactive compounds are Alkaloids, Glycosides, Triterpenoids, Terpenoids Flavonoids, Phenols, Reducing sugars, Saponins, Steroids and Tannins.^[17]

The flowers was investigated against experimentally induced thermal burns in rats, Showed significant improvement in healing of wounds as indicated by increase in collagen hydroxyproline and hexosamine contents.^[18]

While the phytochemical screening of *Cichorium Intybus* root extract: The experiments represented by phytochemical components in the chicory root extracts contains the effective active constituents responsible for eliminating the bacterial pathogens^[19]

The components analysed were alkaloids, volatile oils, fatty acids, emodins, flavonoids, triterpenoids, anthracene glycosides, tannins, phenolics and saponins.^[20,21,19]

Finally; The phytochemical anyalsis of leaves extract of *Portulaca oleracea* showed presence of The Saponin, alkaloid, tannin, flavonoid, cardiac glycoside, terpenoids, protein and starch has been observed as the active phytoconstituents of PO with saponin as the major constituent.^[22]

The high percentage content of saponin, hence its use as an antimicrobial, The presence of tannin, phenolic and flavonoid in this plant shows its potential as an antiviral, antibacterial & antifungal properties.^[23,24,22]

The extract nature & mode of action of the active constituents is quite obscure at this stage but may be these compound act as intracellular bacterial enzymes inhibitor or impair the cell wall synthesizing system of the cell impairment of biological Activities.^[25]

The fact that the some isolate showed Less inhibition toward our extracts, were Gram-negative, may be related to cell wall structure, the cell wall of Gram-negative bacteria acts as a barrier to a number of substances, including antibiotics. This would also explain why medicinal plants tend to be more effective against Gram-positive than Gram-negative cultures.^[26]

In spite of this permeability differences, however, some of the extracts had still exerted some degree of inhibition against gram negative organisms as well.^[27]

There are many published reports on the effectiveness of traditional herbs against Gram-positive and Gram-negative microorganisms, and as a result plants are still recognized as the bedrock for modern medicine to treat infectious diseases.^[6,8]

Plant/Herbal products are considered to be potential agents for healing of wounds and are in great demand due to their wide availability, less toxicity, absence of unwanted side effects after use and being more potent in their crude form.^[6]

PANDEY, MATHUR & EFSTRATIOS *et al*^[17,18,28] observed clear effect of *Calendula Officinalis* on *Pseudomonas aeruginosa*.

Some Gram negative bacteria have been shown to be appreciable susceptibility from *Calendula Officinalis* as in the studies by RIGANE *et al* & by BISSA *et al*.^[29,30]

The Recommendations

The phytoconstituents observed in this study shows the plant's potency for use in producing pharmaceutical bioactive compounds for therapeutic drugs.

So, is possible use or add plants extract with The ointment topical antimicrobials to stimulation repair the damage skin and without any complication.

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