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FORMULATION AND EVALUATION OF GREWIA TENAX EXTRACT OINTMENT AS NATURACEUTICAL DRUG DELIVERY SYSTEMS FOR ANTIMICROBIAL ACTIVITY

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ABSTRACT

The objective of this present study was to produce an ointment formulation containing an *Grewia tenax* extract and evaluate its antimicrobial activity. *Grewia tenax* (Family:Tiliacea) represent as one of the selected plants used as treatment. The alcoholic extract of different part of plant (leaves, stems and fruits) were have antimicrobial effects against different tested microorganisms at different concentrations. The results indicated that the alcoholic leaves extract was more efficacy against tested microorganisms (S. aureus, S. epidermidis, E. coli and P. aeruginosa) with inhibition zones equal (18,19,17and14mm) respectively than other parts extract followed by stem extract then by fruits extract with the inhibition zones (17,18,17, and 13mm) and (17,16,15 and 14mm) against (S. aureus, S. epidermidis, E. coli and P. aeruginosa) respectively. Generally, the antibacterial activity of *Grewia tenax* extract appears to be inhibitory to Gram-positive bacteria more than Gram-negative bacteria. The ointment formulations were prepared which are concentrations (12.5, 6.25, 3.125 and 1.5% w/w) and made compare between base and ethanol extract were evaluated. The formulation ointment containing F1(12.5w/w) extract showed better antimicrobial activity, physicochemical characteristics, and pharmacological parameters than the other ointment formulations. The zone of Gentamycin was similarity with a highly concentration ointment formulation F1(12.5w/w) were evaluated. Among the all-ointment formulations the ointment formulation F1(12.5w/w) containing *Grewia tenax* showed better antimicrobial activity. It was concluded that the extract of *Grewia tenax* F1(12.5w/w) was a better candidate for nutraceutical delivery systems of antimicrobial agents for eradicating common microbial infections.

KEYWORDS: *Grewia tenax*, Ointment, Antimicrobial Activity, Nutraceutical Delivery Systems.

INTRODUCTION

Background of *Grewia Tenax* (Family: Tiliaceae)[1-84]

The plant product or natural products show an important role in diseases prevention and treatment through the enhancement of antioxidant activity, inhibition of bacterial growth, and modulation of genetic pathways. The therapeutics role of number of plants in diseases management is still being enthusiastically researched due to their less side effect and affordable properties. It has been accepted that drugs based on allopathy are expensive and also exhibit toxic effect on normal tissues and on various biological activities. It is a largely accepted fact that numerous pharmacologically active drugs are derived from natural resources including medicinal plants.

Plants spices have been utilized to cure many diseases for a long time. In today's time, despite the fact that synthetic drugs are promptly accessible and exceedingly powerful in curing different diseases, there are persons who still incline toward utilizing traditional pharmaceuticals on account of their less harmful impacts. There is a wide diversity of compounds, particularly auxiliary metabolites, which are confined from plants having antibacterial, anticancer, anti-inflammatory, analgesic, antiviral antitumor, and many other activities to a major or minor extent. Well-known

examples of these phytochemical compounds incorporate phenols, glycosides, flavonoids, phenolic, saponins and stilbenes, cyanogenic glycosides, nitrogen compounds (betalains, amines, alkaloids), tannins, terpenoids, and few different endogenous metabolites.

Grewia tenax (Family: Tiliaceae), commonly known as Gangeti and or guddaim is a valuable plant species in Kachchh region. *G. tenax* is presumed to cure distress of stomach, skin and intestinal infections, fever, diarrhea, cough, dysentery, hepatic disorders, jaundice, rheumatism and have calm antibiotic properties. Leaves branches of *G. tenax* are significant element of traditional medicine for the curing of tonsillitis, trachoma and are used as a poultice to treat swelling. The plant species have free radical scavenging activities which might be in charge of the remedial action against tissue damages. As shown in Figure 1.

Grewia tenax (Forssk.) Fiori is a small-leaved white cross berry that belongs to the Tiliacea family. This fruit-producing deciduous shrub or small tree is prevalent in African and Southeast Asian countries. The tree has a wide distribution in the savannah plantation area in the north and middle of Sudan. *Grewia tenax* has been used in folk medicine in several ways in different countries. The roots have been used to treat jaundice. There is commercial potential in using the fruits in beverages, yogurt, ice cream, and baby food. *Grewia tenax* is used as medicine to treat various diseases including jaundice and hepatic disorders, a decoction prepared from the bark is used as antihelmintic and an alcoholic extract ointment was reported to help in faster wound healing. The fruits, roots and leaves of *G. tenax* were used as food while its juice and fruit decoctions have been used in Africa as thirst quenching drinks in hot weather. *Grewia tenax* is cultivated and distributed in Yemen.

In addition to the fruit, bark infusions are also used in wound healing. There is a paucity of scientific evidence regarding its use for jaundice and other liver disorders. Moreover, the fruits are eaten to treat anemia and chest diseases. The genus *Grewia* is represented in Egypt by three species, *Grewia villosa* Willd, *Grewia tembensis Fresen*, and *Grewia tenax Forssk*. The species is known for its edible fruits, which are nutritionally balanced and rich in iron and calcium. The drupes also contain amino acids, mineral elements (K, Ca, Mn, Fe, Cu and Zn), tannin and pectic substances.

The methanolic extract of *Grewia tenax Forssk*. showed good activity against Pseudomonas aeruginosa. In certain study, the carbohydrate and lipids content were investigated for the first time, in addition to the hepatoprotection activity of the prepared extracts from *Grewia tenax Forssk*. Fruits. The plant has high medicinal values and is widely used for the treatment of various common diseases. *G. tenax* Fiori is reputed to cure upset of stomachs, some skin and intestinal infections, cough, fever, diarrhea, dysentery, jaundice, rheumatism and have mild antibiotic properties. The plant preparations are used for the treatment of bone fracture and for bone strengthening. Its root and fruits are well known household remedy for the treatment of osteoporosis, tissue and wound healing. Leaves and twigs of *G. tenax* are an important component of folk medicine for the treatment of trachoma, tonsillitis, infections and are used as a poultice to treat swelling.

The stem bark and leaves of G. populifolia are reported to constitute some phytochemicals like triacontan-1-ol, α- amyrin, β-amyrin, β-sitosterol, lupenne, erythrodiol botulin and tetratriacont-21-ol-12-one. The plant has been found to contain grewinol, tetratricontane-22-ol-13-one. The seeds contain 5% of bright-yellow oil containing palmitic acid, stearic acid, oleic acid, linoleic acid and unsaponifiable. In preliminary phytochemical studies, plant extracts in different solvents were found to contain diterpenes, glycosides.

Product of Food Importance

Food: The fruits consumed by man and animals contain a large amount of iron and can be made into a refreshing drink. Fruit storage can be extended by drying. The dead leaves are eaten, but only while they remain on the plant. Its fruits are thirst quencher in summer season. A drink is prepared by soaking the fruit overnight, hand-pressing, sieving, and sweetening.

Fig. 1: *Grewia Tenax* Plant (Family: Tiliaceae).

Medicinal Importance

Leaves and twigs of *G. tenax* are important components of folk medicine for the treatment of trachoma, tonsillitis, infections and are used as a poultice to treat swelling. *G. tenax* plant is used for the treatment and prevention of iron deficiency anemia. Porridge, called Nesha, is prepared by boiling fruit pulp of *G. tenax* and millet flour given to lactating mothers. Ointment of whole plant extract applied locally for hard tissue repair and bark paste of G. tenax can be applied as plaster. A preparation of *G. tenax* fruit powder mixed with milk is given for the treatment of bone fracture and swelling.

Effective treatment strategies have been developed for eradicating common microbial infections. Despite the concerning approaches, the ever-increasing incidences of

microbial infections commonly caused by parasites, fungi, viruses, and bacteria are alarming. Several common bacterial infections are caused by bacteria such as Escherichia coli, Streptococcus pneumonia, Klebsiella pneumonia, and Staphylococcus aureus. Bacterial infections further cause several chronic infections and are the reason for the high number of mortalities, globally. Therefore, the discovery of antibiotics is directed as a remarkable achievement in the medical field, from surgery to organ transplant and for various microbial infections and diseases. But bacteria have developed immunity against antibiotics which led to the occurrence of multidrug resistant bacteria. For decades, antimicrobial resistance (AMR) has been growing as a threat to the treatment efficacy of antiparasitic, antifungal, antiviral, and antibacterial drugs. Hence, antibiotic resistance, especially antibacterial resistance (ABR), has turned out as a dilemma in the medical field and public health, for treating patients. *Grewia tenax* extract for their antimicrobial applications to use as alternatives to antibiotics for the benefit of patients.

In this study, ointment formulations containing extract of *Grewia tenax* were prepared for nutraceutical delivery systems of antimicrobial agents for eradicating common microbial infections.

MATERIALS AND METHODS

Plant Materials and Preparation of Extract

The extract of *Grewia tenax* was prepared and gift from (Dr. Salwa M. Raweh, Associate Professor Dr. of Pharmacognosy, Department of Pharmacognosy, Faculty of Pharmacy, Sana'a University, Sana'a, Yemen). Oleaginous base (white soft paraffin), Emollient (liquid paraffin), Emulsifying (cetostearyl alcohol), Ethanol 95% (v/v) (Umco®, Yemen) and Distilled water as a gift from (Modern and Global Pharmaceutical Industry Company-Yemen).

Formulation and Evaluation of *Grewia Tenax* Extract[85-182]

Phytochemical Screening of Extract

The phytochemical screening was conducted on the *Grewia tenax* extract by using many tests of phytochemical such as (Molisch's test, Benedict's test, Xanthoprotein, Ninhydrin, Fecl3, Cupper acetate, Mayer's test, Wagner's test, Dandruff's test, Hager's test, Alkaline reagent and Lead acetate).

Antimicrobial Activity of Extract

Four bacteria were used for the study, Gram positive bacteria include of staphylococcus epidermidis, Staphylococcus aureus and Gram-negative bacteria include Escherichia coli, Pseudomonas aeruginosa. All the tested strains were local isolates and were obtained from AL-Aulaqi laboratory. These bacteria served as test pathogens for antibacterial activity assay. Four different concentrations of each extract of selected plants (12.5, 6.25, 3. 25 and 1.5 mg/ml) were dissolved in purified water (PW) to be used in antimicrobial activity test. Extract solutions were prepared just before carrying out the test. Antibacterial activity of the extracts was determined by agar well diffusion method.

The bacterial suspensions containing 106 CFU/ml of bacteria were spread on petri dishes plates with a sterile swab moistened with the bacterial suspension. In each of these plates, five wells were cut out using a standard corn borer (7mm). About 60µl of each extract was added into different wells (duplicate each concentration), PW was used as a negative control. Positive control antibiotic wells were placed in the plate. All the plates were incubated for 24hrs, at 37°C. After incubation bioactivity was evaluated by measuring the zone of inhibition. The experiment was performed in one of antibiotic standard Gentamycin (10mcg) were used as reference to determine the sensitivity of each bacterial species tested and used as control positive.

Preparation of Ointment Formulations

Ointments were prepared by the fusion method. The white soft paraffin was melted at 70 -75ºC then liquid paraffin was added at 70ºC. After melting, the ingredients were stirred gently. Cetostearyl powder (0.25% W/W) then the mixture was added to previous ingredients.

In water bath with a temperature not exceeding 50°C, the *Grewia tenax* extracts in a concentration of 12.5, 6.25, 3.25 and 1.5% were added to prepare four formulations, F1 (12.5% w/w), F2 (6.25% w/w), F3 (3.25% w/w), and F4(1.5% w/w), respectively. The remaining quantity of purified water was added, and the pH was drop wise adjusted with buffer. The final weight was adjusted with water *quantum statis* (*q.s.*) to 100g as shown in Table 1.

Table 1: Composition of Ointment Formulations.

Ingredients Percentage	F1	F2	F3	F4
***Grewia Tenax* Extract (w/w)**	12.5	6.25	3.25	1.5
Oleaginous Base (White Soft Paraffin) WSP	1.5	3	4	5
Emollient (Liquid Paraffin) LP	2.5	5	3	4
Emulsifying (Cetostearyl Alcohol) CSA	1.5	2	3	4
Distilled Water	q.s.p.	q.s.p.	q.s.p.	q.s.p.

q.s.n. = quantity sufficient to neutralize ointment base, q.s.p = quantity sufficient to prepare 100 grams of ointment

Formulation Ointment Base

The three ingredients for ointment base formulation, which are white soft paraffin liquid paraffin, and cetostearyl alcohol.

Evaluation of Ointment Formulations

Physical Appearance

The ointment formulations were evaluated for their physical parameters.

pH Determination of Ointment Formulations

The pH of the ointments was detected with a digital pH meter. An amount of 0.5g of ointment was dissolved in 50ml of distilled water and stored for two hours. Each formulation's pH was measured in triplicate and the average values were taken.

Viscosity Evaluation of Ointment Formulations

The rheological behavior of different formulations was done by measuring the viscosity. This viscosity expressed in centipoise (cP) was determined by Brookfield viscometer by a modified method. The test sample was taken in a clean and dry 250ml beaker, and the viscosity of the test sample was determined by the standard operating procedure of viscometer using spindle No. 5. This spindle was used for finding the viscosity of the sample at speeds of 25, 50, and 80rpm. Samples were measured at 25 ± 1°C.

Evaluation of Ointment Base

The different formulation bases aimed at choosing the best proportion of ingredients as oleaginous base (white soft paraffin), emollient (liquid paraffin) and emulsifying (cetostearyl alcohol) dependent and independent. These results as shown in Table 2.

Table 2: Results of the Selection Variables.

Independent (Ingredient)	Dependent (Response)
Oleaginous Base (White Soft Paraffin)	Ointment base
Emollient (Liquid Paraffin)	Viscosity
Emulsifying (Cetostearyl Alcohol)	Emulsifying agent

Effect of Formulation Ingredients on Viscosity

Significantly shows that the oleaginous base (white soft paraffin) has the maximum effects on the viscosity of ointment base while the binary effects of emollient (liquid paraffin) and emulsifying (cetostearyl alcohol) have the minimum effect on the viscosity. The results as shown in Table 3.

Table 3: Optimization of Formulations.

Independent (Ingredient)	Abbreviation	Lower Limit %	Upper Limit %
Oleaginous Base (White Soft Paraffin)	WSP	1.25	5
Emollient (Liquid Paraffin)	LP	2.5	5
Emulsifying (Cetostearyl Alcohol)	CSA	1.5	5

Antibacterial Activity of Ointment Formulations

Each formulation was assessed for its antimicrobial effects against the microorganisms on a nutrient agar using a suitable diffusion method. About 0.2 ml of the bacterial test strain was inoculated over a nutrient agar plate with a sterile cotton swab and was allowed to dry. With the help of a cork borer, 6 mm diameter wells were created. Half a milliliter of the *Grewia tenax* extract was introduced into the wells. The plates were placed at room temperature for about one hour. Then the plates were placed in an incubator at 37 °C for 24 hours. Then, the zone of inhibition was checked and recorded Gentamycin was used as standard.

RESULTS AND DISCUSSION

This study evaluated the antibacterial activity of herbal ointments. Four different concentrations of an *Grewia tenax* extract were used to prepare ointment formulations. The formulations were evaluated for the physical parameters.

Phytochemical Screening of Extract

The Molisch's test was negative with all parts of extracts for *Grewia tenax*. While, it showed positive for benedict's test. In the other test of detection proteins and amines the results of the current study showed positive for xanthoprotein test in leaves and fruits but was negative in M. at the same of test for protein in Ninhydrin test it was positive in M and fruits but negative in leaves. The detection of phenol and diterpenes it was positive in leaves and M respectively while was negative for other parts. In alkaloids, it was only positive with Wagner's test in all parts of plant so this extract contains of alkaloids. Detection of Flavonoids it was only positive in Lead acetate in two parts of plant leaves and fruits but negative in Alkaline test at the all parts of plant. As shown in Table 4.

Table 4: The Results of Phytochemical Tests for *Grewia Tenax* Extract.

Test	Ethanolic Extract		
	Leaves	Mix	Fruits
Detection of (CHO)			
Molisch's Test	-ve	-ve	-ve
Benedict's Test	-ve	+ve	+ve
Detection of Proteins and Amines			
Xanthoprotein	+ve	-ve	+ve
Ninhydrin	-ve	+ve	+ve
Detection of Phenols			
Fecl3	+ve	-ve	+ve
Detection of Diterpenes			
Cupper Acetate	-ve	+ve	-ve
Detection of Alkaloids			
Mayer's Test	-ve	-ve	-ve
Wagner's Test	+ve	+ve	+ve
Dandruff's Test	-ve	+ve	+ve
Hager's Test	-ve	-ve	+ve
Detection of Flavonoids			
Alkaline Reagent	-ve	-ve	-ve
Lead Acetate	+ve	-ve	+ve

Antibacterial Activity of *Grewia Tenax* Extract

The results of antibacterial activity of *Grewia tenax* obtained as one solvent extract against four different human pathogenic bacteria and different concentrations. The results of this study showed the alcoholic extract of different part of plant (leaves fruits and mix) were have antimicrobial effects against different tested microorganisms at different concentrations. these results indicated that the alcoholic leaves extract was more efficacy against tested microorganisms (S. aureus, S. epidermidis, E. coli and P. aeruginosa) with inhibition zones equal (18,19,17,14mm) respectively than other parts extract followed by mix extract then by fruits extract with the inhibition zones (17,18,17 and 13mm) and (17,16,15 and 14mm) against (S. aureus, S. epidermidis, E. coli and P. aeruginosa) respectively. All these results were comparing with the zones of reference antibiotic standard (17mm, 16 mm, 15mm and 14mm) against (S. aureus, S epidermidis, E. coli and P. aeruginosa) respectively. Through these results may possible to using instead of antibiotics. These results showed that the ethanolic extract of different parts might be equal or more in the characteristics of antibiotics.

Generally, the antibacterial activity of *Grewia tenax* extract appears to be more inhibitory to Gram-positive bacteria than Gram-negative bacteria. The zones of growth inhibition on the Gram-positive, Gram negative, that used in this research showed the growth inhibition as shown in Table 5 and Figure 2(a,b,c,d). The concentration of *Grewia tenax* extracts was determined by incubating four concentrations (12.5, 6.25, 3.25 and1.5 %) of extracts with a standard inoculum of microbial cultures; however, 12.5% was the highest concentration showed the effects, while placebo showed no growth inhibitory on all microorganisms. Gentamycin was the only antibiotics used as standard for gram positive and gram-negative bacteria. This standard revealed antimicrobial activity with all organisms in ethanolic solvents.

Table 5: The Antimicrobial Activity of *Grewia Tenax* Extract Against Some Pathogenic Bacteria.

Bacteria	Conc												RSTD	Plac
	Leaves				Mix				Fruits					
	12.5	6.25	3.25	1.5	12.5	6.25	3.25	1.5	12.5	6.25	3.25	1.5		
S. Aureus	18	15	11	10	17	14	11	9	17	15	10	8	14	0
S. Epidermidis	19	16	11	10	18	14	12	9	16	13	12	9	15	0
E. Coli	17	15	13	12	17	14	12	11	15	12	11	10	14	0
P. Aeruginosa	14	12	10	8	13	12	10	9	14	12	10	9	14	0

RSTD = Reference Standard (Gentamycin). Plac = Placebo, Conc = Concentration.

Fig. 2a: Staphylococcus Epidermidis Test.

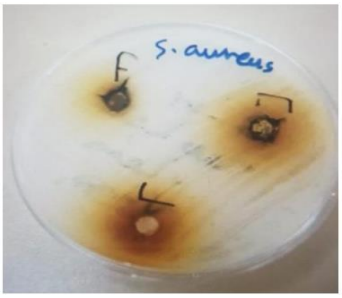

Fig. 2b: Staphylococcus Aureus Test.

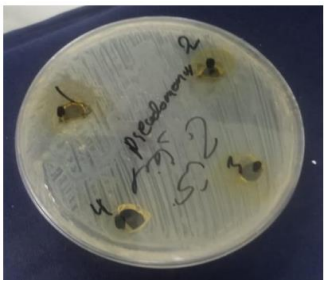

Fig. 2c: Pseudomonas Aeruginosa Test.

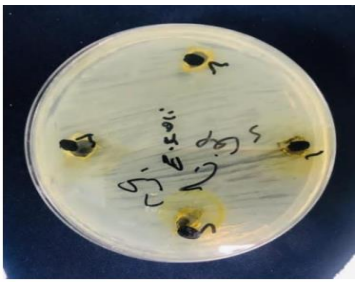

Fig. 2d: E. Coli Test.

Fig. 2: The High Sensitivity Results of *Grewia Tenax* Extract Against Some Pathogenic Bacteria.

During this study, the ointment formulation was tested on the mice. Many concentrations were prepared which are (12.5, 6.25, 3. 25 and 1.5%) and make compare between base and ethanol extract. The results of this study showed a high effective at 12.5%.

DISCUSSION

This study has been focused in particular on the famous medicinal plant used in Yemen. This present study used ethanolic solvents to extract this plant against gram positive and gram-negative bacterium and evaluated obtained extract for antimicrobial activity using well diffusion assay, phytochemical test and formulation. This results showed that the highest antimicrobial activity was with ethanolic solvent with gram-positive bacteria with all concentrations. The *Grewia tenax* indigenous to be used as an excellent dressing for wounds. The results of diameter of the zone of inhibition are shown in Table 5. Plant extracts resulting in 15mm or more growth inhibition zones were considered active and those resulting in less than 15mm were inactive. This current study of ethanolic fraction found a high antimicrobial effective against several gram positive and gram-negative bacteria including S. aureus, S. epidermidis and E. coli but it showed no effective against P. aeruginosa because the inhibition zone was 14mm. In this study, Ethanolic fraction showed better antimicrobial activity against S. aureus, S. epidermidis, E- Coli, and Pseudomonas aeruginosa, (18, 19, 17, and 14mm) in leaves and (17, 16, 15 and 14mm) in fruit respectively, these results showed a high sensitivity against S. aureus.

As shown in Table 5, the results showed that the high antimicrobial activity with all organisms using Gentamycin as standard. The zone of Gentamycin was similarity with a highly concentration (12.5%). In phytochemical screening the extract of this plant has alkaloids, Diterpenes, phenol and Flavonoids it was only positive with Wagner's test in all parts of plant so this extract contain of alkaloids. Detection of Flavonoids it was positive in Lead acetate in two parts of plant leaves and fruits but negative in Alkaline test at the all parts of plant. The effectiveness of medicinal plants in healing diseases is due to the presence of different phytochemical compounds (Alamgeer and Asif, 2018). The different phytochemical compounds present in *G. tenax* could be responsible for the antibacterial activity detected in this study against S. aureus. The lowest minimum inhibitory activity of *G. tenax* crude extract observed against P. aeruginosa. The ointments were creating an optimal process of wound healing. The ointment showed that the oleaginous base (white soft paraffin) has the maximum effects on the oleaginous of ointment base while the binary effects of emollient (liquid paraffin) and emulsifying (cetostearyl alcohol) have the minimum effect.

CONCLUSION

According to the present study, the antimicrobial activity improves with the increasing concentration of the *Grewia tenax* extract. The extract of this medicinal plant has antimicrobial activity with S. epidermidis, S. aureus and E. coli. The different phytochemical compounds present in *G. tenax* could be responsible for the antibacterial activity detected in this study against S. aureus. The results of *Grewia tenax* extract showed a high antimicrobial activity with all organisms using Gentamycin as standard. The zone of Gentamycin was similarity with a highly concentration ointment formulation F1(12.5w/w). Among the all-ointment

formulations the ointment formulation F1(12.5w/w) containing *Grewia tenax* showed better antimicrobial activity. It was concluded that the extract of *Grewia tenax* F1(12.5w/w) was a better candidate for nutraceutical delivery systems of antimicrobial agents for eradicating common microbial infections.

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