INTERNATIONAL JOURNAL OF RESEARCH CULTURE SOCIETY ISSN(O): 2456-6683
Monthly Peer-Reviewed, Refereed, Indexed Journal [Impact Factor: 9.241]
Volume - 9, Issue - 5, May - 2025

DOIs:10.2017/IJRCS/202505014 --:-- Research Paper / Article / Review

DEVELOPMENT OF COTTON GAUZE WITH NANOPARTICLE FROM POMEGRANATE PEEL EXTRACT FOR DIABEIC FOOT ULCERS

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Abstract: *People with diabetes will develop foot ulcers due to neuropathy (sensory, motor and autonomic deficits), ischaemia, or both. The initiating injury may be from mechanical or thermal trauma or from repetitively or continuously applied mechanical stress. Pomegranate belongs the family of Punicaceae. It has delicious shrubs. Pomegranate is used as food and plays a vital role in the medicinal field. Because pomegranate fruit and its peel extraction contain various compounds like antioxidant, phenolic acid, Flavonoids, Tannins etc., that have been found very effective in the health field. This study about the pomegranate (Punica granatum) peel has the ability to heal the diabetic foot ulcers because of its compounds. It was tested by some of the Laboratory techniques such as phytochemical analysis, anti inflammatory, antioxidant activity, TLC, UV Spectroscopy, FTIR Spectroscopy and antimicrobial activity. To enhance the anti inflammatory, antioxidant and antimicrobial activity silver nanoparticle (AgNP3) was added and it given the effective result than the Pomegranate (Punica granatum) peel extract alone. Formulation of combine sterile cotton gauze with Pomegranate (Punica granatum) peel extract and nanoparticle (AgNP3) enhanced antimicrobial properties and faster the wound healing. Pomegranate (Punica granatum) peel extract and nanoparticle (AgNP3) have regenerative effects of tissue-stimulating potential and reduced in infection risk. Nanoparticle prevents bacterial growth, keeping the diabetic foot ulcer environment sterile.*

Key Words: *Pomegranate, phenolic acid, diabetic foot ulcers, wound healing, nanoparticle, antimicrobial.*

1. INTRODUCTION

The most significant global health challenge associated with diabetes mellitus is Diabetic Foot Ulcer (DFU), affecting millions of individuals around the world. These chronic wounds result from the interplay of various factors including peripheral neuropathy, poor vascular supply, immune dysfunction, and prolonged hyperglycemias [1]. The mortality rate associated with diabetic foot ulcers is also high, with studies showing that individuals with DFUs have a two to four-fold increased risk of mortality compared to those without foot ulcers [2]. Cotton gauze is commonly used for wound care due to its absorbent nature, allowing it to soak up fluids from the wound, promote clotting, and maintain a moist environment conducive to healing it also provides a protective barrier against contaminants and helps to keep the wound clean. Pomegranate (*Punica granatum*) peel contains the ability of anti inflammatory, antioxidant and antimicrobial activities which are effectively works on diabetic foot ulcer wound healing. To enhance the properties of diabetic wound healing silver nanoparticles are introduced in the pomegranate (*Punica granatum*) peel extract as silver nitrate. It can more effectively work against the diabetic foot ulcer wounds than the Pomegranate (*Punica granatum*) peel extract alone.

2. METHODOLOGY

Sample collection and Extraction

The fresh Pomegranate was collected from palamuthircholai Tirupur, Pomegranate(*Punica granatum)* peel was separated and dried then grinded using blender and sieved [3].1 gram of Pomegranate(*Punica granatum)*peel powder

mixed with 150ml of distilled water and kept in magnetic stirrer at 50ºC and rpm at 650. (Figure: 1) The obtained sample was filtered and refrigerated at 4ºC.

Figure 1: Extract of Pomegranate (Punica granatum) peel

Qualitative analysis of phytochemical screening

The phytochemical analysis test of Pomegranate (*Punica granatum)* peel extract to observe the analytical colour change, for Tannins,Alkaloids,Steroids,Saponins,Terpenoids,Glycoside,Phenol,Quinones,Proteins,Carbohydrates,Flavonoids,Iodne Alkaloids.

Test for Tannins

To identify the presence of tannins 1 ml of extract was taken and 3 ml of bromine water. The disappearance of colour indicates the presence of tannins.

Test for Alkaloids

To identify the presence of alkaloids add 1 ml of extract and 1 ml of NaOH. Formation of yellow colour indicates the presence of alkaloids

Test for Saponins

Add 1 ml of extract and add 3 ml of water and shake it well. The formation of foam after 10 minutes indicates the presence of saponins.

Test for Steroids

To identify the presence of steroids add 1 ml of extract and add 3 ml of acetic acid and 1 ml of sulphuric acid. The presence of blue or green indicates the presence of steroids.

Test for Terpenoids

To 1 ml of extract and 3 ml of chloroform add 1 ml of acetic acid and 1 ml of sulphuric acid. The red, pink and violet colour indicates the presence of terpenoids.

Test for Glycosides

To identify the presence of glycoside about 1 ml of extract is added with 1 ml of acetic acid and 1 ml of sulphuric acid and 1 ml of ferric chloride was added. The presence of blue or green colour indicates the presence of glycoside.

Test for Phenols

To 1ml of extract, add 1 ml of ferric chloride observance of black or green colour indicates the presence of phenol.

Test for Quinones

To identify the presence of quinones, add about 1 ml of extract and add 1 ml of hydrochloric acid. Observance of green red and pink colour indicates the presence of quinones.

Test for Proteins

To 1 ml of extract, add 1 ml of nitric acid. The observance of golden yellow colour indicates the presence of proteins.

Test for Carbohydrates

To identify the presence of carbohydrates, add 3 ml of Benedict's reagent and add 1ml of extract keep in water bath for 2-3 minutes, observation of red colour indicates the presence of carbohydrates.

Red colour indicates the high concentration of carbohydrates.

Green colour indicates the medium concentration of carbohydrates.

Blue colour indicates the absence of carbohydrates.

Test for Iodine Alkaloids

1ml of extract and 1ml of iodine solution mixed together Blue colour confirms the presence of alkaloids.

Test for Flavonoids

To identify the presence of Flavonoids about 1ml of the extract is added to 1ml of sulphuric acid, the observance of yellow colour indicates the presence of Flavonoids.

Anti inflammatory assay

The Anti inflammatory test was carried out with 10 test tubes with 0.1 to 1ml of bovine Serum Albumin (BSA), and Pomegranate (*Punica granatum)* peel extract was also added. Then 4.78ml of Phosphate buffer solution was added to each tube, and readings were taken by calorimetry at 680nm.

Antioxidant assay

The DPPH method of Antioxidant activity was carried out by using 30ml of methanol with a pinch of DPPH for activation; 15 minutes of dark incubation occurred. Then 6 tubes were taken, and 100µl to 500µl of sample was added in 5 tubes; one tube was served as a blank [4]. Then 3ml of prepared solution was added in the tubes, including the blank. After that, tubes were taken to dark incubation for 30 minutes, and then readings were taken by calorimetry at 580nm.

$$\frac{\text{Radical scavenging activity}}{\text{Inhibition \%}} = \frac{\text{Ab-As}}{\text{Ab}} * 100$$

Thin layer chromatography

The eluent was prepared by mixing methanol and acetic acid in 12:8 ratios then the chamber was closed and leaves it for 1hour to saturate. The extract was placed on TLC plate with 2cm above and then allowed to dry [5].TLC plate was taken to the elution process; if it reached the upper edge of the plate, this process was stopped, and the plate was allowed to dry, then the Rf value was calculated by Retardation Factor Value (Rf)-Distance travelled by solute/Distance travelled by solvent.

Silver nanoparticle incorporated extract

The pomegranate (*Punica Granatum*) peel extract has the ability to heal diabetic foot ulcers and other wounds. Because it contains the components of flavonoids and phenolic compounds which work most effectively on wound healing [6]. Here, to enhance the healing effect of diabetic foot ulcers nanoparticles (silver nitrate) are added to the extract because these nanoparticles more effectively work on any kind of wound healing but especially on diabetic foot ulcers.

UV-Visible Spectroscopy analysis

UV-Vis spectrophotometer and calibrate it using a blank solution (solvent). Scan the sample extract over a range of wavelengths (typically 200-700 nm) to identify absorption peaks, which correspond to the specific bioactive compounds in the extract. Record the absorbance at different wavelengths and analyze the spectra for characteristic peaks, which can be used to identify the presence and concentration of various phenolic compounds or flavonoids.

FTIR Spectrum analysis

To perform FTIR analysis on a liquid pomegranate peel extract, prepare the sample by applying a small drop of the liquid directly onto the ATR (Attenuated Total Reflectance) crystal or a KBr pellet if using transmission mode. Ensure the FTIR spectrometer is calibrated and set to scan the sample in the typical wavelength range of 4000-400 cm^{-1}. Once the sample is in place, initiate the scan, and the instrument will record the infrared absorption spectrum, showing peaks corresponding to the functional groups and molecular vibrations in the sample. After the scan, interpret the spectrum by comparing the observed peaks to known reference data or databases to identify the chemical components in the pomegranate peel extract [7].

Development of diabetic foot ulcer gauze

For diabetic foot ulcer gauze development, the cotton gauze was taken and sterilized using an autoclave or soaking in ethanol method [8]. The sterilized gauze was soaked overnight in Pomegranate (*Punica granatum*) peel extract and nanoparticle-incorporated extract, and then the coated gauze was dried using a hot air oven. This process was carried out for three days; after this process was completed both gauzes were obtained.

Antimicrobial activity

Antimicrobial activity of well diffusion method was used for Pomegranate (*Punica granatum)* peel extract (25µl, 50µl,75µl,100µl) against the organisms of *Escherichia coli, Staphylococcus aureus, Pseudomonas, Aspergillus and Candida* [9]

AATCC test method 147 was carried out for the developed Pomegranate (*Punica granatum*) peel extract-coated Gauze and extract incorporated with silver nanoparticles-coated Gauze. The organisms used against the gauze were *Escherichia coli, Staphylococcus aureus, Pseudomonas* and *Aspergillus.* The coated and uncoated wound gauze was cut into 5*1 cm (length*breath) and placed on each side of a plate and incubated at 37°C for 24 hours. The zone of inhibition was observed and recorded [10].

3. RESULTS

Processing of Pomegranate (*Punica granatum*) peel

The Pomegranate was collected from Tirupur fruit shop and separated the peels then used for exraction process in magnetic stirrer.

Qualitative analysis of phytochemical analysis

According to the results, the peel extract containing tannins, alkaloids, saponins, phenol, protein and flavonoids gives positive results.(Table:1) Pomegranate (*Punica granatum*) peel can be considered a rich source of phytochemicals.

Table:1 Phytochemical analysis

Phytochemical constituents	Results
Tannins	+
Saponins	+
Steroids	-
Terpenoids	-
Glycoside	-
Phenol	+
Alkaloids	+
Quinones	-
Proteins	+
Flavonoids	+
Carbohydrates	-
Iodine Alkaloids	-

‘+’indicates positive, **‘-’**indicates negative

Anti inflammatory assay

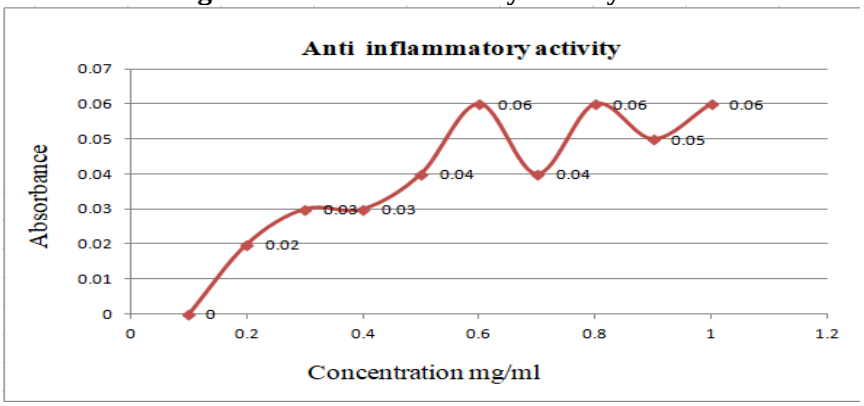
The protein denaturation method was utilized to measure the anti-inflammatory properties of the extract obtained from pomegranate *(Punica granatum*) peel. The pomegranate (*Punica granatum*) peel extract has anti inflammatory properties, which was confirmed by this test; (Table:2) increasing value of anti inflammatory results was observed depending upon the sample.(Figure:2)

Table 2: Anti inflammatory assay for sample

CONCENTRATION (µl)	ABSORBANCE OF (620 nm) OF SAMPLE
0.1	0.00
0.2	0.02
0.3	0.03
0.4	0.03
0.5	0.04
0.6	0.06

0.7	0.04
0.8	0.06
0.9	0.05
1.0	0.06

Figure:2 Anti inflammatory activity



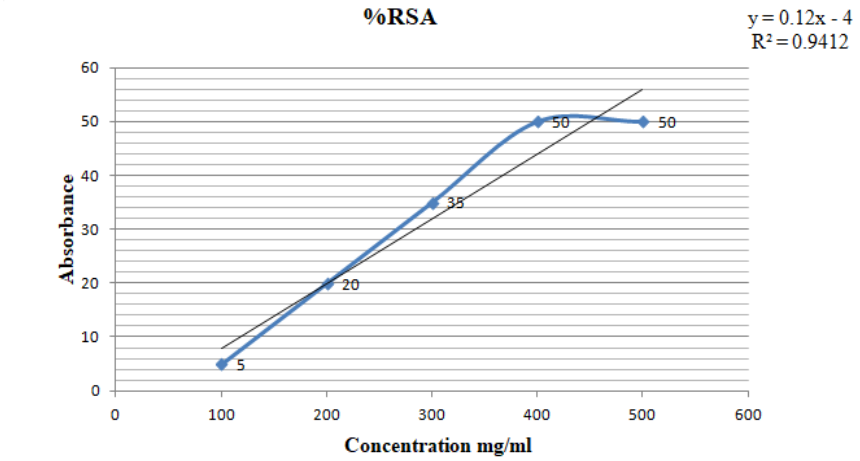
Antioxidant assay

The Antioxidant assay is carried out by DPPH Assay; this test was used here to recognise the antioxidant properties in the Pomegranate (*Punica granatum)* peel extract (Table: 3). Depending upon the increasing amount of extract with DPPH solution, the antioxidant activity was also upgraded.(Figure:3)

Table: 3 Antioxidant assay

CONCENTRATION	CONTROL ABSORBANCE AT 580 nm	SAMPLE ABSORBANCE AT 580 nm	%RSA	IC 50
100	0.20	0.21	5	16.66
200	0.20	0.16	20	
300	0.20	0.13	35	
400	0.20	0.10	50	
500	0.20	0.10	50	

Figure:3 Antioxidant Assay



Thin layer chromatography technique

Thin-layer chromatography is carried out for the identification of pigment
The RF value is calculated by using the formula
Each spot has a retention factor ® expressed as:
RF-distance travelled by the solute/distance travelled by the solvent
RF value of ellagic acid=5.6/5.5
RF value=1.0

Here, the thin-layer chromatography technique is used to separate the ellagic pigment of the Pomegranate (*Punica granatum*) peel extract. This pigment carries phenols and flavonoids these have anti-inflammatory properties. The RF value of this chromatography technique is 1.0.

Antimicrobial activity for extract

The antimicrobial activity was carried out for the pomegranate (*Punica granatum*) peel extract, with the organisms of *Escherichia coli, Staphylococcus aureus, Pseudomonas, Aspergillus and Candida* (Table:4). The well diffusion method was used in extract.

Table:4 Antimicrobial activity for extract

Organisms	25µl of extract	50µl of extract	75µl of extract	100µl of extract
Escherichia coli	15mm	15mm	15mm	16mm
Staphylococcus aureus	22mm	16mm	22mm	22mm
Pseudomonas	6mm	6mm	6mm	8mm
Aspergillus	10mm	10mm	10mm	10mm
Candida	NA	10mm	11mm	11mm

Nanoparticle incorporated in Pomegranate (*Punica granatum)* peel extract

The silver nanoparticle was added to the Pomegranate (*Punica granatum)* peel extract to enhance the antimicrobial, anti-inflammatory and antioxidant properties of the extract.

UV Spectroscopy for Pomegranate (*Punica granatum*) peel extract

The Spectroscopic method has become a powerful tool for secondary metabolite profiling as well as for qualitative and quantitative analysis of pharmaceutical and biological material. The spectroscopy was characterized for the ellagic pigment. The peak was observed in the wavelength range of 200 to700 nm(Table:5). The absorbance for the ellagic pigment at the wavelength of 295nm. Similarly, extract with nanoparticle incorporated wavelength ranges at 638.0, 626.0, 61.0, 489.0, 483.0, 480.0, 446.0, 339.0 nanometers were observed.(Table:6)

Table:5 UV Spectroscopy for Pomegranate peel extract

Wavelength	Absorbance
295.0	4000

Table:6 UV Spectroscopy for nanoparticle incorporated extract

Wavelengh	Absorbance
638.0	3.4461
626.0	3.5272
617.0	3.6126
489.0	3.7747
483.0	3.7545

480.0	3.7423
446.0	3.4078
339.0	2.4859

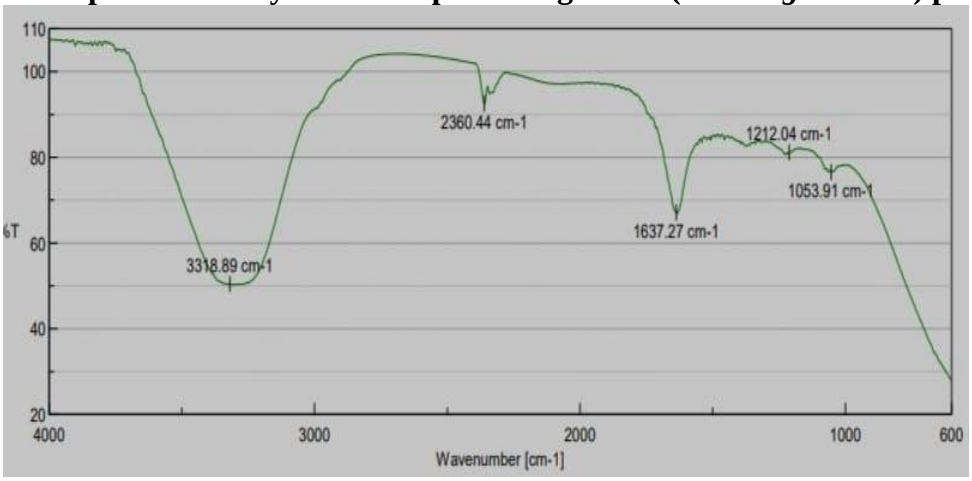
FTIR Spectrum analysis

The Pomegranate (*Punica granatum)*peel extract was analyzed using FTIR to separate the functional groups based on their peak ratios. The results of the analysis confirmed the presence of several functional groups, including alcohol, methane, alkane, alkene, carbonyl, acetylene, nitrile, and ammonia compounds will be present (Table:7). These functional groups were identified by their corresponding peaks at 1053.911cm^{-1}, 1212.04cm^{-1},1637.27cm^{-1},2363.44cm^{-1},3318.89cm^{-1}respectively.(Figure:4)

Table:7 FTIR spectrum analysis

S.No	SAMPLE PEAK	BOND STRENGTH	FUNCTIONAL GROUP	COMPOUND
Peak 1	1053.911 cm^{-1}	Weak	C-O, C-H	Alcohols, Methane group
Peak 2	1212.04cm^{-1}	Weak	C-O,C-C	Alcohols,Alkane
Peak 3	1637.27cm^{-1}	Strong	C=C,C-O-O	Alkene, carbonyl group
Peak 4	2360.44cm^{-1}	Strong	C≡C,C≡N,CH	Acetylene, Nitrile,
Peak 5	3318.89cm^{-1}	Medium	N-H,CH	Ammonia

Figure:4 FTIR spectrum analysis for sample Pomegranate (*Punica granatum*) peel extract



Development of Diabetic foot ulcer gauze

The cotton gauze soaked in Pomegranate (*Punica granatum)* peel extract was in pale yellow and the cotton gauze soaked in nanoparticle- incorporated extract was dark. Those gauzes were obtained at the end of the three days process. (Figure:5)

Figure: 5 Developed cotton gauzes

Antimicrobial activity in developed gauzes

The Pomegranate (*Punica granatum)* peel extract and nanoparticle-incorporated extract-coated gauzes were taken to antimicrobial activity against *Escherichaia coli, Staphylococcus aureus, Pseudomonas and Aspergillus.* AATCC 147 test method was used in prepared gauzes.The nanoparticle-coated gauze gives enhanced antimicrobial activity compared to extract-coated gauze alone. It shows that nanoparticle-incorporated gauze effectively works on diabetic foot ulcers.(Table:8)

Table:8 Antimicrobial activity for both extract coated gauze and nanoparticle incorporated gauze

Organisms	**Zone of inhibition in extract coated gauze**	**Zone of inhibition in nanoparticle incorporated gauze**
Escherichia coli	+	12mm
Staphylococcus aureus	4mm	6mm
Pseudomonas	+	10mm
Aspergillus	+	+

4.CONCLUSION

Pomegranate (*Punica granatum)* peels were gathered, and aqueous extraction was obtained; Phytochemical analysis was observed; anti inflammatory activity was assessed using PBS and BSA method; antioxidant activity was assessed using DPPH method; TLC, UV Spectroscopy and FTIR Spectroscopy were used to analyse the ellagic pigments, bioactive compounds, and functional groups; nanoparticles were added to the extract to improve the healing effect of diabetic foot ulcer; the extract and prepared gauzes antimicrobial activity against *Escherichia coli, Staphylococcus aureus, Pseudomonas, Aspergillus* and *Candida* was then tested; further research is required to standardize the nanoparticle in the coated gauze, and a comparison between prepared and commercial gauze must be conducted.

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