UK Journal of Pharmaceutical and Biosciences
Available at www.ukjpb.com
ISSN: 2347-9442

Evaluation of Wound Healing Activity and Preliminary Phytochemical Screening of *Thespesia populnea* Bark Extracts

Manisha Kothari*, D. K. Jain

SSL Jain PG College, Vidisha (MP)-464001, India

Article Information
Received 20 September 2017
Received in revised form 23 Dec 2017
Accepted 25 December 2017

Keywords:
Thespesia populnea,
Wound healing,
Phytochemical,
Gel

Corresponding Author:
E-mail : manisha16kothari@gmail.com
Mob.: +918989532040

Abstract

The leaves and bark of *Thespesia populnea* are used for the treatment of fracture wounds and as an antiinflammatory poultice applied to ulcers and boils, as a folk medicine. The aim of the present study was to evaluate the wound healing activity and phytochemical screening of *Thespesia populnea* barks extract. The petroleum ether, chloroform, acetone, ethanol and aqueous extract prepared. All the extracts were screened for preliminary phytochemical to determine the nature of secondary metabolite present in bark. Excision wound models were used to evaluate the wound healing activity of extract. The 250 mg and 500 mg of petroleum ether, chloroform, acetone, ethanol and aqueous extract were individually incorporated with 100 g of Carbopol 940 to get 2.5% and 5% (w/w) gel. Preliminary phytochemical investigations of the extracts of barks of *Thespesia populnea* revealed the presence of flavonoids, tannins, phenolic compounds, alkaloids, glycosides, fats and carbohydrates. The order of wound healing activity of various extract were Ethanol extract > Aqueous extract > Acetone extract > Chloroform extract > Petroleum ether extract. The ethanol extracts of exhibited maximum wound healing activity compared to other extracts. The findings could justify, at least partially, the inclusion of this plant in the management of wound healing in folk medicine.

1 Introduction

Wound is defined as the disruption of the anatomic and cellular continuity of tissue caused by chemical, physical, thermal, microbial, or immunological injury to the tissue. Wound healing processes consist of integrated cellular and biochemical cascades leading to reestablishment of structural and functional integrity of the damaged tissue. Various growth factors such as transforming growth factor beta, platelet activation factor, epidermal growth factor, and platelet-derived growth factors seem to be necessary for the initiation and promotion of wound healing.

Various treatment options (analgesics, antibiotics, and nonsteroidal anti-inflammatory drugs) are available for the wound management but majority of these therapies produce numerous unwanted side effects. Presently, several studies have been carried out on herbal drugs to explicate their potential in wound management and these natural remedies proved their effectiveness as an alternative treatment to available synthetic drugs for the treatment of wound[1-3].

Further many developing countries, especially in rural areas, still rely heavily on traditional healer and medicinal plant to meet their primary health care need. This has been mainly attributed to easy availability of herbal medicines and their low cost[4]. Hence, the investigation for reasonably cheap, extensively available, widely accepted and effective wound healing of plant origin; that is equally non invasive in administration, non hormonal in action, nontoxic and relatively long acting in acting continues.

Thespesia populnea (Family: Malvaceae) is a large tree found in the tropical regions and coastal forests in India and cultivated in the gardens. All the parts of the plant used in traditional system of medicine. The bark, leaves, flower and fruits are useful in cutaneous infection such as scabies, psoriasis, eczema, ringworm, and guinea worm. The decoction of the bark

was commonly used for the treatment of skin and liver diseases. A compound oil of bark and capsules is useful in urethritits and gonorrhe.

The bark, root, fruits were used in dysentery, cholera and hemorrhoids. The fruits of the plant are used in Ayurveda for the control of diabetes. The barks and flowers possess astringent, hepatoprotective, antioxidant and antiinflammatory activities in rats. The leaves and bark of this tree are still used to produce oil for the treatment of fracture wounds and as an antiinflammatory poultice applied to ulcers and boils, as a folk medicine. Four naturally occurring quinones viz thespone, thespesone, mansonone-D, and mansonone-H have been extracted from heart wood of the plant[5-7].

Previous studies showed that *Thespesia populnea* contained various chemical constituents including triterpenoids, steroids, flavonoids, anthraquinones, phenolic acids, and alkaloids[8,9]. Certainly, the medicinal activities of the plant are mainly due to the presence of the constitutive secondary metabolites. Further, no report has yet been published on the wound healing activity of bark of *Thespesia populnea*. Hence the present study was aimed to assess preliminary phytochemical screening and wound healing activity of *Thespesia populnea* bark extracts.

2 Materials and Methods

2.1 Plant material

The barks of *Thespesia populnea* was selected for our present work.

2.2 Collection and identification of plant material

The barks of *Thespesia populnea* were collected from the Village Bilantri & Sitamau Dist Mandsaur, M.P. India, medicinal garden of KNK College of Horticulture, Janta Colony, Rajaram Factory, Mandsaur, M.P. India. The plant was authenticated by Dr. S. N. Mishra (Sr. Scientist), K.N.K College of Horticulture. A voucher specimen of the plant was preserved in the herbarium for further reference.

2.3 Preparation of extracts

The powder of the bark of *Thespesia populnea* was packed in the Soxhlet apparatus and successively extracted with petroleum ether, chloroform, acetone, ethanol and distilled water until the completion of the extraction. The extract were filtered while hot, and the resultant extract was distilled in vacuum under reduced pressure in order to remove the solvent completely, and later dried in a desiccator. After that extracts of petroleum ether, chloroform, acetone, ethanol and aqueous were kept in air tight container for further study.

2.4 Preliminary phytochemical tests of extracts

Qualitative chemical tests were performed to determine the presence of alkaloids, carbohydrates, cardiac glycosides, polyphenols, saponins, tannins and terpenoids[10-14].

2.4.1 Test for alkaloids

(a) Dragendorff's test: To 1 ml of the extract, add 1 ml of dragendorff's reagent (Potassium Bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

(b) Mayer's test: To 1 ml of the extract, add 1 ml of mayer's reagent (Potassium mercuric iodide solution). Whitish yellow or cream coloured precipitate indicates the presence of alkaloids.

(c) Hager's test: To 1 ml of the extract, add 3ml of Hager's reagent (Saturated aqueous solution of picric acid), yellow coloured precipitate indicates the presence of alkaloids.

(d) Wagner's test: To 1 ml of the extract, add 2 ml of wagner's reagent (Iodine in Potassium Iodide), Formation of reddish brown precipitate indicates the presence of alkaloids.

2.4.2 Test for Proteins

(a) Biuret test: Added 1ml of 40% sodium hydroxide solution and 2 drops of 1% CuSO4 solution till a blue color was produced, and then added to the 1ml of the extract. Formation of pinkish or purple violet color indicated the presence of proteins.

(b) Ninhydrin test: Added two drops of freshly prepared 0.2% Ninhydrin reagent (0.1% solution in n-butanol) to the small quantity of extract solution and heated. Development of blue color revealed the presence of proteins, peptides or amino acids.

(c) Millon's test: 1ml of test solution was made acidify with sulphuric acid and added Millon's reagent and boiled this solution. A yellow precipitate was formed indicated the presence of protein.

(d) Xanthoproteic test: To 1ml of the extract, added 1ml of concentrated nitric acid. A white precipitate was formed, it is boiled and cooled. Then 20% of sodium hydroxide or ammonia was added. No formation of orange color indicated the absence of aromatic amino acids.

2.4.3 Test for Glycosides

(a) Legal test: Dissolve the extract in pyridine and add sodium nitroprusside solution to make it alkaline. The formation of pink red to red colour shows the presence of glycosides.

(b) Baljet test: To 1ml of the test extract, add 1ml of sodium picrate solution and the yellow to orange colour reveals the presence of glycosides.

(c) Keller-Killiani test: 1gm of powdered drug is extracted with 10ml of 70% alcohol for 2 minutes, filtered, add to the filtrate, 10ml of water and 0.5ml of strong solution of lead acetate and filtered and the filtrate is shaken with 5ml of chloroform. The chloroform layer is separated in a porcelein dish and removes the solvent by gentle evaporation. Dissolve the cooled residue in 3ml of glacial acetic acid containing 2 drops of 5% ferric chloride solution. Carefully transfer this solution to the surface of 2ml of concentrated sulphuric acid. A reddish brown layer forms at the junction of the two liquids and the upper layer slowly becomes bluish green, darkening with standing.

(d) Borntrager's test: Add a few ml of dilute Sulphuric acid to 1ml of the extract solution. Boil, filter and extract the filtrate with chloroform. The chloroform layer was treated with 1ml of ammonia. The formation of red colour of the ammonical layer shows the presence of anthraquinone glycosides.

2.4.4 Test for carbohydrates and sugars

(a) Molisch's test: To 2ml of the extract, add 1ml of α-napthol solution, add concentrated sulphuric acid through the side of the test tube. Purple or reddish violet colour at the junction of the two liquids reveals the presence of Carbohydrates.

(b) Fehling's test: To 1ml of the extract, add equal quantities of Fehling solution A and B, upon heating formation of a brick red precipitate indicates the presence of sugars

(c) Benedict's test: To 5ml of Benedict's reagent, add 1ml of extract solution and boil for 2 minutes and cool. Formation of red precipitate shows the presence of sugars.

2.4.5 Test for tannins and phenolic compounds

(a) Take the little quantity of test solution and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins.

(b) To 1ml of the extract, add ferric chloride solution, formation of a dark blue or greenish black colour product shows the presence of tannins.

(c) The little quantity of test extract is treated with Potassium ferric cyanide and ammonia solution. A deep red colour indicates the presence of tannins.

2.4.6 Test for flavonoids

(a) The drug in alcoholic and aqueous solution with few ml of ammonia is seen in U.V. and visible light, formation of fluorescence indicates the presence of flavonoids.

(b) Little quantity of extract is treated with amyl alcohol, sodium acetate and ferric chloride. A yellow colour solution formed, disappears on addition of an acid indicates the presence of flavonoids.

(c) Shinoda's test: The alcoholic extract of powder treated with magnesium foil and concentrated HCl give intense cherry red colour indicates the presence of flavonones or orange red colour indicates the presence of flavonols.

(d) The extract is treated with sodium hydroxide, formation of yellow colour indicates the presence of flavones.

(e) The extract is treated with concentrated H_2SO_4, formation of yellow or orange colour indicates flavones.

2.4.7 Test for steroids

(a) Libermann-Burchard test: 1gm of the test substance was dissolved in a few drops of chloroform, 3ml of acetic anhydride, 3ml of glacial acetic acid were added, warmed and cooled under the tap and drops of concentrated sulphuric acid were added along the sides of the test tube. Appearance of bluish-green colour shows the presence of sterols.

(b) Salkowski test: Dissolve the extract in chloroform and add equal volume of conc. H_2SO_4. Formation of bluish red to cherry colour in chloroform layer and green fluorescence in the acid layer represents the steroidal components in the tested extract.

2.4.8 Test for fixed oils and fats

(a) Spot Test: Press a small quantity of extracts between the filter paper. Oil stains on paper indicates the presence of fixed oils.

(b) Saponification test: To 1ml of the extract, add few drops of 0.5 N alcoholic Potassium hydroxide along with a drop of phenolphthalein. Heat the mixture on a water bath for 1-2 hours. The formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

2.5 Wound healing activity

2.5.1 Preparation of Wound

The wound site was prepared following the excision wound model. Albino rats (Wistar strain) of both sexes weighing between 180–230 gm were randomly divided into five groups of six animals each. The animals were anaesthetized with diethyl ether and the hairs on the skin of the nape of the neck, shaved with sterilized razor blades. A circle of diameter 400 mm^2 was marked on each of the two sides of the skin. Circular incisions

were then made on the marked areas of the skin surface and the skin carefully dissected out. The area was measured immediately by tracing out the wound area using a transparent tracing paper and the squares counted.

2.5.2 Drug Formulation

The extracts were formulated as gel. The 250 mg and 500 mg of petroleum ether, chloroform, acetone, ethanol and aqueous extract were individually incorporated with 100 g of Carbopol 940 to get 2.5% and 5% (w/w) gel. These gels were applied topically over wounds of animals.

2.5.3 Determination of wound healing rate

Treatment with the gel and standard drug i.e. Soframycin started immediately after incision by application on the wound surface, of the gel, twice daily. The two wound sites on each animal were treated similarly.

Group I served as control groups, administered drinking water

Group II animals were treated with Soframycin

Group III and IV animals were treated with 2.5% and 5% petroleum ether extract gel, respectively

Group V and VI animals were treated with 2.5% and 5% chloroform extract gel, respectively

Group VII and VIII animals were treated with 2.5% and 5% acetone extract gel, respectively

Group IX and X animals were treated with 2.5% and 5% ethanol extract gel, respectively

Group XI and XII animals were treated with 2.5% and 5% aqueous extract gel, respectively

All the gels were applied topically after dressing the wound with methylated spirit. The wound areas were measured while the animals were under anesthesia on the 0, 4th, 8th, 12th and 16th day after surgery. The progressive reduction in the wound area was monitored periodically by tracing the wound margin on paper and the area was measured using graph paper[15-21]. The reduction in the wound size was calculated by the formula:

Wound contraction% = (difference in the area of the wound in Sq.mm between the initial and on a particular post-operative day) × 100/area of the wound in sq.mm immediately after the wound excision

2.5.4 Statistical analysis

The results are expressed as mean ± SEM of six independent experiments. Statistical significance between the groups was evaluated by one-way analysis of variance (ANOVA) followed by Dunet's test. A $P < 0.05$ value was considered as statistically significant.

3 Results and Discussions

3.1 Phytochemical screening

Presence of classes of secondary metabolite may be a useful indicator of both efficacy and potential toxicity; hence test for the presence of phytochemical classes with known bioactivity was done.

Preliminary phytochemical investigations of the extracts of barks of *Thespesia populnea* revealed the presence of flavonoids, tannins, phenolic compounds, alkaloids, glycosides, fats and carbohydrates. The details are presented in table 1.

From the result of phytochemical screening, the petroleum ether extract of barks of *Thespesia populnea* exhibited the presence of fats and oils. The alkaloids and steroids were present in chloroform extracts. The acetone extract revealed the occurrence of alkaloids and flavonoids. Alkaloids, glycosides, carbohydrates, flavonoids, tannins and polyphenol were found in hydroalcoholic extracts of barks of *Thespesia populnea*. Similarly glycosides, carbohydrates, flavonoids, tannins and polyphenol were existing in aqueous extracts of barks of *Thespesia populnea*. The maximum phytocostituents were observed in ethanol extracts of barks of *Thespesia populnea* (Table 1). Further petroleum ether, chloroform, acetone, ethanol and aqueous extracts of *Thespesia populnea* were selected for quantify the alkaloids, flavonoids and polyphenol.

3.2 Wound healing activity

From the table 2 it reveals the improvement of wound healing induced by barks of *Thespesia populnea* of different gel formulation extracts (Petroleum ether, Chloroform, Acetone, Ethanol and Aqueous extracts of 2.5% and 5% w/w gel) treated groups, untreated group (control) and soframycin (standard drug) treated group of animals. The mean percentage closure of wound area was calculated on the 4th, 8th, 12th and 16th post wounding days as shown in table 2. The animals treated with 2.5% petroleum ether extracts gel did not produce significant wound healing activity. While the 5% petroleum ether extract treated animals exhibited significant wound healing activity after 8th days. The 5% chloroform and acetone extract gel treated animals demonstrated significant difference from control group animals on 4th days of treatment of gel. The 2.5% chloroform and acetone extract gel treated animals demonstrated significant difference from control group animals on 8th days of treatment of gel. The both doses of ethanol and aqueous extract gel treated animals demonstrated significant difference from control group animals on 4th days of treatment of gel. The standard group exhibited significant wound healing activity from 4th days of treatment. The rate of wound contraction was found to reach a maximum on the 16th day in the treated groups. The ethanol extracts treated animals showed faster epithelialization of wound (16 days) than the animals treated with other extract. The period of epithelialization was 14 in the case of standard

drug. The order of wound healing activity of various extract were Ethanol extract > Aqueous Extract > Acetone extract > Chloroform extract > Petroleum ether extract. The ethanol extracts of exhibited maximum wound healing activity compared to other extracts.

Table 1: Phytochemicals present in barks of *Thespesia populnea* extracts

Phytoconstituent		Pet. Ether	Chloroform	Acetone	Ethanol	Aqueous
Alkaloids	Dragendorff's test	-	+	-	-	-
	Hager's test	-	+	-	+	-
	Mayers	-	-	+	+	-
	Wagners	-	-	-	+	-
Glycosides	Legal's test	-	-	-	+	+
	Keller killiani test	-	-	-	+	+
	Baljet test	-	-	-	+	-
	Keller-Killiani test	-	-	-	-	-
Carbohydrates	Borntrager's test	-	-	-	+	+
	Molish test	-	-	-	+	+
	Benedict's test	-	-	-	+	+
	Fehling's test	-	-	-	+	-
Tannins and Phenolic compound	$5\%Fecl_3$ solution	-	-	-	+	+
	Lead acetate solution	-	-	-	+	+
	Bromine water	-	-	-	+	-
	Potassium ferric cyanide and ammonia solution	-	-	-	+	+
Flavonoids	Shinoda test	-	-	+	+	+
Steroid test	Liebermann burchard test	-	+	-	-	-
	Salkowski test	-	-	-	-	-
Protein	Biuret test	-	-	-	-	-
	Ninhydrin test	-	-	-	-	-
Fat and oil test	Saponification test	-	-	-	-	-
	Spot Test	+	-	-	-	-

+ = Present, – = Absent

Wound healing is a complex and dynamic process of restoring cellular structures and tissue layers in damaged tissue as closely as possible to its normal state. Wound contraction is a process that occurs throughout the healing process, commencing in the fibroblastic stage whereby the area of the wound undergoes shrinkage. In the maturational phase, the final phase of wound healing the wound undergoes contraction resulting in a smaller amount of apparent scar tissue. The wound healing study exhibited progressive reduction in wound area and enhanced period of epithelization of extract treated rats. The changes in the test groups might be attributed to the potential of the test extracts or its constituents to promote epithelization either by facilitating proliferation or by increasing the viability of epithelial cells[22,23].

Wound has infrequently breaking strength in the commencement which boosts rapidly during healing due to the synthesis and maturation of collagen. Collagen contributes strength and elasticity to recovered skin. As the wound heals, collagen molecules are synthesized and laid down at the wound site. These molecules become cross-linked to form fibers. The findings of study demonstrated the intensity of the repaired wound tissue might be the result of the remodeling of collagen

and the formation of stable intra- and inter-molecular cross linking, which is necessary for maturation of collagen as described by Pather et al.[24] and Abraham et al.[25] on different plant extracts. Consequently, the study suggested that the extracts could proliferate collagen synthesis and perhaps aid in creation of cross linkages as the collagen matures[22,26].

Table 2: Effects of topical application of *Thespesia populnea* extract in excision wound model

Group	0 Days	4 Days	8 Days	12 Days	16 Days	Epethilization period (days)
Control	403.8±2.41 (0%)	323.4±4.46 (19.91%)	228.6±3.91 (13.38%)	152.3±2.28 (62.28%)	85.2±2.75 (78.90%)	28
Standard	410.2±2.35 (0%)	186.7 ±7.42* (54.48%)	72.8±1.51* (82.25%)	21.3±10.29* (94.80%)	0 (100%)	14
PE (2.5%)	390.5±2.91 (0%)	310.8 ±7.62 (20.40%)	214.7±6.43 (45.01%)	141.5±5.12 (63.76%)	71.2±5.12 (81.76%)	26
PE (5%)	405.7±3.21 (0%)	292.6±5.46 (27.87%)	178.3±8.17 (56.05%)	122.7±3.26* (69.75%)	43.6±5.12* (89.25%)	23
CE (2.5%)	415.5±2.91 (0%)	298.1 ±7.62 (28.25%)	161.4±6.43* (61.15%)	101.8±5.12* (75.49%)	53.4±5.12* (87.14%)	23
CE (5%)	402.9±3.21 (0%)	279.4±5.46* (30.65%)	121.5±8.17* (69.86%)	73.2±3.26* (81.83%)	21.1±5.12* (94.76%)	19
AE (2.5%)	395.6±2.91 (0%)	291.5 ±7.62 (26.31%)	152.7±6.43* (61.40%)	91.2±5.12* (76.94%)	28.3±5.12* (92.84%)	22
AE (5%)	406.3±3.21 (0%)	266.2±5.46* (34.48%)	112.5±8.17* (72.38%)	65.3±3.26* (83.92%)	10.7±5.12* (97.41%)	19
EE (2.5%)	413.5±2.91 (0%)	263.7 ±7.62* (36.22%)	132.4±6.43* (67.98%)	72.1±5.12* (82.56%)	0 (100%)	20
EE (5%)	405.8±3.21 (0%)	230.5±5.46* (43.19%)	89.2 ±8.17* (78.01%)	32.6±3.26* (91.96%)	0 (100%)	16
AqE (2.5%)	418.4±2.91 (0%)	285.7 ±7.62* (31.79%)	145.3±6.43* (65.27%)	85.9±5.12* (79.46%)	0 (100%)	21
AqE (5%)	402.6±3.21 (0%)	256.3±5.46* (36.33%)	95.5 ±8.17* (76.27%)	53.8±3.26* (86.63%)	0 (100%)	18

*Significantly different from the control at P<0.05, n=6; PE – Pet. Ether extract, CE – Chloroform extract, AE – Acetone extract, EE – Ethanol extract, AqE – Aqueous extract

The antimicrobial activity of plant extract imparts chief role in enhancing the healing of wound. Kumar and Simon (2016) reported significant antimicrobial activity of leaves of *Thespesia populnea* extracts[27]. The microorganism present surrounding of wound lead to infection, and retards the healing of wound. The antimicrobial agent inhibits the growth of microorganism and keeps the wound free of infections[22]. The alkaloid has inhibitory activity against the gram positive and gram negative bacteria. The findings of phytochemical screening indicate the presence of alkaloid in chloroform, acetone and ethanol extracts. In addition, the antimicrobial activity of the extract of *Thespesia populnea* might be integrated with the wound healing activity. Considering the fact that *Staphylococcus aureus* and *Escherichia coli* are among the commonest strains of microorganisms associated with open wound infections, the alkaloid can be a promising remedy not only for the treatment of such wounds but also in managing infections in which these microorganisms are implicated. The petroleum ether extract was devoid of alkaloids test and produces poor wound healing activity. Additionally, the extracts containing alkaloids exhibited the moderate to good wound healing activity. The ethanol extracts incorporating maximum quantity of alkaloids and produces higher wound healing activity compared to other extracts. The study support the wound healing activity of extract might be due to presence of alkaloids.

The various researchers documented that antioxidant activity of plant extracts promotes healing of wound. The oxidative stress has been implicated in a variety of degenerative processes and diseases. The free radicals initiate the acute and chronic inflammation in the body. The antioxidant compound

suppresses the oxidative stress, and enhances the acute and chronic inflammatory condition[28]. Further it proliferate the wound healing activity.

The phytochemicals namely glycosides, flavonoids, polyphenols, saponins and tannins were among the major phytoconstituents found in the ethanol and aqueous extracts of *Thespesia populnea*. A number of active compounds isolated from plants have been revealed in animal models as active principles responsible for assisting healing of wounds. Previous study on *Terminalia arjuna* showed that tannins enhance wound healing action by improving regeneration and organization of the new tissue[29]. Flavonoids diminish the lipid peroxidation by preventing or slowing the onset of cell necrosis and by improving vascularity. Accordingly, a drug that inhibits lipid peroxidation is believed to increase viability of collagen fibrils by increasing strength of collagen fibres, increasing circulation, preventing cell damage and by promoting DNA synthesis. Flavonoids are also known for their astringent and antimicrobial property[22,30]. The findings of phytochemical screening indicate the presence of flavonoids in acetone, ethanol and aqueous extracts. Moreover, the ethanol extracts incorporating maximum quantity of flavonoids compared to other extracts. The wound healing activity of ethanol extract was more effective compared to other extracts containing flavonoids. Thus, wound healing property of *Thespesia populnea* may attribute it to the additive effect of the alkaloids and flavonoids present in the extract.

4 Conclusion

Thespesia populnea is rich in secondary metabolite such as alkaloid, glycoside, flavonoids, polyphenol etc. Since the alkaloids, flavonoids and polyphenol compounds found to be active against various pharmacological activity. The results shows that ethanol extract of *Thespesia populnea* barks showed significant wound healing activity. The synergism provoked by maximum quantity of alkaloids, polyphenol and flavonoids could be the reason for the heightened wound healing activity of the ethanol extract of *Thespesia populnea* when compare with other groups. These findings could justify, at least partially, the inclusion of this plant in the management of wound healing in folk medicine.

5 Conflict of interest

The authors declare that there are no conflicts of interest.

6 Author's contributions

MK performed the experimental work. DKJ carried out draft the manuscript.

7 References

1. Thakur R, Jain N, Pathak R, Sandhu SS. Practices in Wound Healing Studies of Plants. Evidence-Based Complementary and Alternative Medicine. 2011; 2011: 1-17.
2. Shuid AN, Anwar MS, Yusof AA. The effects of Carica papaya Linn. latex on the healing of burn wounds in rats. Malaysian Journal of Medicine and Health Sciences. 2005; 3(2): 39–47.
3. Jalalpure SS, Agrawal N, Patil MB, Chimkode R, Tripathi A. Antimicrobial and wound healing activities of leaves of *Alternanthera sessilis* Linn. International Journal of Green Pharmacy. 2008; 2(3): 141–144.
4. Shaw D. Risks or remedies? Safety aspects of herbal remedies. J. Roy. Soc. Med. 1998; 91: 294–296.
5. Vasudevan M, Parle M. Pharmacological actions of Thespesia populnea relevant to Alzheimer's disease. Phytomedicine. 2006; 13: 677-687.
6. Vohora SB, Mishra GV. Rational basis of the use of medicinal plants in skin diseases. Indian Drugs. 1998; 35: 1-17.
7. Nagappa AN, Cheriyan B. Wound healing activity of the aqueous extract of Thespesia populnea fruit. Fitoterapia. 2001; 72: 503-506.
8. Akhila S, Rani K. Biosynthesis of gossypol in Thespesia populnea. Phytochemistry. 1993; 33: 335-340.
9. Johnson Inbaraj J, Gandhidasan R, Murugesan R. Cytotoxicity and superoxide anion generation by some naturally occurring quinones. Free Radical Biology and Medicine. 1999; 26: 1072- 1078.
10. Kenwat R, Prasad P, Sahu RK, Roy A, Saraf S. Preliminary phytochemical screening and in vitro antioxidant efficacy of fruit oil of Martynia annua. UK Journal of Pharmaceutical and Biosciences. 2014; 2(1): 16-22.
11. Gupta AK, Ahirwar NK, Shinde N, Choudhary M, Rajput YS, Singh A. Phytochemical Screening and Antimicrobial Assessment of Leaves of Adhatoda vasica, Azadirachta indica and Datura stramonium. UK Journal of Pharmaceutical and Biosciences. 2013; 1(1): 42-47.
12. Purohit P, Bais RT, Singh P, khan S. Assessment of antibacterial activity and phytochemical screening of *Hemidesmus indicus* root extracts. UK Journal of Pharmaceutical and Biosciences. 2014; 2(6): 67-72.
13. El-Feky AM, Aboulthana WM. Phytochemical and Biochemical Studies of Sage (*Salvia officinalis* L.). UK Journal of Pharmaceutical and Biosciences. 2016; 4(5): 56-62.
14. Ravi K, Bharavi K, Ravi KP, Vamsi KB. Phytochemical Screening and Antibacterial Activity of

Ethanol Extract of Leaves and Twigs of *Azadirachta indica* A. Juss. UK Journal of Pharmaceutical and Biosciences. 2015; 3(6): 56-59.

15. Qi Zeng, Hui Xie, Hongjin Song. In Vivo Wound Healing Activity of *Abrus cantoniensis* Extract. Evidence-Based Complementary and Alternative Medicine. 2016; 2016: 1-7.
16. Murthy S, Gautam MK, Goel S, Purohit V, Sharma H, Goel RK. Evaluation of In Vivo Wound Healing Activity of *Bacopa monniera* on Different Wound Model in Rats. BioMed Research International. 2013; 2013: 1-9.
17. Christian A, Solomon BB, Yaw DB, Patrick GA, Martin AY, Kwesi BM. Evaluation of Antimicrobial and Wound Healing Potential of *Justicia flavaand Lannea* welwitschii. Evidence-Based Complementary and Alternative Medicine. 2013; 2013: 1-10.
18. Ipek PS, Ufuk K, Esra KA, Demet Y, Murat A. Assessment of Wound Healing Activity of the Aqueous Extracts of *Colutea cilicica* Boiss. & Bal. Fruits and Leaves. Evidence-Based Complementary and Alternative Medicine. 2011; 2011: 1-7.
19. Morton JJP, Malone MH. Evaluation of vulnerary activity by an open wound procedure in rats. Arch Int Pharmacodyn. 1972;196:117–26.
20. Diwan PV, Tilloo LD, Kulkarni DR. Influence of Tridax procumbens on wound healing. Indian J Med Res. 1982;75:460–4.
21. Agarwal PK, Singh A, Gaurav K, Goel S, Khanna HD, Goel RK. Evaluation of wound healing activity of extracts of plantain banana (*Musa sapientum* var. paradisiaca) in rats. Indian Journal of Experimental Biology. 2009; 47: 32–40.
22. Mechesso AF, Tadese A, Tesfaye R, Tamiru W, Eguale T. Experimental evaluation of wound healing activity of *Croton macrostachyus* in rat. African Journal of Pharmacy and Pharmacology. 2016; 10(39): 832-838.
23. Mukherjee H, Ojha O, Bharitkar YP, Ghos S, Monda S, Kaity S, Dutta S, Samanta A, Chatterjee TK, Chakrabarti S, Mondal NB, Chattopadhyay D. Evaluation of the wound healing activity of *Shorearo busta*, an Indian ethnomedicine, and its isolated constituent(s) in topical formulation. J. Ethnopharmacol. 2013; 149:335-343.
24. Pather N, Viljoen AM, Kramer B. A biochemical comparison of the in vivo effects of *Bulbine frutescens and Bulbine natalensis* on cutaneous wound healing. J. Ethnopharmacol. 2011; 133:364-370.
25. Abraham F, Eyasu M, Tadesse E, Asfaw D, Getinet AM. Evaluation of invivo wound healing activity of methanol extract of Achyranthes aspera. J. Ethnopharmacol. 2012; 143:469-74.
26. Wang J, Ruan J, Cai Y, Luo Q, Xu H, Wu Y. *In vitro and in vivo* evaluation of the wound healing properties of *Siegesbeckia pubescens*: Ethnopharmacological communication. J. Ethnopharmacol. 2011; 134:1033-1038.
27. Kumar NS, Simon N. *In vitro* antimicrobial activities and phytochemical analysis of crude leaf extracts of *Thespesia populnea* (l). Int. J. of Curr. Res. in Life Sci. 2016; 5(3): 566-568.
28. Pesin Suntar I, KupeliAkkol E, Yılmazer D, Baykal T, Kirmizibekmez H, Alper M, Yesilada E. Investigations on the in vivo wound healing potential of *Hypericum perforatum* L. J. Ethnopharmacol. 2010; 127:468-477.
29. Chaudhari M, Mengi S. Evaluation of phytoconstituents of *Terminalia arjuna* for wound healing activity in Rats. Phytother. Res. 2006; 20:799-805.
30. Devipriya S, Shyamala Devi CS. Protective effect of quercetin in cisplatin induced cell injury in the kidney. Indian J. Pharmacol.1999; 31:422-425.