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Evaluation of Antibacterial, Antioxidant, Wound Healing Properties of Different Solvent Fractions of *Adina cordifolia* Leaves in Experimental Animals

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SK and SMV designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SK, HK and CKK managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was conducted to investigate the antioxidant potential, antimicrobial activity and wound healing activity of ethyl acetate, butanol, and aqueous fractions of methanolic extract of leaves of *Adina cordifolia* (Roxb.) a locally used leave in Jharkhand, India.
Place and Duration of Study: Department of Pharmaceutical Sciences, Birla Institute of Technology, Mesra, Ranchi between June 2014 to April 2015.
Methods: Dried powder of the *Adina cordifolia* leaves was extracted with methanol. The methanolic extract was then fractionated with solvents of increasing polarity; ethyl acetate, n-butanol and the residual soluble aqueous fraction. Estimation of total phenolic and total flavonoid contents for the methanol extract and all fractions was done. The free radical scavenging activity

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was studied *in-vitro* by measuring 2,2-diphenyl-1- picrylhydrazyl (DPPH) and hydrogen peroxide scavenging of methanolic extract and all the three fractions. Antibacterial activities were evaluated against four micro-organisms *viz; Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* using agar well diffusion method. The wound healing activity was evaluated by excision and incision wound models.

Results: Among all the three fractions, n-butanol fraction and aqueous fraction possessed high amount of phenolic and flavonoid contents as compared to the ethyl acetate fraction. Subsequently the n-butanol fraction and aqueous fraction also showed better free radical scavenging activity on DPPH and hydrogen peroxide radical than ethyl acetate fraction. Antibacterial activities were evaluated against four micro-organisms in which only the n-butanol fraction and Ethyl acetate fraction had showed effective antimicrobial activity against *Staphylococcus aureus* and n-butanol fraction showed better antimicrobial activity at different concentrations than Ethyl acetate fraction. Excision wound model showed highly significant effect of drugs on percent wound contraction at all the periods under study and at day 12, percent wound contraction was 96.03 ± 0.78 in test drug and 66.16 ± 1.56 in control. In incision wound model, significantly higher tensile strength was observed in standard (5.022 ± 0.052 g/mm^2) and test drug (4.858 ± 0.073 g/mm^2) as compared to control (2.960 ± 0.031 g/mm^2).

Conclusion: This results shows that the n-butanol fraction possess effective wound healing properties due to their antimicrobial and antioxidant activities by possessing the active compounds such as flavonoids (polyphenols), terpenes, alkaloids, saponins.

Keywords: Antibacterial; antioxidant; Adina cordifolia; wound healing.

1. INTRODUCTION

Nature has been a source of medicinal treatments for thousands of years. Plants have formed the basis of traditional medicine systems that have been the way of life for thousands of years. Mostly herbs contain polyphenols which are most powerful natural antioxidants and are highly valued for their antioxidant and anti-aging effects. Antioxidant substances can block the harmful action of the free radicals by scavenging the free radicals and detoxify the organism. Oxidative stress due to the production of free radicals such as superoxide radical (O^{-2}), hydroxyl radical (OH^-), peroxide radical (ROO^-), and nitric oxide radical, is the major cause of a variety of pathological conditions including coronary heart diseases, reperfusion injury, inflammation, diabetes, drug toxicity, carcinogenesis and neurodegenerative diseases such as Parkinson and Alzheimer diseases [1]. Antioxidant compounds like phenolic acids, polyphenols and flavonoids inhibit the mechanism that leads to degenerative diseases [2]. A large number of plants are used by folklore traditions in India for treatment of cuts, wounds and burns. Various research data revealed that plants may worked as healing and regeneration of the tissue by multiple mechanisms. Haldu (*Adina cordifolia*) is deciduous tree over 20 m high belonging to the subfamily Cinchonoideae, family Rubiaceae. It is native to Southern Asia, from India and Srilanka east to southern China and Vietnam. It is found scattered in deciduous forests throughout the greater part of India, ascending to an altitude of 900 m in sub-Himalayan tract. It is also common in forests of South India [3]. *Adina cordifolia* has been reported to have multiple pharmacological properties and has been widely used in treatment. As in traditional uses leaves are applied over swollen portion to remove pain and swelling [4]. Bark is used as an antibacterial, eczema, scabies, bark paste is applied to eczema, scabies, or bacterial infections on the skin. Leaf juice is used to treat boils and eye disorders like conjunctivitis [5]. It displays plethora of potential biological activities such as anticancer [6], antiulcer [7], hepatoprotective [8], anti-inflammatory [9], antifertility [10], antidiabetic [11], antiamoebic [3], antinociceptive [12] *etc.* from its various parts. Furthermore, this plant have also used in curing various ailments such as rheumatism [13], stomachache [14], headache [15], cold/cough [16], toothache [17], fever [18], pain and swelling [4], bacterial infection [5], urinary problems [19], conjunctivitis [20], miscarriage [21] *etc*. Keeping the view of the above mentioned medicinal importance of this plant, we therefore became interested to investigate the wound healing activity of this plant. To the best of our knowledge, the wound healing activity from this plant has not been reported so far till now. In the present paper, we would like to explore the antioxidant, antibacterial activity of the three fractions and wound healing activity of selected fraction.

2. MATERIALS AND METHODS

2.1 Sample Collection and Extraction

The leaves were collected in the month of July and August 2014 from BIT Mesra campus, Ranchi, India. The plant was identified by Dr. S. Jha (Professor, Birla Institute of Technology, Mesra) and authenticated by Central National Herbarium of Botanical Survey of India (CNH/Tech.II/2014/105/250), Kolkata as *Adina cordifolia* Roxb. The leaves at maturity were collected and dried under shade to obtain 1.0 kg dry sample. The dried samples were powdered and used for solvent extraction. For extract preparation, 1.0 kg of dried sample was extracted twice with 2.0 L of 95% methanol at 25°C for 48 h. The extracts were filtered with Whatman No. 1 filter paper and evaporate to dry the filtrate by using rotary evaporator. The extract was suspended in distilled water and partitions were made with increasing polarity of solvents i.e. ethyl acetate,n- butanol and water. Then all fractions were dried by using rotary evaporator, and preserved at 4°C. Then the fractions were subjected to quantitative analysis of phytochemicals and checked for antioxidant and antimicrobial activity.

2.2 Phytochemical Analysis

2.2.1 Qualitative analysis of phytochemicals

The methanolic extract of leaves was subjected to preliminary phytochemical screening. Test for alkaloids (Mayer's test), flavonoids (Alkaline reagent test), carbohydrates (Molish's test), glycosides (Legal's test), saponins, tannins, triterpenoid (Libermann Burchard test), proteins and amino acids, anthraquinones, steroids, phlobatannins and resins were done to check the presence of phyto constituents [22,23,24].

2.2.2 Quantitative analysis of phytochemicals

The total phenol content was determined using Folinciocalteau reagent [25] and the total flavanoid content was estimated using method of Park et al. [26].

2.3 Determination of *In vitro* Antioxidant Activity

2.3.1 DPPH radical scavenging assay

The ability of fractions to scavenge 2, 2'-diphenylpicrylhydrazyl was determined according to the method of Ismail and Hong [27]. For DPPH assay the different concentrations of three fractions i.e. n-butanol, ethyl acetate and aqueous fraction were made. The different concentrations of three fractions were of 100 µg/mL, 150 µg/ml, 200 µg/ml, 250 µg/ml and 300 µg/ml in methanol. The concentration of the standard i.e. DPPH solution was made 0.1 mM by dissolving 3.94 g. of DPPH in 100 ml of methanol. Then after the DPPH solution was added in the three fraction solution in the ratio of 1:1. After addition of DPPH solution, the solutions of three fractions were incubated for half an hour. After half an hour the absorbance values of all the solutions and the control (DPPH solution in methanol) were taken in the UV spectrophotometer at 517 nm. Since DPPH is very light sensitive so that whole procedure was carried out in dark condition.

The percentage of DPPH scavenging of fractions was calculated using formula:

$$\% \text{ scavenged (DPPH)} = [(A_o - A_1)/A_o] \times 100$$

Where,

A_o =Absorbance of control
A_1 =Absorbance of test

2.3.2 Hydrogen peroxide scavenging assay

The ability of fractions to scavenge hydrogen peroxide was determined according to the method of Ruch et al. [28]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined spectropthoto-metrically measuring absorption with extinction coefficient for H_2O_2 of 81 $M^{-1}cm^{-1}$. Fractions (12.5-62.5 µg/ml) in distilled water were added to hydrogen peroxide (0.6 ml, 40 mM). Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without Hydrogen peroxide.

The percentage of Hydrogen peroxide scavenging of three fractions was calculated using formula:

$$\% \text{ scavenged } (H_2O_2) = [(A_o - A_1)/A_o] \times 100$$

Where,

A_o =Absorbance of control
A_1 =Absorbance of test

2.4 Determination of Antibacterial Activity

The antibacterial activity was tested using agar well diffusion according to Irshad et al. [29]. The

strains of bacteria were procured from the microbiology lab of Biotechnology department, BIT Mesra. All the three fractions were tested against *Pseudomonas aeruginosa* (NCIM 2036), *Escherichia coli* (NCIM 2021), *Bacillus subtilis* (NCIM 2193), *Staphylococcus aureus* (NCIM 2122).

2.4.1 Agar diffusion assay

Bacterial isolates were prepared to match 0.5 McFarland standards. The antimicrobial activity was tested against all the three fractions. 1ml of the test culture was inoculated into a sterile plate with 20 ml Muller Hinton molten agar which was made to solidify. 5 wells of approximately 6 mm in diameter were made on the surface of the agar plates using a sterile borer. Stock solution of each fraction was prepared at concentrations of 4 mg/ml, 2 mg/ml, 1 mg/ml, 0.5 mg/ml, and 0.25 mg/ml in DMSO. Each well was filled with 0.10 ml of fraction solution. 0.10 ml of DMSO was taken as negative control and 0.10 ml of Gentamycin served as a positive control respectively. The plates were then incubated at 37ºC for 24 h and zone of inhibition was measured. The clear zone of inhibition around the fractions was measured in millimeter.

2.5 Wound Healing Activity

2.5.1 Experimental animal

Fifteen adult healthy swiss albino mice of either sex, weighing between 20-25 gm were used for the study. Animals were procured from laboratory animal house of Birla Institute of Technology, Mesra (Protocol no PROV/BIT/PH/IAEC/38/2014). The animals were house in standard condition (temperature 240±2 with 50-60% relative humidity and a 12 hours light dark cycle). The entire animal had free access to water and normal diet. The study was approved by Institutional Animal Ethical Committee (IAEC) and was in accordance with the guideline of the Committee for the Purpose of Control and Supervision of Experimental Animal (CPCSEA).

2.5.2 Experimental methods

Three groups of animals containing five each were used for each of the excision and incision wound models. The animals of group I was considered as the reference standard and was treated with 0.2% (w/w) Megaheal gel (Aristo Pharmaceuticals Pvt Ltd), the animals of group II served as the control. The animals of group III were treated 5% gel of fraction of *Adina cordifolia.*

2.5.2.1 Excision wound model

The mice were inflicted with excision wound as described by Morton and Malone under light ether anesthesia [30]. The skin of the impressed area was excised to full thickness on the dorsal thoracic region of the rats to obtain a wound area of about 500mm^2. The drugs were topically applied daily till the complete epithelialization starting from the day of operation. The parameters studied were wound closure and time of epithelialization. The wounds were traced on mm^2 graph paper on the days of 3rd, 5th, 7th, 9th and 12th. The wound closure was measured at regular intervals of time to see the percentage of wound closure and epithelialization time that indicates the formation of new epithelial tissue to cover the wound.

2.5.2.2 Incision wound model

In incision wound model [31], 6 cm long paravertebral incisions were made through full thickness of the skin on either side of the vertebral column of the rat. Care was taken to see that incision was at least 1 cm lateral to vertebral column. The wounds were closed with interrupted sutures of 1 cm apart using a surgical thread (No. 000) and curved needle (No. 11). The wounds were left undressed and drugs were topically applied to the wound once a day, till complete healing. The skin breaking strength of the 10-day-old wound was measured by continuous constant water technique of Lee and Tong [32]. The skin breaking strength is expressed as the minimum weight (in grams) of water necessary to bring about the gapping of the wound.

2.6 Histopathological Studies

At day 12 the experiment was terminated and the wound area was removed from the animals for histological examination. Five micrometer thick sections were stained with haematoxylin and eosin for histopathological analysis. The tissue samples were evaluated for the histopathological changes such as fibroblast proliferation, collagen formation and angiogenesis. The results were compared with the control groups.

2.7 Statistical Analysis

The data was analyzed statistically using One-way Analysis of Variance (ANOVA). The data

were expressed as Mean±SE. *P*-values less than 0.05 imply significance [33].

3. RESULTS AND DISCUSSION

3.1 Qualitative Analysis

The methanolic extract showed the presence of various phytochemicals which can be attributed to have antioxidant, antibacterial and wound healing properties. The test showed the presence of nearly all the polyphenols that were tested. Alkaloids, flavonoids, tannins, saponins, phlobatannins, triterpenoids, glycosides, were present in the plant which is clearly shown in Table 1.

Table 1. Qualitative analysis of phytochemicals in the methanolic extract

Phytochemicals	Methanolic extract
Alkaloids	+
Flavonoids	+
Carbohydrate	+
Phenolis compounds	+
Triterpenoids	+
Saponins	+
Tannins	+
Phlobatannins	+
Resin	-
Steroides	-
Glycosides	+
Quinones	-

+: Presence; -: Absence

3.2 Quantitative Analysis

Total phenolic and flavonoid contents: The total phenolic content was recorded in Table 2 in *Adina cordifolia* leaves fractions (determined as gallic acid equivalents or GAE), ranged between 58.71±3.12 mg/g and 29.82±2.51 mg/g dry weight of fraction. The butanol extract showed the highest total phenolics (58.71±3.12mg GAE/g fraction), whereas the phenolic contents of Ethyl acetate fraction was much smaller (29.82±2.51 mg GAE/g), which is in agreement with other similar reports [34,35]. The antioxidant property of the compounds was well correlated with the content of their phenolic compounds [36]. Phenols contain good antioxidant, antimutagenic, and anticancer properties [37]. Flavonoids are the naturally occurring polyphenolic compounds representing one of the most prevalent classes of compounds in vegetables, nuts, fruits, and beverages such as coffee, tea, and red wine [38]. The present study showed flavonoids contents in the range of 41.13±2.11 mg to 27.11±2.29 mg as rutin equivalents/g fraction. The highest amount was observed in the aqueous fraction (41.13±2.11 mg/g) followed by the methanol extract (39.94±3.02 mg/g). Halliwell reported that plants rich in flavonoids are potential sources of natural antioxidants that would add to the overall antioxidant capacity of an organism and inhibit lipid peroxidation [39]. The antioxidant property of the compounds was well correlated with the content of their phenolic compounds [36]. Phenols contain good antioxidant, antimutagenic, and anticancer properties [37]. Therefore, the result suggested that phenolic acids and flavonoids may be the major contributors for the antioxidative properties and inhibitory actions toward the oxidative reaction *in vitro* and *in vivo*.

3.3 *In-vitro* Antioxidant Activity

The *in vitro* antioxidant activity was tested against all the three fractions by two *in vitro* methods. The results showed that the free radicals were scavenged with increased concentration of the extracts up to a given concentration. The scavenging effect of plant fractions on DPPH radical was in the following order n-butanol fraction > ethyl acetate fraction > aqueous. For DPPH radical scavenging the highest IC50 value was shown by Ethyl acetate fraction (74.03 g/ml), Aqueous fraction (38.99 g/ml), lowest being n-butanol fraction (32.96 g/ml) with the standard value as (14.67 g/ml). The H_2O_2 scavenging activity showed that n-butanol fraction showed more scavenging activity than the other fractions. The highest IC_{50} value for H_2O_2 scavenging activity was showed by ethyl acetate fraction (33.27 g/ml), Aqueous fraction (31.86 g/ml), lowest being n-butanol fraction (23.35 g/ml) with the standard value as (7.512 g/ml). The capability of these fractions to eliminate hydroxyl radicals appears to directly relate to the inhibition of lipid peroxidation, and acts as scavengers of active oxygen species by breaking free radical chains. The reducing power of the plant extracts increases with increasing concentration which shows that that the antioxidant compounds are electron donors and can reduce the oxidized intermediates of the lipid per oxidation process. All the fractions exhibited potent antioxidant activity in our study. The presence of the polyphenols in the plant is likely to be responsible for the free radical scavenging effects observed. These plant phenolics are a

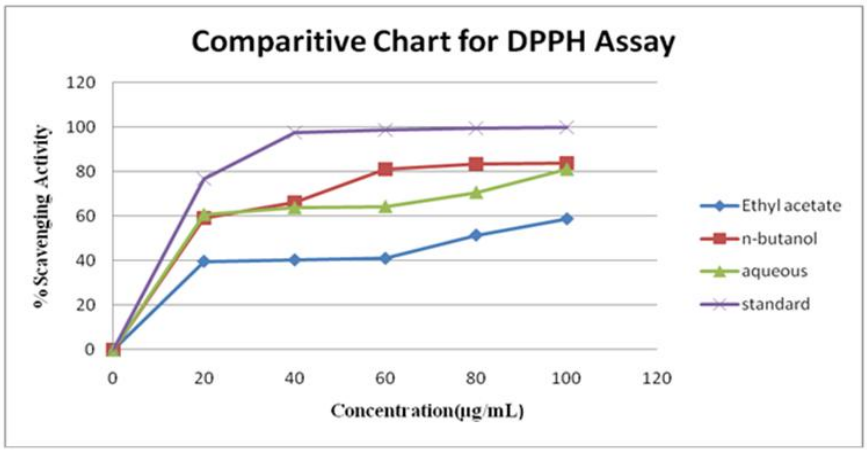
major group of compounds that act as primary antioxidants or free radical scavengers. The degree of reduction in absorbance measurement is indicative of the radical scavenging power of the fraction [40].

The presence of tannins and flavonoids present in the plant is very significant as these secondary metabolites have been found to act as free radical scavenger [41]. They exert their antioxidant property by increasing the activity of catalase and glutathione peroxidase, which detoxify hydrogen peroxide by converting it to oxygen and water [42]. The relatively moderate antioxidant property of the fraction may contribute the wound healing property by mopping up excessive production of free radicals such as superoxide during wound healing process and the activity may be due to the presence of polyphenols including tannins and flavonoids present in the extract [41].

Table 2. Total phenolic and flavonoid content of *Adina cordifolia* fractions

Plant fraction	Total phenolics (mg GAE/g)	Total flavonoids (mg rutin equivalent/g)
Methanol extract	45.32±2.67	39.94±3.02
Ethyl acetate fraction	29.82±2.51	27.11±2.29
n-Butanol fraction	58.71±3.12	37.59±1.92
Aqueous fraction	39.24±2.19	41.13±2.11

Each value in the table is represented as mean ± SE (n=3)



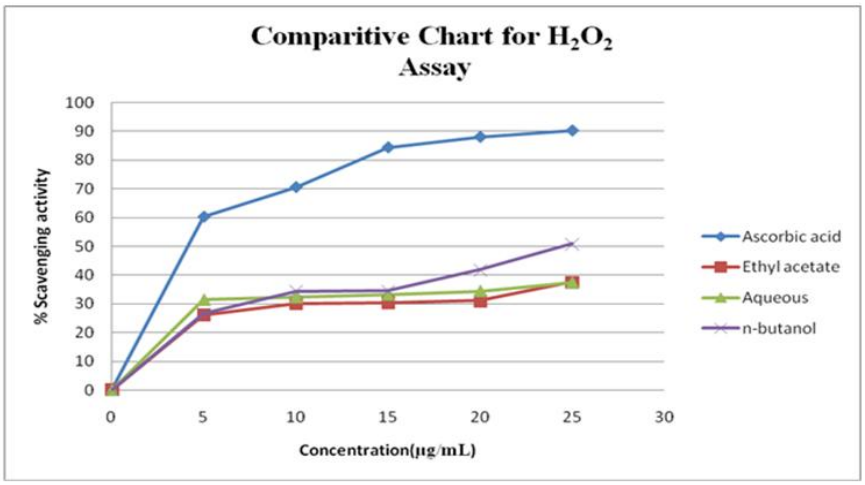


Fig. 1. IC_{50} values of fractions and standard (Ascorbic acid)

3.4 Antimicrobial Activity

Wound healing may be hampered by the microbial activity that is present on the wounds. However, to date, widespread opinion among wound care practitioners is that aerobic or facultative pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Betahemolytic streptococci* are the primary causes of delayed healing and infection in both acute and chronic wounds. Such opinion has been formed on the basis of referenced comments and studies performed largely during the last two decades that have investigated the role of microorganisms in wound healing [43]. So the antimicrobial activity was tested against four organisms namely *Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis* and *Staphylococcus aureus,* but only in case of *Staphylococcus aureus,* the fractions showed positive result in terms of microbial inhibition presented in Table 3. Out of three fractions only the n-butanol fraction and Ethyl acetate fraction had showed effective antimicrobial activity against *Staphylococcus aureus* and n-butanol fraction showed better antimicrobial activity at different concentrations than the Ethyl acetate fraction.

Table 3. Zone of inhibition (in mm) of the three fractions against different microorganisms

Fraction	Concentration (mcg/ml)	Zone on inhibition (in mm)			
		S. aureus	***P. aeruginosa***	***E. coli***	***B. subtilis***
n-butanol fraction	0.25	---	---	---	---
	0.5	---	---	---	---
	1	---	---	---	---
	2	7.3±0.11	---	---	---
	4	8.5±0.14	---	---	---
Ethyl acetate fraction	0.25	---	---	---	---
	0.5	---	---	---	---
	1	---	---	---	---
	2	6.1±0.09	---	---	---
	4	6.6±0.11	---	---	---
Aqueous fraction	0.25	---	---	---	---
	0.5	---	---	---	---
	1	---	---	---	---
	2	---	---	---	---
	4	---	---	---	---

Values are mean, ± SE of three determinations

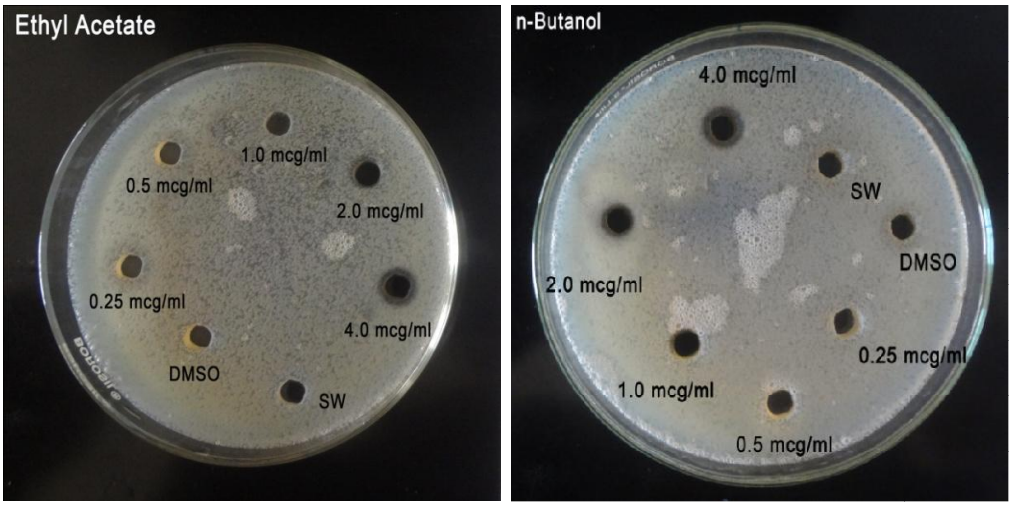


Fig. 2. Zone of inhibition (in mm) of n-butanol fraction and ethyl acetate fraction

A number of organisms have been found to infect wounds and some of them including *P. aeruginosa, S. aureus and E. coli* may lead to chronic or unhealed wounds [44]. Thus the plant extract may contribute to wound healing by inhibiting these organisms that may be present in wounds. Flavonoids have been found to exhibit antimicrobial activity and therefore may significantly help to prevent or reduce wound infections [45].

3.5 Wound Healing Activity

After checking for *in vitro* antioxidant activity and antibacterial activity of the fractions, the wound healing activity of the selected fraction carried out. As per the results of the antioxidant and antimicrobial study it was concluded that the n-butanol fraction showed most potent activity in both among all. So assessment of wound healing was carried out with n-butanol fraction.

3.5.1 Excision wound model

Analysis of variance presented in Table 4 showed highly significant effect of drugs on percent wound contraction at all the periods under study after wounding. Further critical difference test (Table 5) indicated significantly higher percent of wound contraction in standard (21.54±0.67%) than test (16.77±0.41%) and followed by control (5.69±0.32%) after 3rd day of wounding. The differences among all the three drugs differ significantly. However, after 3rd day of wounding i.e. at 5th, 7th, 9th and 12th day, the difference between standard and test was non-significant statistically but both the drugs were significantly superior to control at all the periods mentioned above (Table 5). At 12th day after wounding, significantly higher percent of wound contraction was noticed in standard (98.16 ± 0.52%) and test (96.03 ± 0.78%) than control (66.16 ± 1.56%).

Further effect of various drugs shown through Fig. 3 clearly indicated higher percent of wound contraction in standard and test in comparison to control.

3.5.2 Histopathological studies

The histopathological evaluation revealed that the lesser epithelialization and lesser collagen formation in control group delayed the healing process in control group. Tissue regeneration was much greater in standard and test group. Treatment of wounds with 5% gel fraction of *Adina cordifolia* was associated with enhanced formation of epidermis, deposition of connective

Table 4. Analysis of variance showing the effect of various drugs on the percent wound contraction at different periods

Sources of variation	d.f.	3rd day		5th day		7th day	
		M.S.	F	M.S.	F	M.S.	F
Between drug	2	330.69	275.05**	839.59	160.03**	785.34	253.51**
Within drug	12	1.20		5.25		3.09	

Sources of variation	d.f.	9th day		12th day	
		M.S.	F	M.S.	F
Between drug	2	1925.14	251.07**	1605.28	291.65**
Within drug	12	7.67		5.51	

** - *P < 0.01; M.S. = Mean square, F= Fisher value, d.f. = Degree of freedom*

Table 5. Average wound contraction activity (%) of different drugs

Sl. no.	Post wounding days	Drugs			
		Standard (Megaheal gel contain silver colloid)	Control (Simple gel base without any drug)	Test (n-Butanol fraction gel)	C.D. value
1.	3rd	21.54 ± 0.67[a]	5.69 ± 0.32[b]	16.77 ± 0.41[c]	1.51
2	5th	56.44 ± 0.64[a]	32.77 ± 1.45[b]	53.74 ± 0.79[a]	3.16
3	7th	71.07 ± 0.92[a]	47.97 ± 0.79[b]	68.68 ± 0.61[a]	2.42
4.	9th	90.09 ± 0.79[a]	54.85 ± 1.76[b]	86.69 ± 0.92[a]	3.82
5	12th	98.16 ± 0.52[a]	66.16 ± 1.56[b]	96.03 ± 0.78[a]	3.23

Means under same superscript in a row did not differ significantly
C.D. value= Critical difference value

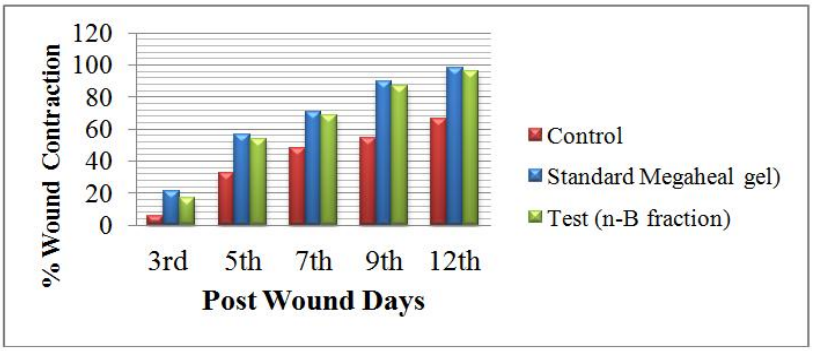


Fig. 3. Percent wound contraction of various drugs at different periods

tissue and faster re-modeling when compared to that of control and vehicle groups. The histology of granulation tissue showed almost complete healing with more fibroblast, increase of collagen tissue and increased number of blood vessels.

3.5.3 Incision wound model

Analysis of variance (Table 7) showed highly significant effect of drugs on tensile strength of healed tissues. Further, critical review of data (Table 8) indicated significantly higher tensile strength in standard (5.022 ± 0.052 g/mm^2) and test (4.858 ± 0.073 g/mm^2) than control (2.960 ± 0.031 g/mm^2), the difference between former two drugs was non-significant statistically.

On the basis of present findings, it was concluded that the test drug prepared in the present plan of work was comparable to standard drug and both are far superior to control one in both the models. Many studies indicate that plant products are potential agents for wound healing and largely preferred because of absence of unwanted side effects and their effectiveness [46]. The wound healing activity exhibited by most plants is due to the synergistic or additive actions of their constituents. In present study, we show probably for the first time that the topical application of n-butanolic fraction of methanolic extract of *Adina cordifolia* promoted wound healing activity in excision and incision wound models. Rout S.K. and Kar D.M. also reported that the ethylacetate and chloroform fractions of methanolic extract of Ipomea carnea posses wound healing activity in excision and incision wound model [47]. Sheeba et al. also reported topical application of methanol extract of *C. occidentalis* in promoting wound healing activity in excision and incision models in rats. Flavonoids present in the plant may increase the viability of collagen fibrils by causing an increase in the strength of collagen fibers [48]. This reduces cell damage by promoting DNA synthesis [49]. Flavonoids have been found to exhibit antimicrobial activity and therefore may significantly help to prevent or reduce wound infections [45]. Presence of tannins in the plant may cause an increase in wound contraction and increase the rate of epithelialization due to their astringent and antimicrobial property [49]. Alkaloids present in the plant may also increase cell proliferation and thus increase the rate of wound healing [50]. A number of organisms have been found to infect wounds and some of them including *P. aeruginosa, S. aureus* and *E. coli* may lead to chronic or unhealed wounds [44]. Thus the plant extract may contribute to wound healing by inhibiting these organisms that may be present in wounds.

Table 6. Tensile strength of the healed tissues treated with different drugs

Sl. no.	Test animals	Tensile strength (g/mm^2)		
		Standard	Control	Test
1	TA-1	5.091	3.057	4.968
2	TA-2	4.970	2.990	4.590
3	TA-3	5.090	2.870	4.840
4	TA-4	4.840	2.960	4.910
5	TA-5	5.120	2.930	4.990

Table 7. Analysis of variance showing the effect of various drugs on the tensile strength of healed tissues

Sources of variation	S.S	d.f.	M.S.	F	C.D. value
Between drug	13.127	2	6.564	441.31**	0.169
Within drug	0.178	12	0.015		

** *- P < 0.01; M.S.= Mean square, F= Fisher value, d.f.= Degree of freedom; C.D. value= Critical difference value*

Table 8. Average tensile strength (g/mm^2) of the healed tissues treated with different drugs

Sl. no.	No. of animals	Treatment	Tensile strength (g/mm^2)
1	5	Standard (Megaheal gel)	5.022 ± 0.052[a]
2	5	Control (Gel base without drug)	2.960 ± 0.031[b]
3	5	Test (n-B fraction gel)	4.858 ± 0.073[a]

Means under same superscript did not differ significantly

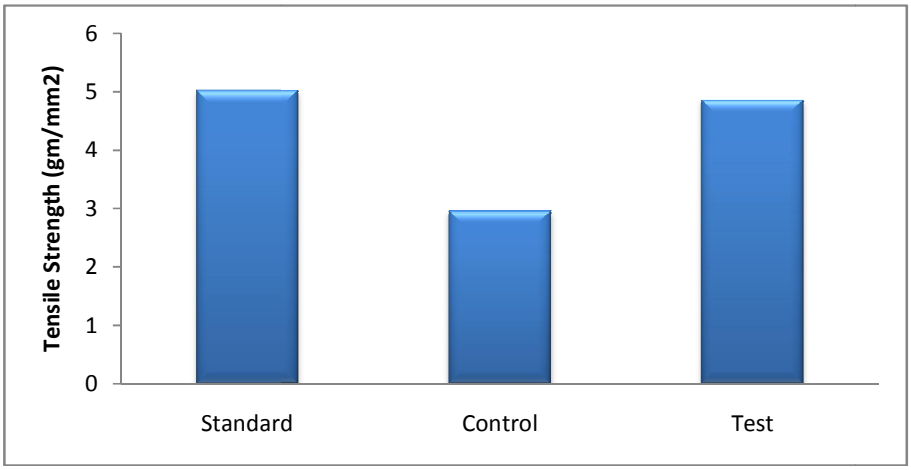


Fig. 4. Average tensile strength (g/mm^2) of the healed tissues treated with different drugs

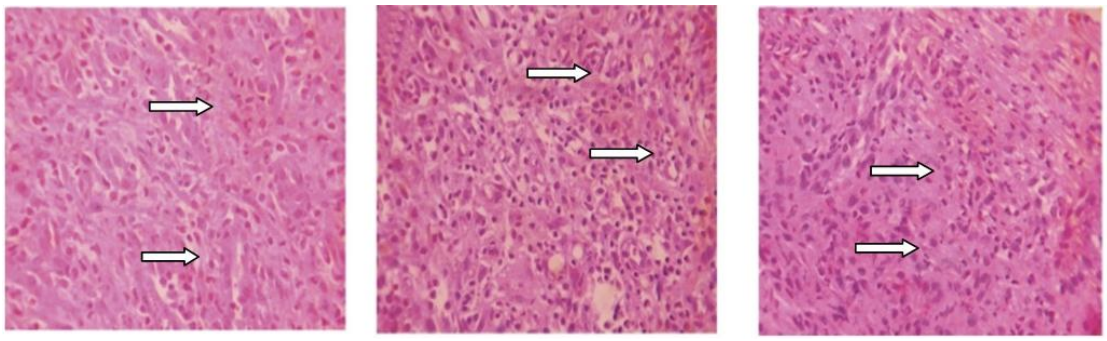


Fig. 5. Histipathological evaluation of wound tissues in excision wound model of *Adina cordifolia* (n-butanol fraction) (a). Histopathological section of wounded tissue of control mice showing incomplete healing with less epitheliazation indicating macrophages and lesser collagen formation (b). Histological section of wounded tissue of standard mice showing complete healing with raised epitheliazation indicating macrophages and leading collagen formation showing complete healing of wound (c). Histological section of wounded tissue of treated mice with 5% n-butanol fraction gel showing increased collagenation with less macrophages

4. CONCLUSION

Our study showed that n-butanol fraction gel found to be effective in the recovery of the wound healing by dose dependent manner. The drug has equally shown better and similar wound healing property as compared with Megaheal gel (Silver Colloid). The result may be attributed to the phytoconstituents present in it which may be either due to their individual or cumulative effect that enhanced wound healing. Further study on the fractionation of active components and the mutual effect of these plant extract machinery on infecting microbial species may provide a better understanding of the infection management in the process of wound healing.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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