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To Fabricate and Evaluate the Wound Healing Activity of Herbal Medicated Plasters Containing the Extracts Obtained from *Tridax Procumbens* and *Azadirachta Indica*

Nikhil M. Satpute

Nagpur College of Pharmacy, Wanadongri, Hingna road, Nagpur-441110.

Ravi Kalsait

Datta Meghe Medical College, Shalinitai Meghe Hospital and Research Centre, Nagpur | Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences Sawangi (Meghe) Wardha

Abhinav Bais

Datta Meghe Medical College, Shalinitai Meghe Hospital and Research Centre, Nagpur | Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences Sawangi (Meghe) Wardha

Narendra Dighade

Datta Meghe Medical College, Shalinitai Meghe Hospital and Research Centre, Nagpur | Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences Sawangi (Meghe) Wardha

Komal Meshram

Datta Meghe Medical College, Shalinitai Meghe Hospital and Research Centre, Nagpur | Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences Sawangi (Meghe) Wardha

Dhruba Hari Chandi

Datta Meghe Medical College, Shalinitai Meghe Hospital and Research Centre, Nagpur | Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences Sawangi (Meghe) Wardha

Abstract---The present research work is to fabricate medicated plaster containing herbal extracts for wound healing properties. Till date no herbal medicated plaster have been prepared and evaluated using the extracts obtained from the plants *Neem* and *Coatbutton*. The objective of the present study is to provide the herbal medicated plasters to the

society. The major advantage of this medicated herbal plaster is its safety, efficacy, portability and efficiency. There is much more study such as Collection of plant, Extraction of plant materials and their antimicrobial test, Evaluation of qualitative and quantitative test, Estimation of total phenolic content, Antioxidant study, Antimicrobial study, Estimation of patch test, Antimicrobial studies of patch test. The plant extracts were quantitatively analysed for total flavonoids content, total phenolic content, and antioxidant activity. Methanolic extract of *Azadirachta indica* and *Tridax procumbens* has shown presence of high content of flavonoids and total phenolic content. Also the Azadirachtin content in the neem extracts was determined. All the six extracts AIME, AIPE, AIAE, TPME, TPPE and TPAE were subjected to antimicrobial activity. In patch test FII batch shows the excellent antimicrobial activity. FII patch was selected for fabrication of plasters which was then subjected to the pharmacological study.

Keywords---antimicrobial test, antioxidant study, coat button, neem, patch test, qualitative, quantitative test.

Introduction

Wound: A wound is a form of injury in which the skin is torn, cut, or cut in a very short time. A wound is an injury that causes the skin to swell or tear. A lesion is defined as a disruption of cellular development and anatomic formation, with or without microbial contamination, which occurs as a result of accidental or cutting with sharp sharp objects. It may be due to physical, chemical, thermal, microbiological, or physiological factors. Tissues are exploited.

Healing: Healing is a normal process, in which the body heals itself from tissue injury, but the rate of healing is sluggish and the risk of microbial infection is high.

Wound Healing: Wound healing is a cellular and biochemical mechanism that restores compromised tissue's natural structure and functions. Wound curing synthetic/semi-synthetic products such as ointment, tincture, cream, powder, and others are available on the market.

Plant profile

Coatbuttons

Synonym – Kambermodi, Dagadi pala

Biological source- It is the species of flowering plant of the daisy family *Tridax procumbens* Linn.

Family – Asteraceae

Geographical source– It is a common weed found throughout India, America, Tropical Africa, Asia and Australia.

Description - It is a 12-24cm tall vegetable with a few 6-8cm leaves growing on roadsides, walls and plains all over the world. They are available during the year.

Simple, opposite, artificial, lanceolate or ovate leaves Hair in both areas, 3-7 cm long with mustache leaves, base wedge shaped, short petals Leaves like arrow, hairy and short. The upper one-layer epidermis of polygonal tabular cells is approximately 40-70 m by 15 to 30 m and a single layer of cylindrical cell palisade approximately 18 to 30 m in diameter and -60 to 70 m wide, spongy parenchyma 2-4 layered, spolyhedral or cellametric cell standing, spongy parenchyma 2-4 layered, spolyhedral cell or isodiametric standing.

Chemical constituent

Phytochemical studies have revealed the presence of secondary metabolites of alkaloids, carotenoids, dexamethasone, luteolin, flavonoids, tannins and leaf extract proteins, luteolin, glucoluteolin, quercetin and quercetin -soquercetin extracted from flowers and fumaric acid, setosterol and tannin in one part of the plant extract. Tridax dissociation is characterized by methyl 14 oxoacagaecunoate, 3-methyl-non adecylbenzene, heptacosanyl cyclohexane carboxylate, 1- (2,2, dimethyl-3-hydroxypropyl) isobutyl phthalate, 12-hydroxytetracos-15-one, 32- meth 30- ozotetraatriacont-31-en-1-ol and β -amyrin, β -amyrone, fucosterol and β -sitosterol, arachidic, behenic, lauric, lineoic , linolenic, myristic, palmitic and stearic acid.

Uses

- Antimicrobial properties of *T. procumbens* are commonly used.
- It's used to stop bleeding from scratches, fractures, and burns.
- The antiseptic, insecticidal, and parasitocidal properties of the leaf gel are all present.
- The leaves have been used to treat bronchial catarrh, dysentery, and diarrhea, as well as to restore hair.
- The herb extract causes reflex tachycardia which has a temporary hypotensive effect on normal blood pressure.

Neem

Synonym – Nimb

Biological source – Neem consists of all aerial parts of plant known as *Azadirachta indica*.

Family - Meliaceae

Geographical source – It is found in India, japan, Pakistan, Bangladesh, shri lanka, thialand, South and East Africa Maharashtra.

Chemical constituent– Active compounds that can be extracted from neem, including *Azadirachtin*. Secondary metabolites are alkaloids, flavonoids, triterpenoids, phenolic compounds, Carotenoids, steroids and ketones. Other compounds are salannin, volatile oil, Nimbidin, Nimbin, Nimbinine, sterols, Nimbolide.

Description – Alternate, exstipulate, imparipinnate leaflet 20-25 cm in length, lanceolate closely clustered toward the end of branches. The leaves have serrate margine, green colour and bitter in taste.

Uses

- Antibacterial, antifungal, antiviral.

Materials and Methods

Collection of crude drugs

The Leaves of the tree *Azadirachta indica* were collected from the college campus and *Tridax procumbens* were collected from the Bhivkund Lake, Mohgao hingna. The Neem and kambermodi plants was authenticated by senior botanist Dr. Dongarwar, Botany department, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, with specimen voucher No.10189 and 10190 resp. The authentication certificate was given by the botany department, shown in figure. The authenticated plants were used for the research work. The leaves were shade dried and coarse powder was subjected to the extraction process.

Extraction of plant materials

The dried powdered leaves (200g) were subjected to the successive soxhlet extraction method. The solvents used for extraction were petroleum ether (40-60), acetone and methanol (Fisher scientific). The extracts were evaporated to dryness and the resulting extracts were stored in air tight container at 4°C. The obtained extracts were referred as AI-PE, AI-AE, AI- ME, TP-PE, TP-AE, and TP-ME. The color, nature and percentage yield of all these six extracts were calculated. It is observed that percentage yields of all the extracts were found to be high in Methanol as compared to the other solvents.

Table 1
Color, Nature and Percentage yield of the extracts

Sr. No	Extract	Color	Nature	% Yield
1	AI – PE	Yellowish brown	Semi-solid	3%
2	AI – AE	Dark green	Dry powder	6%
3	AI – ME	Yellowish green	Dry powder	10%
4	TP – PE	Yellowish brown	Semi-solid	4%
5	TP – AE	Dark green	Dry powder	7%
6	TP – ME	Dark green	Dry powder	9%

Evaluation of isapgol husk powder (polymer)

Morphological characters: The polymers were studied for its colour, odour and texture by visual inspection. The organoleptic characters of isapgol husk were analysed.

Table 2
Organoleptic properties of isapgol husk powder

Sr. No.	Properties	Result
1	Colour	Creamish white

2	Odour	Odorless
3	Taste	Tasteless
4	Shape	Amorphous
5	Texture	Smooth
6	Touch	Smooth

Solubility: The solubility of polymer was carried out in water, methanol, ether, and acetone. About 0.1 gm of polymer was dissolved in solvent with vigorous shaking at room temperature.

Table 3
Solubility of polymer

Sr. No.	Solvent	Solubility
1	Ether	Insoluble
2	Acetone	Insoluble
3	Choloform	Insoluble
4	Methanol	Insoluble
5	Ethanol	Insoluble
6	Cold water	Soluble
7	Hot water	Soluble

Microbial contamination determination

The microbial contamination was checked by preparing agar media containing beef extract peptone, NaCl, distilled water and agar and was sterilized using autoclave. All the petri dishes and test tube were sterilized with acetone or in hot air oven. The agar media was poured in the petri dishes aseptically and carefully. After cooling the agar solution in the petri plate the well were made. Required dilution of specimen were prepared and transferred into the well with the sterile pipette. Inoculated plates were incubated in the incubator at 370 for 24hr.

Table 4
Physicochemical parameter of Polymer

Sr. No	Physicochemical parameter of Polymer	Result
1	Swelling index	12.54
2	pH	6.99 – 7.00
3	Microbial contamination	No growth was observed

Micromeritic evaluation

Table 5
Micromeritic evaluation of Isapgol polymer

Sr. No	Characterization Parameter	Result
1	Bulk density	0.56 gm/ cm ³
2	Tapped density	0.80 gm/ cm ³
3	Carr's consolidation index	22.14
4	Hausner ratio	1.24

Qualitative evaluation (preliminary phytochemical studies)

Many crude medicines contain a natural combination of pharmaceutical agents that have beneficial effects. One factor may be responsible for the intended therapeutic outcome, while the others may be responsible for increasing activity or reducing side effects. These medicinal effects are so desirable and well-known that it's pointless to try to produce a comparable combination artificially. Thus, it is important from a pharmaceutical standpoint to have a better understanding not only of the therapeutically active portion, but also of the inert material extract that has been subjected to preliminary phytochemical screening for the identification of chemical constituents present in them.

Table 6
Preliminary phytochemical screening of *Tridax procumbens* and *Azadirachta Indica*

Sr. No	Test	Test reagent	AI – PE	AI – AE	AI– ME	TP - PE	TP - AE	TP- ME
1	Carbohydrate	Molisch test	-	-	+	-	-	+
2	Protein	Biuret test	-	-	+	-	-	+
3	Alkaloids	Drangendorff test	-	-	+	-	+	+
		Mayer's test	-	-	+	-	-	+
		Hager's test	-	-	+	-	-	+
4	Steroids	Salkowski test	+	-	+	+	-	+
		Liebermann Burchard test	+	-	+	-	-	+
5	Saponin	Foam test	+	-	-	+	-	+
6	Flavonoids	Shinoda test	-	-	+	-	-	+
7	Tannins	5% Ferric chloride Dilute iodine solution 10% Lead acetate solution	-	-	+	-	-	+
			-	-	+	-	-	+
8	Glycoside	Brontrager's test	-	+	+	-	-	+

Quantitative phytochemical analysis

Estimation of total flavonoid content

Total flavonoids content of the methanolic extracts were determined by the following method:

Preparation of calibration curve: 1.5 ml of 95 percent alcohol, 0.1 ml of 10% aluminum trichloride in methanol g l, and 1 ml of sodium acetate were added to 0.5 ml of concentrate, and the amount was changed to ml of purified water. After leaving the mixture at C for a few minutes, the absorption was measured at 254 or 366 nm. Absorbance vs. concentration was plotted on the scale. Many of the tests were done in triplicate. The sum of flavonoids in plant extract was measured in rutin equivalents (RE).

Table 7
Absorbance of Rutin

Sr. No	Concentration of Rutin ($\mu\text{g/ml}$)	Absorbance at 415 nm
1	10	0.408
2	20	0.824
3	40	1.28
4	60	1.70
5	80	2.20
6	100	2.62

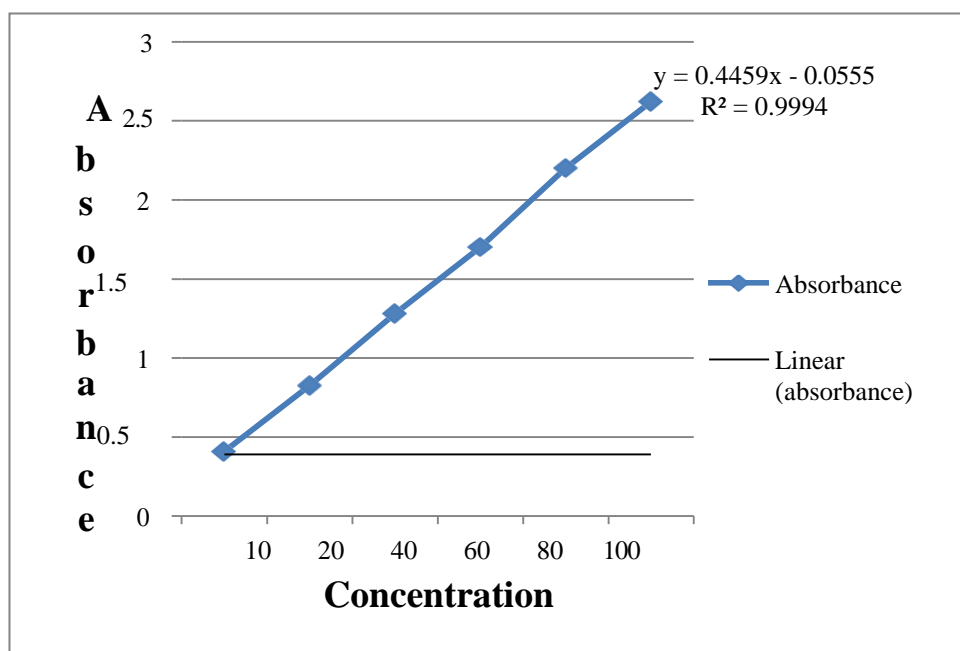


Figure 1. Calibration curve for Rutin

Table 8
Total flavonoids content of *Tridax procumbens* and *Azadirachta indica* plant extracts

Sr. No	Absorbance of TP – ME	Mean	RE ($\mu\text{g/mg}$)
1	0.288		
2	0.298	0.302	0.80($\mu\text{g/mg}$)
3	0.320		
	Absorbance of AI-ME		
1	0.224		AZE ($\mu\text{g/mg}$)
2	0.218	0.21	0.59($\mu\text{g/mg}$)
3	0.208		

Estimation of total phenolic content

The extract (10mg/10ml) was dissolved in water, from that it was diluted to get 100ug/ml concentration. 0.1 mL of the latter solution was moved to a 10 mL volumetric flask, along with 1.0 mL of Folin-Ciocalteu reagent, and thoroughly combined. After 3 minutes, 3.0 mL of 20% sodium carbonate was added, and the amount was made up with purified water. The mixture was then left to rest for 2 hours with occasional shaking. Using an equation derived from the regular gallic acid table, the concentration of total Phenolic compounds in the extract was calculated as mg of gallic acid equivalent.

Standard curve (Gallic acid)

Gallic acid (10mg) was dissolved in 10ml purified water and pipetted out to obtain concentrations of 50, 100, 150, 250, and 500ug/ml. The contents of the flask were thoroughly mixed after adding 1.0ml of Folin-Ciocalteu reagent. After 3 minutes, 3.0 mL of 20% sodium carbonate was applied, and the amount was made with purified water, the mixture was allowed to sit for 30 minutes to 2 hours with occasional shaking. At 760nm, the absorbance of the blue color that formed was measured.

Table 9
Absorbance of Gallic acid

Sr. No	Concentration of Gallic acid (µg/ml)	Absorbance at 760nm
1	10	0.128
2	20	0.240
3	40	0.380
4	60	0.520
5	80	0.670
6	100	0.815

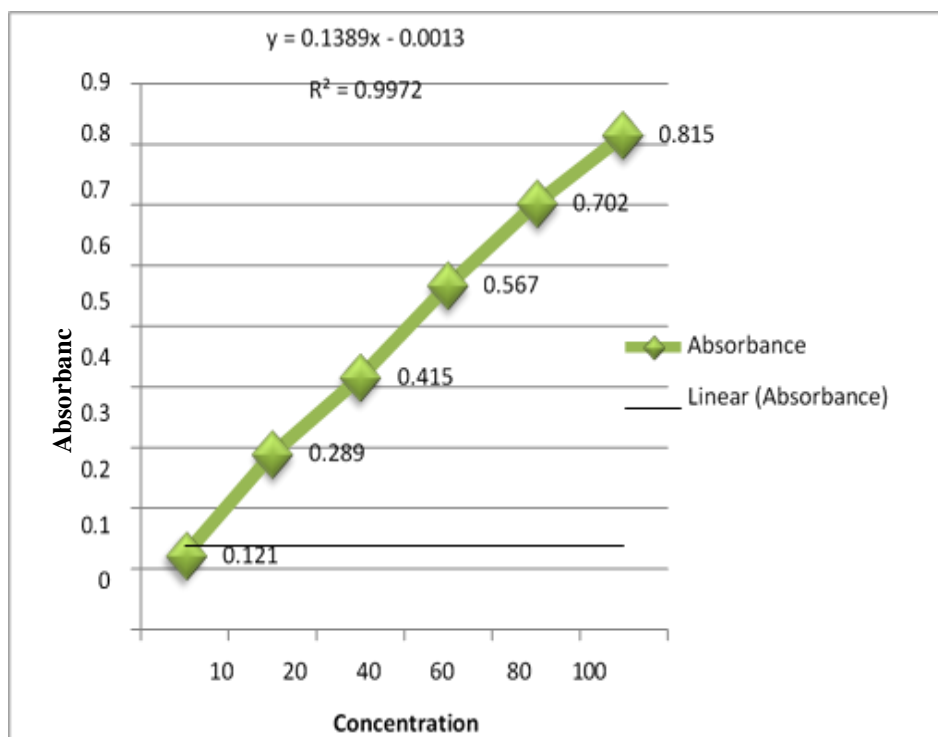


Figure 2. Calibration curve for Gallic acid

Table 10

Total phenolic content in extracts of *Tridax procumbens* and *Azadirachta indica*

Sr. No	Absorbance of TP – ME	Mean	GAE ($\mu\text{g}/\text{mg}$)
1	0.098	0.148	1.21($\mu\text{g}/\text{mg}$)
2	0.120		
3	0.128		
	Absorbance of AI-ME		GAE ($\mu\text{g}/\text{mg}$)
1	0.092	0.116	0.97($\mu\text{g}/\text{mg}$)
2	0.124		
3	0.132		

DPPH radical scavenging activity

Electron in an atom occupies space known as orbital. Each orbital can hold a maximum of two electrons spinning in opposite direction, as in the case with most biological molecule, which can be termed as non-radicals. On the contrary, a free radical contains one or more unpaired of electron in their orbital. Radicals can react with other molecules in several ways. Thus when two radical meet, they can be combined their unpaired electrons (denoted by o) and join to form a covalent bond: $\text{A}\cdot + \cdot\text{A} \rightarrow \text{A-A}$

Table 11
DPPH absorption (%) inhibition of standard ascorbic acid

Sr. No	Concentration($\mu\text{g}/\text{ml}$)	Absorbance at 517nm	% inhibition	IC50
1	50	0.384	43.69%	276 $\mu\text{g}/\text{ml}$
2	100	0.382	43.98%	
3	200	0.354	48.09%	
4	400	0.328	51.90%	
5	600	0.288	57.37%	
6	800	0.274	59.38%	
7	1000	0.254	62.75%	

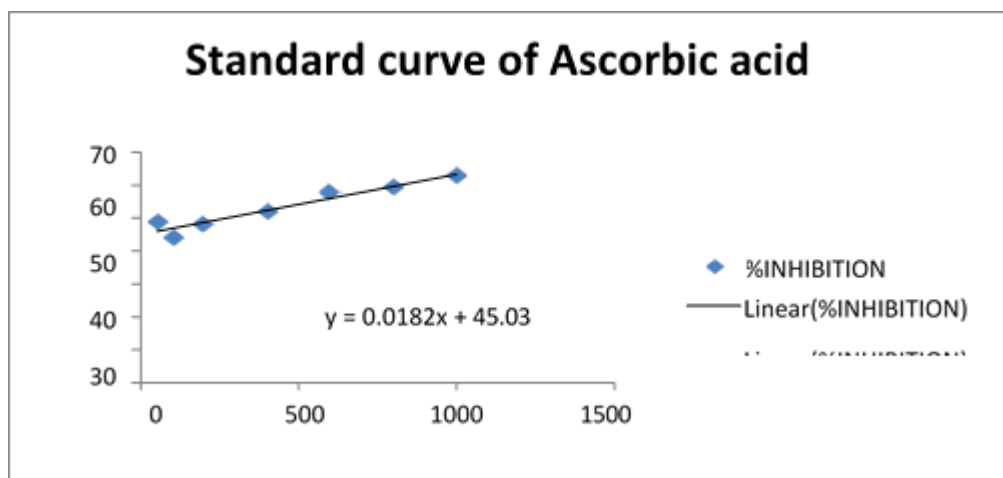


Figure 3. Standard curve of ascorbic acid

Table 12
DPPH absorption % inhibition of methanolic extract of *Azadirachta indica*

Sr. No.	Concentration ($\mu\text{g}/\text{ml}$)	Absorbance at 517nm	% inhibition	IC50
1	50	0.378	44.57	297 $\mu\text{g}/\text{ml}$
2	100	0.372	45.45	
3	200	0.368	46.06	
4	400	0.354	48.09	
5	600	0.248	59.23	
6	800	0.228	69.63	
7	1000	0.216	68.32	

Table 13
DPPH absorption % inhibition of methanolic extract of *Tridax procumbens*

Sr. No.	Concentration ($\mu\text{g}/\text{ml}$)	Absorbance at 517nm	% inhibition	IC50
1	50	0.388	43.10	364 $\mu\text{g}/\text{ml}$
2	100	0.384	43.69	
3	200	0.368	46.04	
4	400	0.354	48.09	
5	600	0.282	58.65	

6	800	0.262	61.58
7	1000	0.242	64.51

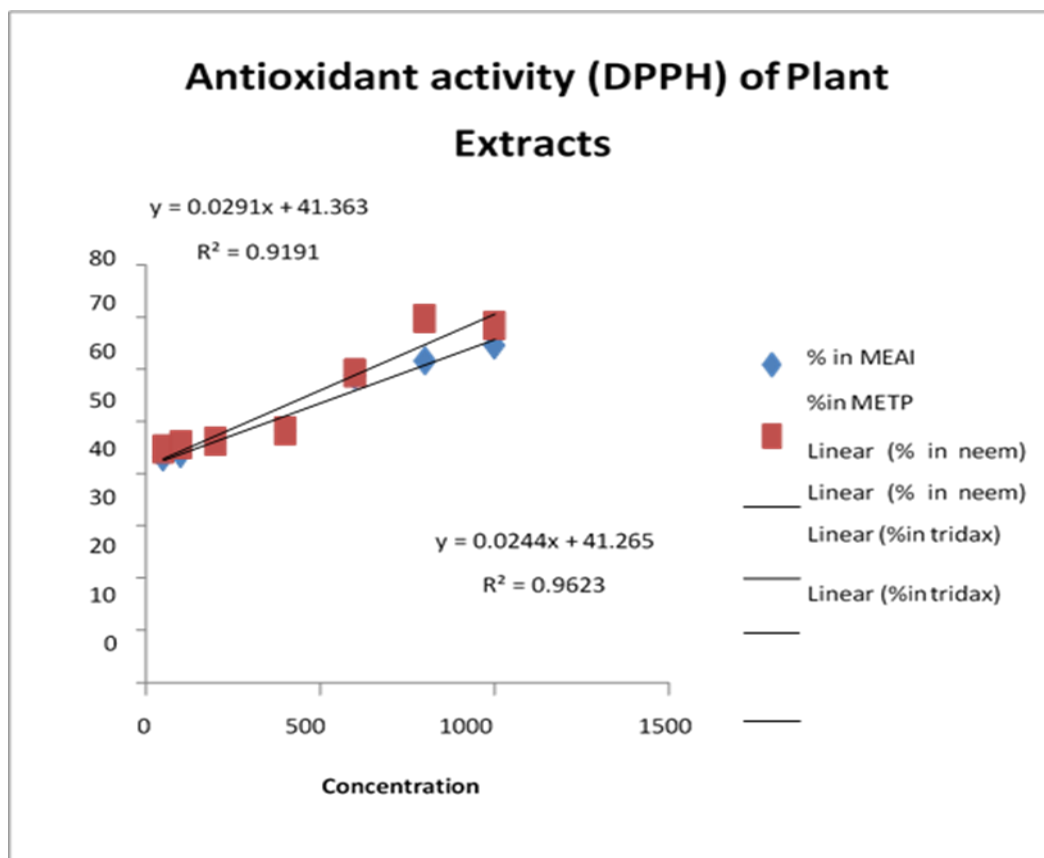


Figure 4. Antioxidant activity (DPPH) of plant extracts

Antimicrobial studies: (Christudas S. and Francine U., 2015, Kale V., 2001)

The lowest concentration of a compound required to prevent the development of microorganisms is known as the minimum inhibitory concentration. Using a medium-sized culture prepared for experimental filtration or microdilution assay, small amounts of inhibitory concentrations are set. Extraction was performed at various locations (200 mg / ml, 150 mg / ml, 100 mg / ml, 50 mg / ml, and 25 mg / ml). The antimicrobial study of *Tridax procumbens* and *Azadirachta indica* leaves by using agar-well diffusion method were compared with standard antibiotic i.e. Neomycin.

Bacterial strains such as *Escherichia coli*, *Staphylococcus aureus* were used for the study. The microbes were maintained in Nutrient agar Broth and continuously sub-cultured.

Preparation of Medium

Nutrient agar medium was prepared by dissolving 2 g of nutrient agar in 100 ml nutrient brot. Heat t e mixture at about c to dissolve the agar completely. Plug the flask with cotton and sterilized the medium by autoclaving at 121°C for 15 min. It was cooled and poured in petridishes to solidify.

Determiration of antibacterial activity (Agar well Diffusion method)

Agar plates are injected with test kits by spreading inoculums viruses on the surface of the media. The springs (8mm wide) were beaten with agar. Petroleum ether, acetone and Methanol extracted with different concentrations were made under antimicrobial study. Plates are placed at 37 ° C for 24 hours. The antibacterial activity was tested by measuring the width of the inhibitory area.

Table 14
Growth inhibitory effect of plant extract against Staphylococcus aureus

Sr. No	Concentration (µg/ml)	AI-PE	AI-AE	AI-ME	TP - PE	TP -AE	TP -ME
1	Standard (Neomycin)	1.93	1.88	1.93	1.93	1.88	1.93
2	0.2	1.93	2.60	2.06	2.58	2.80	1.93
3	0.15	1.90	2.54	2.06	2.52	2.74	1.88
4	0.1	1.86	2.46	1.96	2.48	2.60	1.86
5	0.05	1.87	2.36	1.86	2.38	2.54	1.83
6	0.025	1.88	2.26	1.88	2.18	2.38	1.87

Method of preparing patches

Dots are adjusted in the form of solvent dispersion. (Ref) The polymer was taken with a beaker with a low solvent value i.e. water. It was first heard by movement at low rpm and later at high speed. The plasticizer was added and blended evenly and the drug was combined with the endurance stimulus and the volume was made Films were removed using a sharp blade by inserting the edges of the film. The dried films are wrapped in butter paper and stored in a sealed container away from light and in a cool place.

Table 15
Formula for preparing the patch

Sr. No	Ingredient	Activity	FI (1/2)	FII (1/3)	FIII (1/4)
1	Polymer	As base	0.25gm	0.25g	0.25g
2	Extract AI-ME	Sec. metabolites	0.93g	0.62g	0.46g
3	TP -ME	Sec. metabolites	0.91	0.61g	0.45g
4	AI - PE	Steroidal activity	0.93g	0.62g	0.46g
5	Glycerin	As plasticizer	0.4ml	0.4ml	0.4ml
6	Water	As vehicle	10ml	10ml	10ml

Evaluation of patch Thickness of the patch

The patch size was measured at different points using a digital micrometer and the average size was calculated.

Weight uniformity

The weight uniformity determination, prepared patches were dried at 60°C for 4 hrs before testing, three films of the size 10mm diameter were weight individually using digital balance and average weight was calculated with \pm std deviation.

Percentage moisture content

Prepared films were measured individually and stored on desiccators containing calcium chloride mixed in RT 24 hours. After 24 hours the film was re-weighed and the moisture content was calculated by the given formula. Percentage moisture content = $\frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100$. The result of all the evaluation parameter performed on the FI, FII and FIII patches has been given in the table.

Table 16
Evaluation of patch

Sr. No	Test	Control	FI(1/2)	FII(1/3)	FIII(1/4)
1	Thickness (mm)	0.168	0.184	0.234	0.254
2	Weight uniformity (g)	0.08 \pm 0.01	0.08 \pm 0.02	0.079 \pm 0.04	0.083 \pm 0.23
3	Folding endurance	390 \pm 9.5	325 \pm 18.5	386 \pm 5.03	350 \pm 22.4
4	Percentage moisture Content (%)	1.28	1.14	1.10	1.18

Determination of active phytoconstituents (Rutin and Azadirechtin) in FII patch

The medicated FII patch (1cm \times 1cm) was dissolved in phosphate buffer pH 7.4. Then the solutions were filtered using Whatman filter paper. The collected filtrate was analyzed for the % phytoconstituents by UV spectrophotometer at 254 and 366 nm.

Table 17
The results of % Rutin present in the patch F-II

Sr. No.	Absorbance of Rutin at 254 nm	Rutin present in the (1cm)patch ($\mu\text{g}/\text{ml}$)	Absorbance of Azadirechtinat 366nm	Azadirechtin present in the (1cm) patch ($\mu\text{g}/\text{ml}$)
1	0.311	0.82	0.221	0.62

Antimicrobial studies of patch FII

This FII patch was then subjected for the antimicrobial studies. It was found that the patch FII shows significant result when compared with the standard neomycin.

Fabrication of herbal medicated plaster

The FII patch which was evaluated for its physicochemical properties and antimicrobial activity was finally used for fabrication of herbal medicated plaster by the following method.

Preparation of herbal medicated plaster: The woven fabric was cut into suitable dimension 7.5×2 cm (length \times width). Wound pad of 2×1 cm size was prepared and fixed on adhesive woven fabric. Then prepared herbal patch was covered over wound pad. The backing plastic material having same size was fixed over the herbal wound healing plaster.

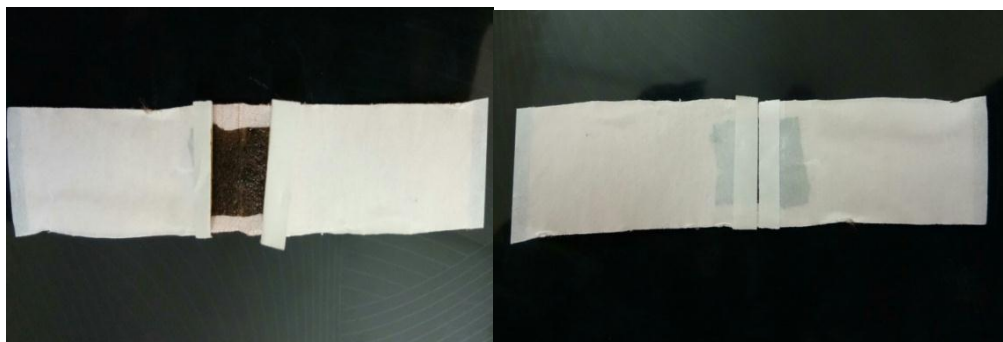


Figure 5. Fabricated patch

Wound healing activity

The above medicated herbal plaster fabricated containing FII patch was now evaluated for its wound healing activity using incision model.

Incision wound healing model

It also represents the model of a surgical wound wound. This model was used to measure strength after wound healing. Two pieces of para vertebral length of 6 cm are made of skin and muscles at a distance of about 1.5 cm from the center of each side of the vertebral column. After the hole is made the separated skin is kept together and sewn in 1 cm intervals of the affected tissue using a surgical cord and a curved needle. Wounds were left untouched and a wooden herbal mask was attached to the top. Sutures were removed on day 8. The strength of the wound cracking was measured on day 10 by continuous flow of water. Mice were killed before and during the creation of the wound. The back hairs of the animals were trimmed. Animals were randomly divided into groups of three animals each.

Table 18
Grouping of animal

Sr. No	Group	Treatment
1	Group I	Vehicle control
2	Group II	Standard treated (Neomycin ointment 5% w/w)
3	Group III	Prepared patch FII



Figure 6. Incision



Figure 7. Stitches



Figure 8. Patch applied to the animal

Parameter evaluated in Incision model was

- Tensile strength
- Scar area

Tensile strength of incision wound model

The strength of a wound is measured by its volume per area. The force of a wound is the force required to break a wound regardless of its size. It varies in skin size. Strong wound strength occurs in about 80% of injuries. A wound that is healing can reach almost 80% of its strength in an inaccessible skin.

Procedure

The rate of wound healing is directly proportional to the gain of muscle strength. Mice were secured to the work table and a line was drawn on both sides of the wound 3 mm away from the wound. Two alligator forceps are firmly attached to the front line. One of the forceps was fixed, and the other was attached to a polypropylene container with free-standing stems with a thread fastened to the

pulley. Water was allowed to flow out of the pond slowly and into the container. Gradual weight gain is transferred to the wound area that pulls the edges of the wound. There and as soon as the wound opened, the flow of water was stopped and the volume of water collected in the container (approximately equal to its weight) was noted. The mean value gives the breaking power to a given group. The strength of the treated wounds (2.5% and 5% w / w ointments) compared with the control and groups treated normally.



Figure 9. Measuring the tensile strength

Table 19
Results of incision wound model

Sr. No	Group	Tensile strength (g)
1	Control	210±6.89
2	Standard	384±11.24
3	Test (patch FII)	328±14.38

Tensile strength which is an important parameter in incision wound model was measured and the results are shown in table no.12 (Fig. 9.6). Wound in the control group had minimum strength of 210 ± 6.89 g. The mean strength of the group treated with prepared patch significantly increased in a dose dependent manner compared to control group. The mean tensile strength of wound treated with standard neomycin is 384 ± 11.24 g) and test patch FII was 328 ± 14.38 g) FII patch showed that they were highly significant ($P < 0.01$) when compared to the control group, which was almost equipotent to that of standard drug (384 ± 11.24 g). In incision wound study, the test drug promoted healing of re sutured incision wound as evidenced by significant increase ($p < 0.01$) in the tensile strength of test group. The increased tensile strength might be due to increased proliferation and transformation of fibroblast cells into my fibroblasts. In incision wound model, there was a significant increase ($p < 0.01$) in the tensile strength of test group compared to the control group and comparable to the standard group. This observation confirms that the FII patch has excellent wound healing properties in terms of the strength of the wound healing tissue. In wound injury, an increase in the strength of the hardened wounds may be due to an increase in collagen concentration and the strengthening of the fibers. The healing tissues include collagen, which is part of the growing cell. The organized collagen molecules are placed in the wound site and are connected to form files. Wound strength is found in both collagen regeneration and the formation of crosslin's intra- and intermolecular solid into fibers.

Scar area

In incision wound model, when cut was given margin was traced after wound creation by using transparent paper and area measured by centimeter scale. Wound contraction was measured on 8th day when sutures were removed. And finally on 10th day medicated herbal medicated plaster was removed and scar was measured again. The observation reveals that the scar area was lesser in standard and in test as compared to the control group. A better healing pattern with complete wound closure was observed in standard and test group within 10 days while it was about 22 days in control group.



Figure 10. Day 1 (Incision of animal)



Figure 11. Day 8 (Removal of sutures)



Figure 12. Day 10 (After removal of plaster the scar was observed)

Discussion

Formulation and evaluation of herbal medicated plasters was performed. The plants *Azadirachta indica* and *Tridax procumbens* has been investigated in a systemic way for presence of total polyphenols, total flavanoids, azadirachtin content and its antioxidant activity. The extracts with significant results were used for the preparation of patches. Fresh leaves were collected from adjoining area of Nagpur. Extraction process was done by soxhlet extraction by successive method using different solvents such as Petroleum ether, Acetone and Methanol. All the six extracts were semisolid and dry powders. They were evaluated for its colour, nature and percentage yield.

On preliminary phytochemical screening it was found that the dried leaves of *Azadirachta indica* and *Tridax procumbens* contains carbohydrates, tannins, flavonoids, alkaloids, glycosides and steroids. The plant extracts were

quantitatively analysed for total flavonoids content, total phenolic content, and antioxidant activity. Methanolic extract of *Azadirachta indica* and *Tridax procumbens* has shown presence of high content of flavonoids and total phenolic content. Also the Azadirachtin content in the neem extracts was determined.

The polymer was prepared by using Isapgol seeds by drying, crushing and sieving. Physicochemical parameter of polymer such as solubility, pH, swelling index and micromeritic properties were also evaluated. The medicated patch was prepared by using AIME, TPME, and AIPE extract, with suitable concentrations. After that prepared patch was evaluated by different parameters such as thickness of patch, weight uniformity, Folding endurance and percentage moisture content

All the six extracts AIME, AIPE, AIAE, TPME, TPPE and TPAE was subjected to antimicrobial activity. The extract i.e. TPME and AIME has shown better antibacterial activity against *S. aureus*. In Antimicrobial activity it was found that newly developed herbal medicated plasters containing *Azadirachta indica* and *Tridax procumbens* extracts had an inhibitory effect on the *S.aureus*. It also showed significant zone of inhibition compared with control sample. In this way herbal medicated plaster shows comparatively significant antimicrobial activity than control sample. The number of batches having different concentrations of herbal drugs was prepared and evaluated. It was found that the FII batch shows the excellent antimicrobial activity.

FII patch was selected for fabrication of plasters which was then subjected to the pharmacological study. Pharmacological screening was done by using Incision wound healing model. FII patch posses the maximum wound healing activity by using the methanolic extracts of AIME, TPME and AIPE. Thus, the wound healing activity of these extracts may be due to the presence of secondary metabolites such as tannins, flavonoids, steroids and due to its antioxidant property which may prevent free radicals which are related to wound healing. The present study shows that newly developed herbal medicated plasters was successfully designed, developed and assessed for antimicrobial activity against control Neomycin. Hence herbal plasters could be used safely as better wound dressing.

Conclusion

The present study concludes that the *Tridax procumbens* and *Azadirachta indica* leaves extracts shows the presence of phytoconstituents which are responsible for its antioxidant property. The result on estimation of antioxidant activity of extracts proves its action on free radicals. The quantitative estimation of flavonoids and phenolic content leads to conclusion that the extract will be beneficial for wound healing activity. Herbal dosage forms of *Tridax procumbens* and *Azadirachta indicahave* shown good elegance and appearance. It is an excellent effort to design and develop the herbal medicated plasters having satisfactory zone of inhibition and antimicrobial activity compared with standard antibiotic i.e. Neomycin. The recovery was observed in the wound healing parameter. Indicate that the extract of plant part i.e. methanolic extract of leaves AIME and TPME are effective in wound healing model. These herbal medicated plasters have an excellent antimicrobial activity. This study revealed that the

developed herbal wound plaster can be a suitable dosage form for wound healing activity on human also.

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