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Evaluation of antibacterial, antioxidant and wound healing properties of seven traditional medicinal plants from India in experimental animals

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ABSTRACT

Objective: To evaluate the ethanolic and aqueous extracts of *Azadirachta indica, Emblica officinalis, Terminalia bellirica, Terminalia chebula, Curcuma longa, Cleome gynandra, Triticum aestivum, Vitis vinifera L* – Black Raisins (Zante Currants) and brown raisins (Sultanas) for *in vitro* antioxidant, antibacterial and wound healing activity. **Methods:** The free radical scavenging activity was studied *in vitro* by measuring DPPH, reducing power, hydrogen peroxide scavenging and total antioxidant assays of these plant extracts. Antibacterial activities were evaluated against five microorganisms using agar well diffusion method. The wounds were created on the skin of the rabbits by crushing the *Paederus fuscipes* beetles and applying the pederin which produced inflammation and wound after two days. **Results:** Antibacterial activities were evaluated against five microorganisms in which *A. indica, C. longa, T. bellirica, E. officinalis* and *T. chebula* showed significant activity with a MIC of 1.562 mg/ml, 3.125 mg/ml respectively. The plant extracts of brown raisins *Vitis vinifera*, *A. indica, T. bellirica, E. officinalis* and *T. chebula* showed remarkable antioxidant activity. The topical application of individual and combination of plant extracts on wounds caused significantly faster healing (83%) in wound area as compared to the commercial ointment (76.6%). **Conclusions:** This results shows that the combination of these plant extracts possess effective wound healing properties due to their antimicrobial and antioxidant activities by possessing the active compounds such as flavonoids (polyphenols), terpenes, alkaloids, saponins.

1. Introduction

Nature has been a source of medicinal treatments for thousands of years and plant-based systems continue to play an essential role in the primary health care of 80% of the world's underdeveloped and developing countries[1]. 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. Antioxidants are widely used as ingredients in dietary supplements and are exploited to maintain health and prevent oxidative stress-mediated diseases. Antioxidant compounds like phenolic acids, polyphenols and flavonoids inhibit the mechanism that leads to degenerative diseases[2].

Under certain conditions, oxygen can seriously affect our well being through the formation of reactive oxygen species (ROS) representing both free radical and non-free radical species which leads to the potential deleterious effects such as atherosclerosis, ischaemic heart disease, aging, inflammation, diabetes, immunosuppression, neurodegenerative diseases, cancer and other diseases[3]. Therefore, antioxidants with free radical scavenging activities may have great significance in the prevention and therapeutics of free radical mediated diseases.

Wound healing occurs in three stages: inflammation, proliferation, and remodelling. Collagen is the major component which strengthens and supports the extracellular tissue. In India, medicines based on herbal origin have been the basis of treatment and cure for healing of wounds and inflammation[4]. In folklore, medicinal

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plants containing polyphenols have been used for their wound healing properties, instead of using the current methods like antibiotics which has unwanted side effects. These compounds are capable of promoting rapid re-epithelialisation of acute wounds and burns and have antimicrobial properties. Medicinal plants have been reported to be very beneficial in wound care, promoting the rate of wound healing with minimal scarring to the patient[5]. *Paederus* dermatitis is a peculiar irritant contact dermatitis caused when beetles of the genus *Paederus* are crushed on the skin, which releases a strong acids (pH 1-2) pederin causing swelling and burning sensations on the part of the skin affected[6].These acids on the skin should be neutralized for the effective treatment of the wound on the skin. Combined drug (CD) is used topically on a variety of body surfaces which is a viscous semisolid preparation. The vehicle of a drug contains a base in which the drug is dissolved, suspended or emulsified[7].

The search for natural compounds rich in wound healing properties, antioxidant, anticancer and antimicrobial properties is increasing. Therefore, in this study, we have prepared an drug by using the extracts of *Azadirachta indica*, *Emblica officinalis*, *Terminalia bellirica*, *Terminalia chebula* which is known as Triphala, *Curcuma longa*, *Cleome gynandra*, *Triticum aestivum*.

Antibacterial and antioxidant activities was evaluated on the plant extracts of *Azadirachta indica*, *Emblica officinalis*, *Terminalia bellirica*, *Terminalia chebula* which is known as Triphala, *Curcuma longa*, *Cleome gynandra*, *Triticum aestivum*, *Vitis vinifera* L - Black Raisins (Zante Currants) and brown raisins (Sultanas).

2. Materials and methods

2.1. Sample collection and extraction

All the plant leaves were collected from Coimbatore district, Tamil Nadu, India during the month of December, 2010. The voucher specimens were identified and authenticated by Dr. V.S. Ramachandran, Reader, Department of Botany, Bharathiar University. (Herbarium No: KU1142, KU6528, KU8264, KU2020, KU9677, KU4012, KU1170, KU1264, KU2281). The raisins were collected from Coimbatore. 25 g of the raisins was weighed and made into a thick paste using 250 ml of distilled water and extract was prepared using solvent extraction process. For the extraction of other plant leaves, they are shade dried and powdered; they were extracted successively with ethanol in a soxhlet extractor for 18-20 h. The extracts were concentrated to dryness under reduced pressure and controlled temperature (40- 50 ℃) in a rotavapor. Then the extract was subjected to phytochemical analysis and checked for antioxidant and antimicrobial activities.

2.2. Phytochemical analysis

2.2.1.Qualitative analysis of phytochemicals

The ethanol extracts of the plants and water extract of raisins obtained were subjected to preliminary phytochemical screening. Test for alkaloids (Mayer's test), flavonoids (Alkaline reagent test), carbohydrates (Molischs test), glycosides (Legals test), saponins, tannins, phytosterol (Salkowski test), triterpenoid (Libermann Burchard test), proteins and amino acids (Ninhydrin test), biuret test, anthraquinones, steroids, catachol, reducing sugars (Fehling's Test), acidic compounds, lipids/fats, phlobatannins and resins were done to check the presence of phyto constituents[6,8,9].

2.2.2. Quantitative analysis of phytochemicals

The total phenol content was determined using Folin-ciocalteau reagent and the total flavanoid content was estimated using aluminium chloride method[10,11]. Estimation of ascorbic acid was done according to the method of Sadasivam and Balasubramanian[12].

2.3. Determination of *in vitro* antioxidant activity

2.3.1. DPPH radical scavenging assay

The procedure was followed by the method of Sanchez-Moreno et al and Narendhirakannan et al[13,14].

2.3.2. Hydrogen peroxide scavenging assay

The ability of the extracts to scavenge hydrogen peroxide was determined according to the method of Ruch et al[15].

2.3.3. Assay of Reducing Power

The total reducing power of the extracts was determined according to the method of Chang et al and Tevfik et al[16,17].

2.3.4. Total Antioxidant Capacity

The total antioxidant capacity assay of the extracts was followed by the method of Preito et al[18].

2.4. Determination of antibacterial activity

The antibacterial activity was tested using agar well diffusion and broth dilution methods according to Arshad H and Lino A, et al[19,20]. The MTCC cultures were obtained from kovai medical centre, Coimbatore, Tamil Nadu. All the plant extracts were tested against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeroginosa*.

2.4.1. Agar well diffusion method

The antimicrobial activity was tested against (ethanol) leaves of *Azadirachta indica*, *Emblica officinalis*, *Terminalia bellirica*, *Terminalia chebula* (Triphala), *Cleome gynandra*, *Curcuma longa*, *Triticum aestivum*, *Vitis vinifera* L - Black Raisins (Zante currants) and brown raisins (Sultanas). 1 ml of the test culture (107 CFU/ml) 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 plant extract was prepared at concentration of 50 mg/ml in ethanol. Each well was filled with 0.10 ml of the plant extracts. 0.10 ml of ethanol was taken as negative control and 10 μg of streptomycin served as a positive control respectively. The plates were then incubated at 37 ℃ for 24 h and zone of inhibition was measured. The results were then tabulated[21].

2.5. Wound Healing activity

2.5.1. Animals

Adult wild type rabbits weighing 1000-1250 g were used in this study. They were procured from Coimbatore. They were housed under controlled conditions with 12 h day/night cycles. The experiments were designed and conducted in accordance with International Guidelines for Animal Care. The animals were housed individually in stainless steel cages. Food and water were provided ad libitum.

2.5.2. Experimental methods

Paederus fuscipes beetles were collected using aspirators in the night time following the rainy season in the Karunya university campus. They were caught and transferred to screw capped vials. Only female insects were used to extract the fluid pederin which carrys the symbiotic bacteria responsible for pederin biosynthesis. The hairs on the skin of the animals′ back were shaved with a sterilized razor blade. 1.5 X 2 cm site was marked with skin pen where the induction of the wound (blisters) was done by direct rubbing of *Paederus* (pederin). The area was disinfected using spirit as standard disinfectant. Clean, blister-free wounds were made by the application of pederin on the site by crushing the *Paederus* insect. Infection was caused over the skin surface of rabbits and the wound appeared after 24- 48 h.

2.5.3. Formulation of the extracts

The combination of ethanolic extracts of *Azadirachta indica*, *Emblica officinalis*, *Terminalia bellirica*, *Terminalia chebula*, *Curcuma longa*, *Cleome gynandra*, *Triticum aestivum* plants were taken in a ratio randomly. *Vitis vinifera* was not included in this study. The individual plant extracts were tested for the antibacterial effect against *Pseudomonas aeroginosa* (endosymbiont) which produces the acid pederin inside the species[22]. Formulation of the drug was done by incorporating the active ingredients. *Azadirachta indica* extract 1g, *Curcuma longa* extract 1g, *Cleome gynandra* extract 1g, *Triticum aestivum* extract 1g, Triphala extract 1g, Yellow Beewax 5% which was the combined drug and each extract was used separately as a drug to treat the wound.

The combined drug base was prepared by fusion method. Formulation of drug was done by incorporating the active ingredients in the base by triturating using mortar and pestle. The prepared drug was stored at room temperature. Another set of drugs were made by adding each extract separately to the base to check the activity of each extracts. The formulated drug was evaluated by using the parameters like: spreadability, irritancy, diffusion and stability. Nuforce ointment was used as standard drug and the group with Beeswax application served as control.

The drug base was smeared onto the surface of the wound to a thickness of about 1mm. It was applied topically once daily using sterile cotton swabs until complete epithelialisation had taken place. The progressive decrease in the wound area was monitored daily. The size of the wound was also measured using a scale daily and the wound area was calculated. The wound contraction was calculated by percentage of wound contraction=[(Initial wound size - specific day wound size) / Initial wound size]×100[23].

2.6. Histopathological studies

At day 9 the experiment was terminated and the wound area was removed from the animals for histological examination. The tissue was processed in the routine way for histological evaluation. Five micrometer thick sections were stained with haematoxylin and eosin, for histopathological analysis. The tissue samples were evaluated for the following histological criteria; the extent of re-epithelisation, the maturation and organization of the epidermal squamous cells, the degree of the tissue formation. The results were compared with the control groups.

2.7. Statistical analysis

All the grouped data were statistically evaluated with SPSS/10 software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant difference (LSD) test. P values of less than 0.05 were considered to indicate statistical significance. All the results were expressed as mean±SD for three experiments in each.

3. Results

The preliminary qualitative phytochemical screening of *Azadirachta indica*, *Emblica officinalis*, *Terminalia bellirica*, *Terminalia chebula* (Triphala), *Cleome gynandra*, *Curcuma longa*, *Triticum aestivum*, *Vitis vinifera* L - Black Raisins (Zante Currants) and brown raisins (Sultanas) 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, anthroquinones, phlobatannins, triterpenoids, lipids/fats, catechol, steroids were present in nearly all the plants which is clearly shown in Table 1. Quantitative analysis (Table 2) was also done for total phenols, carbohydrates and ascorbic acid which are a potent antioxidant. *Vitis vinifera* (brown raisins) showed more phenols than the other plants. *Vitis vinifera* (Black and

Table 1.
Qualitative analysis of phytochemicals in the plant extracts

Phytochemicals	*Cleome gynandra*	*Azadirachta indica*	*Curcuma longa*	Black raisins	Triphala	Brown raisins	*Triticum aestivum*
Alkaloids	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+	+
Glycosides	+	–	+	+	+	+	+
Phytosterols	+	–	+	–	–	–	–
Triterpenoids	+	+	+	+	–	+	–
Proteins	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+
Anthroquinones	+	+	+	+	+	+	+
Phlobatannins	+	+	+	+	+	+	+
Lipids/fats	+	+	+	+	+	+	+
Catechol	+	+	+	+	+	+	+
Reducing sugar	+	+	+	+	+	+	+
Acidic compounds	–	–	–	+	–	+	–
Steroids	+	+	+	+	–	+	+
Resins	–	–	–	–	–	–	–

+ : presence; –: absence

Table 2.
Quantitative analysis of the extracts

Extract	Phenol(mg/g)	Carbohydrates(mg/g)	Ascorbic acid(mg/g)
Curcuma longa	76.70±10.09	300 ± 19.9	0.34±0.22
Cleome gynandra	164.0±15.9	510 ± 22.2	0.19±0.14
Azadirachta indica	101.1±12.1	440 ± 19.2	0.36±0.10
Triphala	192.5±18.4	220± 13.5	0.11±0.09
Black raisins	176.5±16.2	520± 21.8	1.01±0.11
Brown raisins	285.4±21.5	406± 14.3	1.19 ±0.18
Triticum aestivum	278.9±19	440±16.2	0.44±0.11

Values are mean ± SD of three determinations.

brown raisins) also had more amount of carbohydrates and brown raisins showed more amount of ascorbic acid.

3.1. *In vitro* antioxidant activity

The *in vitro* antioxidant was tested against all the plant ethanolic extracts by four *in vitro* methods. The results showed that the free radicals were scavenged with increased concentration of the extracts up to a given concentration. The H_2O_2 scavenging activity showed that black and brown raisins showed more scavenging activity than the other plant extracts. All other extracts showed *in vitro* activity near normal to the standard ascorbic acid. The IC50 value for *Triticum aestivum* (59.2 μg/ml), *Azadirachta indica* (60.60 μg/ml), Triphala (56.81 μg/ml), *Curcuma longa* (57.31 μg/ml), *Cleome gynandra* (58.96 μg/ml), Black raisins (59.88 μg/ml) lowest being Brown raisins (54.34 μg/ml) with the standard showing (58.82 μg/ml) (Figure 1). The total antioxidant capacity was measured in the plant extracts which showed IC50 value as *Triticum aestivum* (92.59 μg/ml), *Azadirachta indica* (66.66 μg/ml), Brown raisins (104.16 μg/ml) , *Curcuma longa* (74.62 μg/ml), *Cleome gynandra* (76.92 μg/ml), Black raisins (108.69 μg/ml) lowest being Triphala (53.96 μg/ml) with the standard showing (58.82 μg/ml) (Figure 2). For DPPH radical scavenging the highest IC50 value was shown by *Cleome gynandra* (147.05 μg/ml), *Triticum aestivum* (131.57 μg/ml), *Azadirachta indica* (135.13 μg/ml), *Curcuma longa* (140.84 μg/ml), triphala (131.57 μg/ml), Brown raisins (131.57 μg/ml), lowest being Black raisins (128.20 μg/ml) with the standard value as (126.58 μg/ml) (Figure 3). 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 peroxidation process (Figure 4).

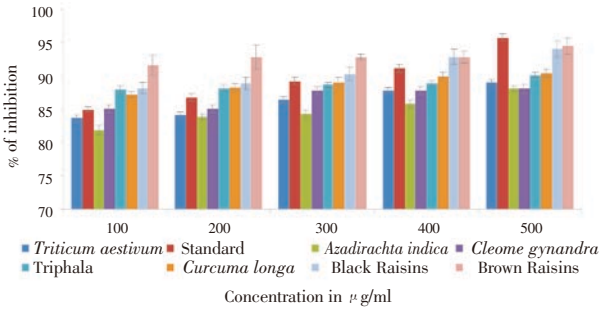

Figure 1. The effects of the plant extracts and the standard ascorbic acid on the scavenging of H_2O_2.
Values are mean ± SD of three determinations.

Table 3.
Represents zone of inhibition (mm) of all the different plants which is measured against five micro organisms.

Plant extract(μg/ml)		Zone of inhibition (mm)				
		E. coli	*K. pneumoniae*	*E. faecalis*	*S. aureus*	*P. aeruginosa*
C. gynandra	50	0	2±0.05	2±0.1	1±0.1	0
	100	1±0.01	3±0.15	4±0.05	2±0.05	1±0.1
	150	1±0.1	4±0.02	4±0.2	3±0.02	2±0.05
	200	2±0.2	4±0.17	5±0.02	4±0.15	4±0.11
	250	2±0.03	6±0.19	6±0.17	6±0.15	5±0.12
Triphala	50	2±0.1	2±0.1	2±0.1	2±0.1	2±0.1
	100	4±0.10	4±0.05	3±0.05	4±0.1	4±0.15
	150	5±0.15	5±0.02	4±0.17	5±0.05	5±0.15
	200	6±0.17	6±0.1	5±0.15	6±0.2	6±0.17
	250	7±0.11	7 ±0.2	6±0.15	8±0.2	7±0.05
C. longa	50	2±0.11	3±0.05	2±0.1	2±0.1	2±0.1
	100	3±0.11	4±0.05	3±0.05	4±0.17	3±0.12
	150	4±0.12	4±0.1	5±0.1	5±0.17	4±0.11
	200	5±0.15	5±0.4	5±0.15	6±0.2	5±0.10
	250	6±0.17	6±0.2	6±0.15	8±0.17	6±0.15
T. aestivum	100	1±0.12	1±0.12	2±0.05	1±0.1	1±0.1
	200	2±0.11	2±0.15	2±0.05	2±0.05	2±0.05
	300	2±0.10	2±0.15	3±0.1	3±0.02	3±0.05
	400	3±0.5	3±0.17	4±0.1	4±0.2	3±0.1
	500	4±0.11	3±0.12	4±0.1	4±0.2	4±0.10
A. indica	50	8±0.2	6±0.2	6±0.1	10±0.2	10±0.15
	100	10±0.2	6±0.1	8±0.2	10±0.15	12±0.15
	150	12±0.25	8±0.15	10±0.25	12±0.15	14±0.12
	200	13±0.3	9±0.17	10±0.3	13±0.17	15±0.12
	250	14±0.2	10±0.4	12±0.3	14±0.12	16±0.17
(*Vitis vinifera*)	50	1±0.1	1 ±0.1	1 ±0.1	2 ±0.1	2±0.1
Brown Raisin	100	4 ±0.15	3 ±0.1	2 ±0.15	3 ±0.17	4 ±0.01
	150	5 ±0.17	6 ±0.1	4 ±0.17	5 ±0.15	8 ±0.10
	200	7 ±0.2	10 ±0.1	8 ±0.17	8 ±0.3	10 ±0.17
	250	10±0.3	12±0.1	10±0.2	10±0.4	12±0.17
(*Vitis vinifera*)	50	2 ±0.1	1 ±0.1	1 ±0.02	1 ±0.1	1 ±0.1
Black Raisins	100	6 ±0.15	4 ±0.1	2 ±0.02	3 ±0.1	4 ±0.05
	150	10 ±0.2	6 ±0.1	4 ±0.05	5 ±0.2	5 ±0.05
	200	12 ±0.3	9 ±0.1	5 ±0.05	8 ±0.5	8 ±0.1
	250	14±0.5	12±0.1	7±0.1	10±0.5	10±0.15
Streptomycin		12±0.2	12±0.15	10±0.2	14±0.17	18±0.5
Control		3±0.1	4±0.05	6±0.1	2±0.05	4±0.2

Values are mean ± SD of three determinations.

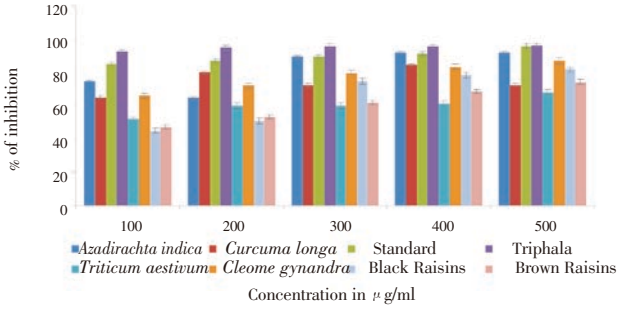

Figure 2. Determines the total anti-oxidant capacity of the plant extracts and the standard ascorbic acid.
Values are mean ± SD of three determinations.

3.2. Antimicrobial activity

Wound healing may be hampered by the microbial activity that is present on the wounds. The organism *Pseudomonas aeroginosa* grows as an endosymbiont on the *Paederus fuscipes* which produces the acid pederin causing the blister dermatitis on the skin. So the antimicrobial activity was tested against five organisms and the results of the antimicrobial activity by the agar well diffusion method of the plant extracts were presented in Table 3. All the plant extracts showed effective antimicrobial activity against the five microorganisms. *Azadirachta indica*, Triphala, *Curcuma longa*, *Vitis vinifera* (Black and Brown raisins) showed high antimicrobial activity than the other plant extracts followed by *Cleome gynandra* whereas *Triticum aestivum* showed the least antimicrobial activity. The

Table 4.
Minimum inhibitory concentrations of the plant extracts against the pathogenic organisms.

Plant extract(mg/ml)		Minimum inhibitory concentration				
		E. coli	*K. pneumoniae*	*E. faecalis*	*S. aureus*	*P. aeruginosa*
C. gynandra	25.000	+	+	+	+	+
	12.500	+	+	+	+	+
	6.250	+	+	−	+	−
	3.125	−	−	−	−	−
	1.562	−	−	−	−	−
	0.781	−	−	−	−	−
Triphala	25.000	+	+	+	+	+
	12.500	+	+	+	+	+
	6.250	+	+	+	+	+
	3.125	+	+	−	−	−
	1.562	+	−	−	−	−
	0.781	−	−	−	−	−
C. longa	25.000	+	+	+	+	+
	12.500	+	+	+	+	+
	6.250	+	+	+	+	+
	3.125	+	+	+	+	−
	1.562	+	−	−	−	−
	0.781	−	−	−	−	−
T. aestivum	25.000	+	+	+	+	+
	12.500	+	+	+	+	+
	6.250	+	−	−	−	−
	3.125	−	−	−	−	−
	1.562	−	−	−	−	−
	0.781	−	−	−	−	−
A. indica	25.000	+	+	+	+	+
	12.500	+	+	+	+	+
	6.250	+	+	+	+	+
	3.125	+	+	+	+	+
	1.562	+	+	−	+	+
	0.781	+	−	−	−	−
(*Vitis vinifera*)	25.000	+	+	+	+	+
Brown Raisins	12.500	+	+	+	+	+
	6.250	+	+	+	+	+
	3.125	+	+	−	−	+
	1.562	−	−	−	−	−
	0.781	−	−	−	−	−
(*Vitis vinifera*)	25.000	+	+	+	+	+
Black raisins	12.500	+	+	+	+	+
	6.250	+	+	+	+	+
	3.125	−	−	−	−	−
	1.562	−	−	−	−	−
	0.781	−	−	−	−	−
MHB alone[a]		NG	NG	NG	NG	NG
MHB+plant extract[a]		NG	NG	NG	NG	NG
MHB+Test organism[b]		GO	GO	GO	GO	GO

a: negative control; b: positive control; MHB: Mueller Hinton broth; NG: No growth; GO: Growth observed.

minimum inhibitory concentration of all the plant extracts against the microorganisms is shown in Table 4. *Azadirachta indica* showed the highest MIC for E.coli (0.781mg/ml) followed by *Curcuma longa* and Triphala (1.562 mg/ml). Again *Azadirachta indica* showed the highest MIC for *K. pneumonia* (1.562 mg/ml) which was followed by Brown

raisins, *Curcuma longa* and Triphala (3.125 mg/ml). For *E. faecalis*, *Azadirachta indica* and *Curcuma longa* showed effective activity at 3.125 mg/ml. *S. aureus* was inhibited maximum by *Azadirachta indica* (1.562 mg/ml) and *Curcuma longa* (3.125 mg/ml). *P. aeruginosa* which is the main cause for the production of pederin is inhibited by *Azadirachta indica* (1.562 mg/ml) followed by other plant extracts.

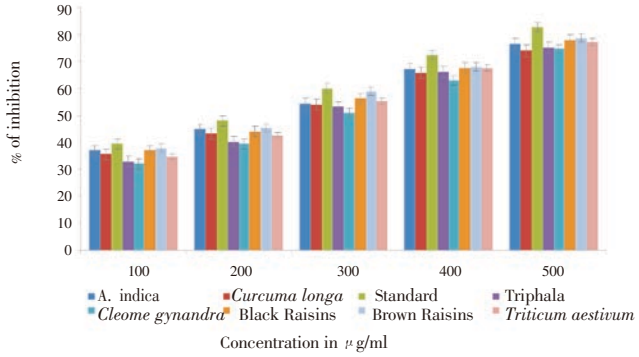

Figure 3. DPPH free radical scavenging activity of the different plant extracts and the standard ascorbic acid.
Values are mean ± SD of three determinations.

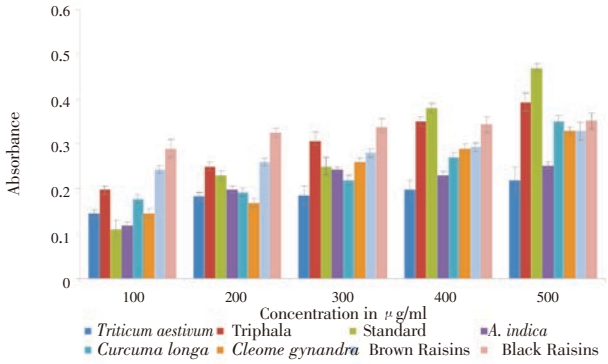

Figure 4. The reducing abilities of the plant extracts and the standard ascorbic acid.
Values are mean ± SD of three determinations. The absorbance was plotted against concentration of the sample.

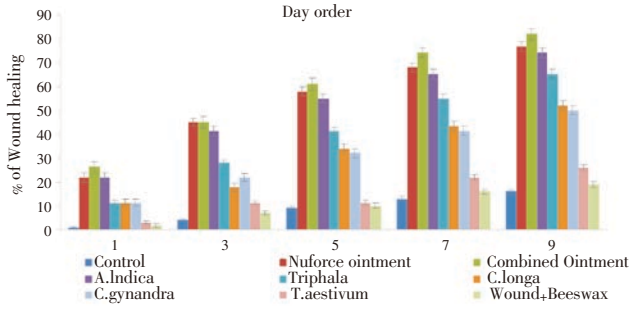

Figure 5. Wound healing activity of the separate and combined drug in rabbits
Values are mean ± SD of three determinations.

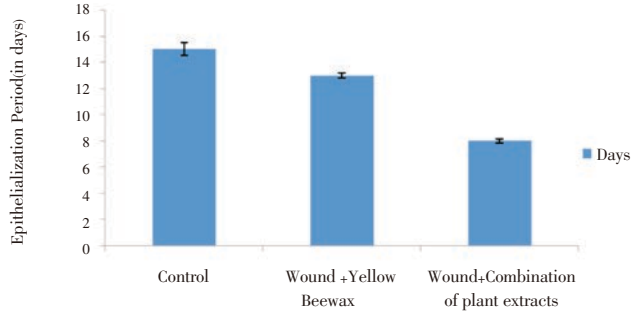

Figure 6. Epithelialization of the wound area in rabbits.
Values are mean ± SD of three determinations.

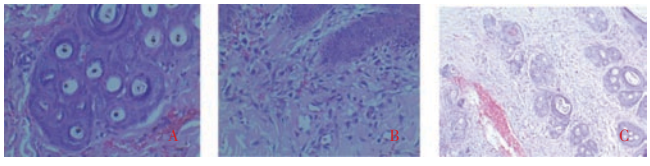

Figure 7. Histopathological observations showing the control sites and experimental sites with and without the treatment of the drug on the skin of the animal.
A: Control sites–Normal skin; B: Experimental sites – wound alone, reticular degeneration of the epidermis and scattered acantholytic cells; C: Experimental sites – wound treated, formation of new keratinocytes, edematuos tissue with PMN leukocyte, Fibroblast proliferation increased.

3.3. Wound healing activity

After checking for *in vitro* and antibacterial activity of the extracts, the wound healing activity of the extracts from the plants of *Azadirachta indica*, *Emblica officinalis*, (*Terminalia bellirica*, *Terminalia chebula*) together called as Triphala, *Curcuma longa*, *Cleome gynandra*, *Triticum aestivum*. All these plants are owed to have an efficacy of wound healing properties combined with antimicrobial properties. A total number of 200 beetles were collected in our campus in Coimbatore. *Paederus fuscipes* species was predominant in this area. After the application of live *Paederus* (pederin) on the control and medication treated rabbits, there was no dermal reaction for the first two days on the skin of the rabbits. After the second day erythema progressed in all the rabbits. Pathological reactions like diffuse erythematous and dermal desquamative lesions were seen predominantly. Early lesions showed epidermal necrosis with a layer of suprabasal cells. Acantholytic cells were present which were seen scattered. Intraepidermal neutrophils and reticular degeneration are the features seen with superficial keratinocytes. After the application of the medication to the treated rabbits, crusting and hardening of the wound was evident. The treated wounds started to heal and wound sites were decreasing. After the 6th day protrusion of the scab above the wound was seen. Maturing new keratinocytes were seen during the healing process and at last the crust, some acanthosis and perivascular infiltrates were seen in the treated ones. The control sites showed the protrusion of the scabs even on the 9th day while the scabs had fallen off in the medication treated wounds showing granulation tissue turning from red to pale color. By the 10th day hair follicles were seen growing on the skin. The control site had the wound area alone without any treatment. The

wound healing activity of the drug that was used separately and in a combined form is shown in Figure 5. The drug prepared with *Azadirachta indica* individually showed good healing effect when compared to other individual plant extract drug preparations followed by Triphala. The epithelialisation of the wound was observed on different days for different wound area which is shown in Figure 6. The histopathological observations are shown in Figure 7.

4. Discussion

Many traditional plants remedies are known in folk medicine and used for treatment and some have been validated by scientific studies to actually exert biological action against wound healing or its complications[24]. This study therefore provided bases to the folkloric use of different plants as a remedy for skin disease caused by the pathogens. It also justifies the folklore medicinal uses about the therapeutic values of these plants as curative agent and therefore, the purification and characterization of the phytochemicals that can be isolated from these plants will be useful as a chemotherapeutic agent[25]. Oxidative stress has been shown to play an important role in the development of wound healing and blood regulation. Indeed, increase in total antioxidant status has been shown to be important in recovery from wound. All the plants exhibited potent antioxidant activity in our study. The presence of the polyphenols in all the plants 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 extract[26].

Phytochemical test, quantitative analysis, in-vitro antioxidant test, anti-microbial analyses are some of the basic tests done. Quantitative estimation showed more amounts of phenols present in all the plants. Neem showed high anti microbial activity when compared to other plants. Extracts from Turmeric, Neem, African spider plant, Triphala, Wheatgrass showed varying antioxidant (free radical scavenging) activities when compared with the standard ascorbic acid. The H_2O_2 scavenging activity was found to be more in neem extract. Triphala and turmeric showed high reducing power activity. Wheatgrass and turmeric was found to have high total antioxidant capacity. *Azadirachta indica* and *Vitis vinifera* (Brown raisins) showed more antioxidant activity than the other plant extracts. The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen-donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable molecule. The plant extracts are made to react with the stable radical DPPH, in ethanol solution. The decrease in the absorbance at 517 nm shows the reduction capability of DPPH radical[27].

The results suggest that the antioxidant activity of these plants may contribute to their claimed wound healing property. Possibly, the constituents like triterpenoids and alkaloids of the turmeric, neem, Triphala, and African spider plant may play a major role in the process of Wound healing. However, triterpenoids and flavonoids are known to promote the wound-healing process mainly due to their astringent and antimicrobial property, which seems to be responsible for wound contraction and increased rate of epithelialisation[28,29]. Wound healing, a complex sequence of events, is initiated by the stimulus of injury to the tissues. A positive stimulus may result from the release of some factors by wounding of tissues. It has been found that plants have high cicatrizing and vulnerary properties. Impairment at the cellular level may be prevented by different antioxidants which reduce damage caused by free radicals[30].

This study deals with a new combination of seven medicinal plants against blisters and infection caused by an insect, *Paederus fuscipes*, family Staphylinidae, order Coleoptera. This *Paederus fuscipes* is nocturnal and attracted by incandescent and fluorescent lights and accidentally comes into contact with humans. Pederin is released when it is crushed onto the skin of the human's accidentally which causes dermatitis[31,32]. Further wound healing property of each plant have been determined which shows Neem with high wound healing power followed by turmeric, Triphala, African spider plant and wheatgrass. Wheatgrass has less effect compared to all other plants. Through the visual and histological observation throughout the wound healing period we experienced the sequential changes and normalization of the repair of skin wound in our rabbit model and it took 9 days for epithelialisation period. Hence the drug formulated has high wound healing effect against the *Paederus* dermatitis as it promotes wound contraction and shortens epithelisation period. The drug had its pharmacological properties, and it was treated for *Paederus* dermatitis, and it has proved better than traditional methods for its remarkable analgesic, rapid wound-healing and potent anti-infective effects.

Conflict of interest statement

We declare that we have no conflict of interest.

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