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RESEARCH ARTICLE

Formulation and Efficacy Assessment of a Polyherbal Wound Healing Formula from *Heliotropium Indicum* and *Nephrolepis Biserrata*

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ABSTRACT

Background: Heliotropium indicum and *Nephrolepis biserrata* are common medicinal plants used in folk medicine for treating many diseases, including wounds in most African countries such as Ghana. Previous separate preliminary studies on these two herbs have shown that they have wound-healing abilities. However, their combined effects concerning wound healing remain unknown. Many studies also have identified diverse phytocompounds in the leaves of these two medicinal plants. *Objective:* The present study describes the development of a polyherbal wound-healing formulation from leaves of *H. indicum* and *N. biserrata* and further assessed their wound-healing effects using both *in vivo* and *in vitro* wound healing models. *Materials and methods:* After the herbs leaf collection and confirmation, ethanol was used to extract phytocompounds from their dried leaves and subjected to phytochemical screening using standard methods. Subsequently, the antioxidant and free radical scavenging properties of the crude extracts were assessed. The weight: weight proportions were used to prepare the polyherbal formula and were tested using an excision wound model. Wound contraction and wounding days were used as endpoints to assess the degree of wound-healing effects. *Results:* Extracts of *N. biserrate* and *H. indicum* exhibited antioxidant activity with a half-maximal inhibitory concentration (IC_{50}) value of 324.1 and 301.1 μg/mL in the 2,2-diphenyl-1-picrylhydrazyl radical scavenging method, 745.6 and 350.1 μg/mL in the nitric oxide synthase assay, and 806.4 and 180.5 μg/mL in the ferric reducing assay, respectively. The total phenolic content in *N. biserrata* extract was 52.271 mg gallic acid equivalent per gram of dry extract, significantly higher than 32.170 recorded in *H. indicum* extract. Similarly, *N. biserrate* extract exhibited higher total flavonoid content of 594.537 compared with 357.471 for *H. indicum* extract. None of the formulations gave 100% complete wound healing on the 18th day of treatment. However, the monotherapy formulations performed better than the polyherbal formulations. The 10% dosage formulation of *H. indicum* and the 20% dosage of 1:2 *H. indicum* and *N. biserrata* formulation recorded the highest wound healing activity of 97.4 and 93.8% among the polyherbal treatments.

Keywords:
antioxidant; excision wound; phenolic content; polyherbal; wound healing

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INTRODUCTION

Wounds not only increase morbidity and mortality but also decreases the quality of life.[1] They are associated with several human diseases and injuries of various kinds.[2–5] Wounds may be associated with pain, microbial infections, unpleasant smell, loss of self-esteem and confidence, and disfiguration of affected body parts.[6,7] Some specific types of wounds, such as diabetic sores, are untreatable in many instances and leave these unfortunate patients with a lifelong problem that causes social and psychological confusion.

The prospects of providing effective wound healing pharmacotherapy are in part related to the nature of the wound, the level of microbial infection, genetic predisposition, and the wound healing therapy effectiveness.[8–11] Currently, many conventional wound healing therapies are in use, including chemotherapy.[3,10,12–14] But these therapies are not only ineffective but also are expensive and present detectable toxic reactions.[15,16] However, indigenous wound healing systems employing several herbs continue to be popular among locals and are proven effective so far. Therefore, there is a high probability of discovering a potential wound healing therapeutic agent from these medicinal plants.

The wound healing medicinal herbs (*Cochorus olitorius, Combretum dolichopetalum,* and *Heliotropium indicum*) from the Volta region of Ghana, were previously researched for their property as claimed by local folks. Interestingly, our preliminary *in vitro* and *in vivo* results also confirmed these claims. The herbs in our primary study were investigated as individual herbs concerning their wound healing and antioxidant properties.[17,18]

Synergism allows interaction between phytocompounds or two or more drugs to enhance their therapeutic effects.[19,20] This reflects in most indigenous healing systems where many herbs are combined in an appropriate proportion to form polyherbal mixtures and potions for disease treatment. On this basis, we hypothesize that wound healing herb combined to form a polyherbal formula may aid in the treatment of wounds. This study formulates and assesses wound healing ointments of *Heliotropium indicum* and *Nephrolepis biserrata* used locally for the wound treatments.

METHODOLOGY

STUDY LOCATION

The research was carried out in two phases (1 and 2) in the Department of Chemistry, Organic Research laboratory, and Department of Laboratory Technology Research Laboratory at the University of Cape Coast, Ghana.

Phase 1 involved phytochemical and the determination of the chemical constituents and antioxidant potentials of the selected herbs. Phase 2 involved formulations of wound healing ointment of different concentrations and *in vivo* wound healing tests using excision wounds.

PHASE 1

Plant materials and sample treatment

The fresh leaves of *C. dolichopetalum* were collected from Jukwa near Cape Coast in the Central Region during December 2013. Leaf parts of *N. biserrata* and *H. indicum* were collected in large quantities from the surrounding bushes around UCC in September 2018. The samples were authenticated by Mr. Felix Fynn and Mr. Otoo, the curators of the herbarium, Department of Environmental Sciences, School of Biological Sciences, with the UCC voucher specimen no. CCG 4873 (May 13, 1982) for *H. indicum* and CCG 259 (March 12, 2016) for *N. biserrata*.

The samples were washed under tap water, rinsed with deionized water, and dried at room temperature. The dried leaves were pulverized into a fine powder and were divided into two parts. The first part of about 10 g of each plant was combined in different proportions and mixed with Shea butter to formulate wound healing ointment at different doses. Twenty grams of the other part was extracted with 250 mL (90% v/v) methanol by cold maceration (48 h), followed by filtration through Whatman no. 1 filter paper. The resultant filtrate was concentrated in a vacuum and the extract obtained was placed in a refrigerator for phytochemical screening and antioxidant activity.

Phytochemical screening

Previously described standard qualitative methods such as Liebermann Burchard's test for steroids, Salkowski's test for triterpenes, Gelatin test for tannins, Molisch's test for carbohydrates, etc. were used to identify the chemical composition of the plants.[21,22]

Assessment of total phenolic content (TPC)

The TPC in gallic acid equivalence (GAE) was estimated by the Folin Ciocalteu method with slight modifications.[23] A dilute concentration of the plant extract (125 μL of 1 mg/mL) was mixed with 375 μL Folin-Ciocalteu reagent (1 in 10 dilutions with water) and incubated for 5 min before adding 375 μL of 7% sodium carbonate. The reaction mixture was then made to 2.5 mL with distilled water and incubated at room temperature for 2 h for color development. The phenolic content was calculated by measuring the optical density at 765 nm using a spectrophotometer (T70 PG Instruments). A standard curve of gallic acid was prepared in the range 0–300 μg/mL (Figure 1), from which the TPC of the extracts was estimated and expressed as GAE. The experiment was repeated thrice for each concentration.

Total flavonoid concentration

The total flavonoid content was determined as described by Zhishen et al. [24] with few modifications. A thousand microliters of the extract were added to 4 mL of 50 % ethanol, followed by 300 μL of 5% sodium nitrite, and was left to stand for 5 min. Later 300 μL of 10% aluminum chloride was also added. After 6 min, 2000 μL of 1M sodium hydroxide was added to the mixture and was immediately diluted with 3300 μL distilled water and thoroughly mixed. Subsequently, absorbance

versus reaction blank was measured at 510 nm with quercetin in ethanol as standard. The total flavonoid content of extracts was estimated from the standard curve (Figure 2) and expressed as quercetin equivalence (QE).

Ferric reducing antioxidant power assay

The reduction of ferric ions by the extract was determined using the ortho-phenanthroline procedure as previously reported.[25]The reaction mixture contained 1 mL of the plant extract, 0.5 mL of *O*-phenanthroline (in methanol) t, and 1 mL of 200 μM ferric chloride. The mixture was incubated at room temperature for 10 min and the absorbance was read at 510 nm. A concentration range of 50–1000 μg/mL for both extracts with standard gallic acid and ascorbic acid were recorded. Later, a graph of absorbance against concentration was plotted for analysis.

Nitric oxide (NO) scavenging assay

The NO scavenging ability of the plant extract was estimated using the Griess Illosvory reaction.[26] The Griess Illosvory reagent contained 0.1 % (w/v) naphthyl ethylenediamine dihydrochloride. Plant extracts and standard ascorbic acid and gallic acid at different concentrations (0-1500 μg/mL) were prepared to 0.25 mL and placed in separate test tubes. Sodium nitroprusside (10mM; 0.5mL) and 0.125 mL of sodium phosphate buffer (pH 7.4) was added and incubated at 25°C for 180 min. Subsequently, 0.25 mL sulfanilic acid reagent (0.33% in 20% glacial acetic acid) was added and allowed to stand for another 5 min. The procedure was completed by further addition of 0.25 μL of 0.1 % Griess Illosvory reagent. Later all test tube contents were mixed well and allowed to stand at 25°C for 30 min. The nitrite concentration was evaluated at 546 nm by preparing a control set up (buffer with all other components of reaction mixture intact). The percentage NO^- scavenging abilities were calculated using Equation (1):

$$\%\,\text{inhibition} = \frac{Ac - At}{Ac} \times 100, \quad (1)$$

where ***Ac*** = absorbance of the control and ***At*** = absorbance of the test (extract/standard)

DPPH radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity was determined using the method reported by Brand-Williams et al. and Blois.[26,27] The purple/violet DPPH fades to a yellow color in the presence of substances with antioxidant properties. In the determination of the extractability against DPPH radical, a DPPH solution (0.5 mM; 1mL) was added to 1 mL of various sample concentrations (50–500 μg/mL) and standard gallic acid in methanol. A control setup was prepared with 1ml each of methanol and DPPH solution. Reaction solutions were thoroughly mixed and incubated at room temperature for 30 min in the dark. Absorbances were spectrophotometrically measured at 517 nm. The percentage radical activity was calculated from using Equation (2):

$$\%\text{inhibition} = \frac{Ac - At}{Ac} \times 100, \quad (2)$$

where ***Ac*** = absorbance of the control and ***At*** = absorbance of the test (extract/standard).

A graph of the percentage of inhibition against concentration in μg/mL was plotted from which half-maximal inhibitory concentration (IC_{50}) values were calculated. All experiments were conducted in triplicates and the results were reported in mean ± standard deviation.

PHASE 2

Experimental animals

Healthy adult Sprague-Dawley rats (120–200 g) of either sex were purchased from Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana and maintained at the Animal Experimentation Unit of the School of Biological Sciences, University of Cape Coast, Cape Coast. The animals were fed with commercial pellet diet (GAFCO, Tema, Ghana), water ad-libitum, and maintained under ambient laboratory conditions. The study followed both institutional and national guidelines on the use and care of animals in scientific experimentation. All protocols involving animals were approved by the institutional review board (IRB), University of Cape Coast.

EXCISION WOUND MODEL

This model was used to establish wound healing in animals as previously described.[28] Briefly, each animal was anesthetized by intraperitoneal administration of 90 mg/kg ketamine. The fur on the dorsal region of each animal was shaved and the animal was fixed on the surgery table in ventral posture for surgical preparation. A circular surgical full-thickness wound was created on the anterior-dorsal side of each animal. The animals were divided into 24 groups of five rats per group. The various groups were treated as follows: Group 1 (5 % of povidone iodine), Group 2 (shea butter), Group 3 (negative control), Group 4 (10 % of *H. indicum* ointment), Group 5 (20 % of *H. indicum* ointment), Group 6 (40 % of *H. indicum* ointment), Group 7 (10 % of *N. biserrate* ointment), Group 8 (20 % of *N. biserrate* ointment), Group 9 (40 % of *N. biserrate* ointment), Group 10 (10 % of 1 *N. biserrate*:1 *H. indicum* ointment), Group 11 (20 % of 1 *N. biserrate*:1 *H. indicum* ointment), Group 12 (40 % of 1 *N. biserrate*:1 *H. indicum* ointment), Group 13 (10 % of 1 *N. biserrate*:2 *H. indicum* ointment), Group 14 (20 % of 1 *N. biserrate*:2 *H. indicum* ointment), Group 15 (40 % of 1 *N. biserrate*:2 *H. indicum* ointment), Group 16 (10 % of 1 *N. biserrate*:4 *H. indicum* ointment), Group 17 (20 % of 1 *N. biserrate*:4 *H. indicum* ointment), Group 18 (40 % of 1 *N. biserrate*:4 *H. indicum* ointment), Group 19 (10 % of 1 *N. biserrate*:2 *H. indicum* ointment), Group 20 (20 % of 1 *N. biserrate*:2 *H. indicum* ointment), Group 21 (40 % of 1 *H. indicum*:2 *N. biserrate* ointment), Group 22 (10 % of 1 *H. indicum*:4 *N. biserrate* ointment), Group 23 (20 % of 1 *H. indicum*:4 *N. biserrate* ointment), and Group 24 (40 % of 1 *H. indicum*:2 *N. biserrate* ointment). The rats were treated once daily with the formulations starting from the day of wounding until complete epithelialization.

Wound contraction and wound closure time were assessed to calculate the wound-healing property. The wound area was measured by immediate placement of transparent paper

over it and traced out. The percentage of wound closure was estimated for each group using the wound's measured diameter at the beginning of the experiments and on days 0, 2, 4, 6, 8, 10, 12, 14, 16, and 18 post wounding.

STATISTICAL ANALYSIS

Data were expressed as mean ± standard deviation of triplicate measurements. GraphPad Prism Version 8.4.3 for Windows (Graphpad Software, San Diego, CA) was used for all statistical analysis. Significant differences between means of the various treatment groups were obtained using one way analysis of variance. $P \leq 0.05$ was considered significant in all analyses.

RESULTS

PHYTOCHEMICAL ANALYSIS

Table 1 present the results of the qualitative phytochemical analysis carried out on *H. indicum* and *N. biserrata*. The results showed the presence of alkaloids, triterpenoids, and flavonoids. Both the plants showed negative for saponins and tannins. However, *H. indicum* gave a positive test for cardiac glycosides while *N.* biserrate was negative.

Quantitative determination of phenolic and flavonoid contents was carried out on the methanol extracts of *H. indicum* and *N. biserrata*. The results were not significantly high (Table 2).

Table 1 Results of phytochemical screening on *H. indicum* and *N. biserrate*.

Test	*H. indicum*	*N. biserrate*
Saponin (foam test)	–	–
Tannins and phenols ($FeCl_3$ test)	Inconclusive	Inconclusive
Tannins and phenols (lead acetate test)	–	–
Alkaloids (Dragendroff's test)	+	+
Alkaloids (Hager's test)	–	–
Alkaloids (Mayer's test)	+	+
Triterpenoids (Salkowaski test)	+	+
Triterpenoids (Liebermann Burchard test)	+	+
Flavonoids	+	+
Cardiac glycosides	+	–

N. biserrata recorded significantly higher GAE (52.271) and QE (594.537) than *H. indicum* (32.170 ; 357.471).

ANTIOXIDANT ACTIVITY

The antioxidant test showed that *H. indicum*, *N. biserrata*, and the two standards (ascorbic- and gallic acid) caused a concentration-dependent increase in antioxidant activity (Figures 3–5). However, at equipotent concentrations, the two standards had a better antioxidant activity versus *H. indicum* and *N. biserrata*, except in iron-reducing assay; gallic acid had lower antioxidant activity than both herb samples.

WOUND HEALING ASSAY

The mean percentage of wound closure was calculated on days 0, 4, 6, 10, 12, 16, and 18 postwounding days by measuring the wound's diameter to determine the wound healing activity. Results obtained on 10, 12, 16, and 18 postwounding days are shown in Table 3. None of the treatments gave 100% complete wound healing on the 18th day of treatment. The monotherapy treatments performed better than the polyherbal formulations. The 10% dosage formulation of *H. indicum* recorded the highest percentage of wound healing (97.4), followed by 20% dosage formulation of *N. biserrata* (95.7) and 10% dosage formulation of *N. biserrata* (91.2). 10% *H. indicum* (97.4%), 20% *N. biserrata* (95.7%), and 20% 1:2 *N. biserrata* and *H. indicum* (93.8%) performed better than shea butter. However, for the polyherbal formulation, the 20% dosage of 1:2 *N. biserrata* and *H. indicum* formulation recorded the highest percentage of wound healing activity (93.8%). Only nine formulations, including shea butter, performed better than the negative control. However, 17/22 formulations excluding shea butter and the negative control performed better than the standard drug, povidone. No effective wound healing with 40% formulations was observed in both monotherapy or combination therapy, except with 1:4 *H. indicum* and *N. biserrata* combined formulation. The wound healing activities were not dose-dependent in all the treatments (Table 3 and Figure 2).

DISCUSSION

The results clearly showed that the two plants exhibited high scavenging ability which is comparable to ascorbic and gallic acid. However, the IC_{50} recorded in *H. indicum* (180.5) extract indicated a better antioxidant activity than *N. biserrata* extract and the standards. No significant difference was observed

Table 2 Antioxidant activityand total phenolic and flavonoid contents estimated in *H. indicum* and *N. biserrata* compared with standard acids.

	Estimated IC_{50} values				
Sample	**DPPH**	**Iron chelating**	**Nitric oxide scavenging**	**Total phenolic Content (GA)**	**Total flavonoid content (QE)**
H. indicum	301.1^{a}	350.1^{a}	180.5^{a}	32.170^{a}	357.471^{a}
N. biserrata	324.1^{b}	$\sim 806.4^{b}$	745.6^{b}	52.271^{b}	594.537^{b}
Gallic acid	131.6^{c}	300.2^{c}	594.4^{c}	–	–
Ascorbic acid	–	531.8^{d}	732.6^{bd}	–	–

$^{a-d}$Values that do not share the same letter in a column are significantly different using one way analysis of variance at 95% confidence intervals.

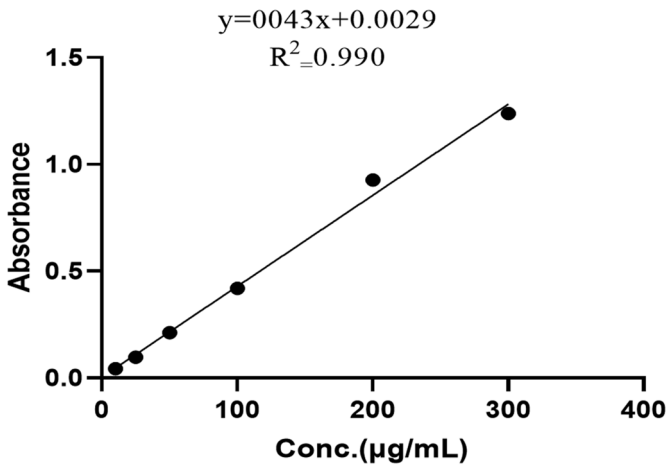

Figure 1 Standard curve of gallic acid for interpolation of unknown phenolic content of extracts.

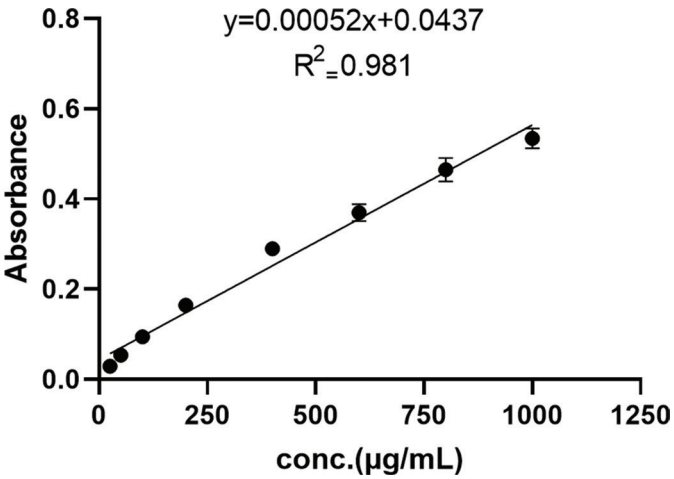

Figure 2 Standard curve of quercetin for interpolation of unknown flavonoid in extracts.

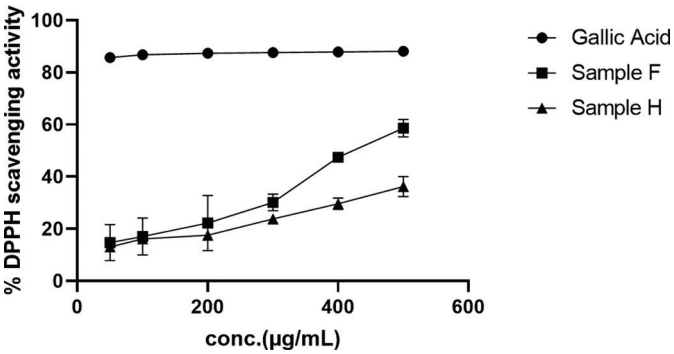

Figure 3 Percentage of DPPH scavenging capacity of *H. indicum*, *N. biserrate*, and standard gallic acid at varying concentrations.

in the antioxidant activity of *N. biserrata* and ascorbic acid. NO is an inhibitor of physiological processes and plays the role of effectors molecules in diverse biological systems. The accumulation of NO is linked to the reduction of melatonin secretion that leads to impaired resistance to free radicals, which may cause many devastating disease conditions and accelerate aging. Ascorbic acid is a standard antioxidant used to scavenge free radicals in the plasma and cell membranes. The significantly better antioxidant activity exhibited by *H. indicum* than ascorbic acid is of much interest ($P < 0.0001$).

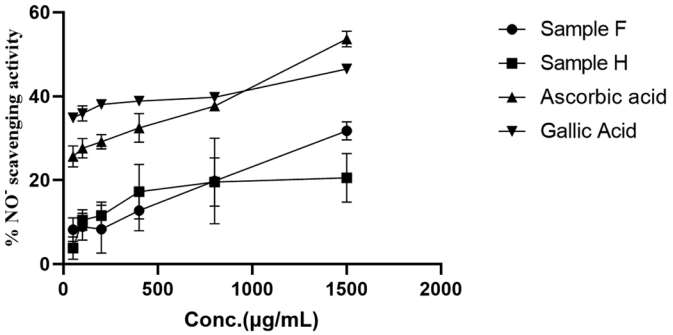

Figure 4 Percentage of nitric oxide radical scavenging activity of samples *H. indicum* and *N. biserrata* with gallic acid and ascorbic acid as standards at varying concentrations.

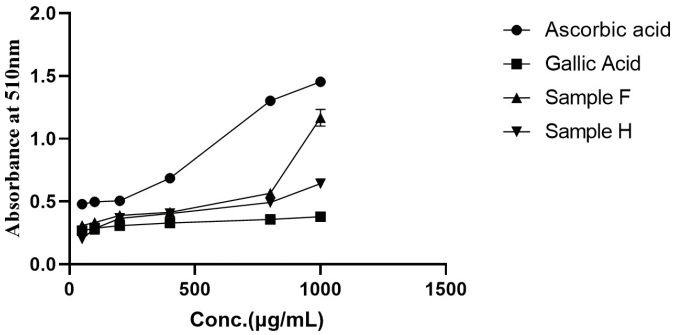

Figure 5 Iron chelating ability of *H. indicum* and *N. biserrata* with standard acids.

The IC_{50} is the concentration of an antioxidant-containing substance required to scavenge 50% of the initial DPPH radicals. The lower the IC_{50} value, the more potent a substance is at scavenging DPPH and implies a higher antioxidant activity. The DPPH assay showed that both extracts are potential primary sources of antioxidants since they exhibited the capabilities to scavenge DPPH free radicals. *H. indicum* was more potent than *N. biserrata* but less potent than standard antioxidant gallic acid ($P < 0.001$).

Similarly, the ferric reducing ability of *H. indicum* was significantly ($P < 0.0001$) better than ascorbic acid and *N. biserrata*. The reductive capacity of a compound is a measure of its ability to donate electrons and this reducing capacity correlates well with potential antioxidant activity.[29,30] The ferric reducing assay of the extracts and the standard showed an increase in a concentration-dependent manner, indicating the antioxidant activity of all the compounds. This assay also indicated that *H. indicum* was a better free radical scavenger than ascorbic acid.

Significant promotion of wound-healing activity was observed with the various formulations in the excision wound model. From the results, all the formulations on the plant extracts displayed some significant level of wound healing activity which to some extent confirmed the folkloric potential uses of the plants for wound healing. The antioxidant activities exhibited by the two plants reflected the relatively moderate wound healing potentials recorded for the formulations. This model also showed the better wound healing capacity of *H. indicum* on the 18- day postwounding. This result agreed with the previous study of Shiddhuraju et al.[31] The observation of antioxidant activity and wound healing property of plants

Table 3 Wound closure in diameter and % wound contraction of topical application of various formulations on excision wound model.[a]

Treatment group	Mean wound contraction (%) on postwound healing days					
	0	6	10	12	16	18
H10%	25.67±1.04 (0)	19.5±0.5 (24.0)	9.00±0.87 (64.9)	7.17±0.29 (72.1)	2.67±0.58 (89.6)	0.67±0.58 (97.4)
H20%	26.17±3.06 (0)	20.3±3.2 (22.4)	10.5 ± 1.7 (59.9)	7.67±1.89 (70.7)	3.50±1.32 (86.6)	3.0 ± 1.3 (88.5)
H40%	25.0 ±1.8 (0)	17.67±1.04 (29.3)	11.2 ± 1.9 (55.2)	8.67±1.04 (65.3)	4.00±0.50 (84.0)	3.5 ± 0.5 (86.0)
F10%	22.8± 3.5 (0)	19.3±5.7 (15.4)	11.2 ± 5.8 (50.9)	8.0±4.4 (64.9)	4.00±2.65 (82.5)	2.0 ± 1.8 (91.2)
F20%	23.3 ±5.20 (0)	17.8±1.1 (23.6)	9.83 ± 1.53 (57.8)	7.00±1.50 (69.9)	2.50±1.00 (89.3)	1.0± 1.0 (95.7)
F40%	23.0 ±1.80 (0)	19.33±2.08 (15.9)	12.50 ± 2.7 (45.6)	10.00±1.50 (56.5)	5.17±0.76 (77.5)	4.5 ± 0.0 (80.4)
1F:1H10%	25.0± 2.5 (0)	19.3±2.75 (22.8)	12.3±4.07 (50.8)	9.8±2.84 (60.8)	4.67±1.6 (81.3)	2.0±1.73 (92.0)
1F:1H20%	24.5± 2.18 (0)	20.0±2.29 (18.4)	13.0±6.26 (46.9)	10.67±5.0 (56.4)	5.67±2.8 (76.9)	2.0±3.46 (91.8)
1F:1H40%	26.8 ±3.51 (0)	22.33±1.26 (16.7)	15.67±5.5 (41.5)	12.67±4.9 (52.7)	7.33±2.8 (72.6)	7.0±3.12 (73.9)
1F:2H10%	25.7± 1.04 (0)	21.50±1.50 (16.3)	12.50±3.50 (51.4)	9.67±3.01 (62.4)	5.83±2.08 (77.3)	3.17±3.25 (87.7)
1F:2H20%	24.33±0.58 (0)	20.0±1.0 (17.8)	12.50±4.33 (48.6)	10.33±4.07 (57.5)	5.67±2.57 (76.7)	1.50±2.60 (93.8)
1F:2H40%	26.8± 0.76 (0)	23.17±0.76 (13.5)	16.17±5.39 (39.7)	13.33±5.11 (50.3)	8.17±2.75 (69.5)	6.83±2.08 (74.5)
1F:4H10%	24.3 ±1.53 (0)	20.5±2.18 (15.6)	13.00±3.28 (46.5)	10.83±2.9 (55.4)	7.17±2.36 (70.5)	2.00±3.46 (91.8)
1F:4H20%	23.2± 1.66 (0)	19.33±2.93 (16.7)	11.83±3.40 (49.0)	9.67±2.75 (58.3)	6.17±2.36 (73.4)	2.33±3.21 (90.0)
1F:4H40%	23.8 1.04 (0)	19.50±1.8 (18.1	12.83±1.04 (46.1)	11.00±1.0 (53.8)	7.50±0.50 (68.5)	5.00±0.50 (79.0)
1H:2F10%	26.2 0.58 (0)	22.0±1.50 (16.0)	11.50±2.29 (56.1)	9.83±1.89 (62.5)	6.67±1.26 (74.5)	3.17±3.01 (87.9)
1H:2F20%	24.7± 1.26 (0)	21.00±1.32 (15.0)	11.67±3.75 (52.7)	9.17±2.93 (62.9)	6.67±1.89 (73.0)	3.67±2.47 (85.1)
1H:2F40%	23.0 ±0.87 (0)	20.50±0.50 (10.9)	10.33±2.36 (55.1)	8.83±1.04 (61.6)	5.50±0.50 (76.1)	4.67±1.04 (79.7)
1H:4F10%	23.7± 2.52 (0)	20.67±2.57 (12.8)	9.50±2.18 (59.9)	8.17±1.53 (65.5)	5.00±1.0 (78.9)	3.00±1.8 (87.3)
1H:4F20%	25.7 ±1.10 (0)	22.17±2.08 (13.7)	13.33±2.5 (48.1)	11.17±1.9 (56.5)	7.50±2.0 (70.8)	5.67±1.6 (77.9)
1H:4F40%	25.2 ± 2.8 (0)	21.50±2.8 (14.7)	11.5±2.29 (54.4)	9.83±2.02 (61.0)	6.83±1.26 (72.9)	1.67±2.89 (93.4)
povidone (5%)	24.2 ±0.29 (0)	19.67±1.89 (18.7)	12.00±3.04 (50.4)	10.67±3.62 (55.9)	7.50±3.04 (69.0)	3.83±1.26 (84.2)
Negative control	27.8 ±2.75 (0)	22.0±1.5 (20.9)	12.67±1.04 (54.4)	10.83±0.76 (61.0)	7.00±0.87 (74.8)	3.00±1.32 (89.2)
Shea butter	25.7 ±1.76 (0)	20.00±2.18 (22.2)	9.67±0.76 (62.4)	8.50±1.00 (66.9)	5.17±0.58 (79.9)	1.67±0.76 (93.5)

[a]Mean ± standard deviation (n = 3) of wound diameter and percentage (%) wound contraction at days of postwounding.
F: *N. biserrate*; H: *H. indicum*.

used in the current study corroborated the statement that antioxidant activity and wound-healing property coexists in many plant species.[32]

The wound healing potentials of both the plants studied are relatively lower versus a similar study conducted on the *Amaranthus spinosus*.[33] However, a relatively enhanced wound contraction induction was observed versus the *Elaeis guineensis* leaf extract study.[34]

The preliminary phytochemical analysis on the plant extracts showed the presence of flavonoids, triterpenoids, and alkaloids. These observed phytochemical constituents may be responsible for the antioxidant and wound healing activity. Flavanoids and triterpenoids are known phytoconstituents with antimicrobial properties, that are responsible for wound contraction to promote wound healing.[35] The observed wound-healing activity of these plants may be attributed to the phytoconstituents present.

CONCLUSION

The efficacy assessment performed in this study indicated 10% *H. indicum* and 20% 1:1 *H. indicum* and *N. biserrate* as the best wound healing formulations. We also have demonstrated significant pieces of evidence showing the antioxidant activity and wound healing properties of *H. indicum* and *N. biserrate*, justifying their traditional use in folkloric medicine for wound healing.

AUTHOR CONTRIBUTIONS

VYAB, AB) and DOA conceptualized and designed the research. VYAB coordinated in the collection of the plant samples, extraction, and conducted the phytochemical analysis, as well as the antioxidant activity tests. VYAB wrote the draft manuscript.

AB, DOA, and DNK were responsible for all the experimental designs and analysis on wound healing properties. AB and DOA revised the manuscript critically for important intellectual content. All authors were involved in the interpretation of data and final approval of the manuscript for publication.

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REFERENCES

1 Macioch T, Sobol E, Krakowiecki A, Mrozikiewicz-Rakowska B, Kasprowicz M, Hermanowski T. Health related quality of life in patients with diabetic foot ulceration - translation and Polish adaptation of Diabetic Foot Ulcer Scale short form. Health Qual Life Outcomes. 2017; 15(1):15.

2 Aiyer SN, Shetty AP, Kanna R, Reddy S, Rajasekaran S. Enterococcus faecalis causing delayed spondylodiscitis in a case with retained intraspinal bullet. J Spine Surg. 2016; 2(4):328–33.

3 Karlakki SL, Hamad AK, Whittall C, Graham NM, Banerjee RD, Kuiper JH. Incisional negative pressure wound therapy dressings (iNPWTd) in routine primary hip and knee arthroplasties: A randomised controlled trial. Bone Joint Res. 2016; 5(8): 328–37.

4 Ricci E, Cutting KF. Evaluating a native collagen matrix dressing in the treatment of chronic wounds of different aetiologies: A case series. J Wound Care. 2016;25(11):670–8.

5 Lullove EJ. Use of a dehydrated amniotic membrane allograft in the treatment of lower extremity wounds: A retrospective cohort study. Wounds. 2017; 29(11):346–51

6 Minniti CP, Eckman J, Sebastiani P, Steinberg MH, Ballas SK. Leg ulcers in sickle cell disease. Am J Hematol. 2010; 85(10):831–3.

7 Alavi A, Kirsner RS. Hemoglobinopathies and leg ulcers. Int J Low Extrem Wounds. 2015; 14(3): 213–6.

8 Chudobova D, Cihalova K, Guran R, Dostalova S, Smerkova K, Vesely R, et al. Influence of microbiome species in hard-to-heal wounds on disease severity and treatment duration. Braz J Infect Dis. 2015; 19(6):604–13.

9 Stanirowski PJ, Wnuk A, Cendrowski K, Sawicki W. Growth factors, silver dressings and negative pressure wound therapy in the management of hard-to-heal postoperative wounds in obstetrics and gynecology: A review. Arch Obstet Gynaecol. 2015; 292(4):757–75.

10 Mutluoglu M, Uzun G, Bennett M, Germonpre P, Smart D, Mathieu D. Poorly designed research does not help clarify the role of hyperbaric oxygen in the treatment of chronic diabetic foot ulcers. Diving Hyperb Med. 2016; 46(3):133–4.

11 Vatankhah N, Jahangiri Y, Landry GJ, Moneta GL, Azarbal AF. Effect of systemic insulin treatment on diabetic wound healing. Wound Repair Regen. 2017; 25(2):288–91

12 Anghel EL, Kim PJ, Attinger CE. A solution for complex wounds: The evidence for negative pressure wound therapy with instillation. Int Wound J. 2016; 13 (Suppl 3):19–24.

13 Rahimnejad M, Derakhshanfar S, Zhong W . Biomaterials and tissue engineering for scar management in wound care. Burns Trauma. 2017;5:4.

14 Shao W, Wu J, Wang S, Huang M, Liu X, Zhang R. Construction of silver sulfadiazine loaded chitosan composite sponges as potential wound dressings. Carbohydr Polym. 2017; 157:1963–70.

15 Sarangdhar M, Kushwaha A, Dahlquist J, Jegga A, Aronow B. Using systems biology-based analysis approaches to identify mechanistically significant adverse drug reactions: Pulmonary Complications from combined use of anti-TNFalpha agents and corticosteroids. AMIA Jt Summits Transl Sci Proc. 2013; 2013: 151–5.

16 Hoffman KB, Dimbil M, Kyle RF, Tatonetti NP, Erdman CB, Demakas A, et al. A drug safety rating system based on postmarketing costs associated with adverse events and patient outcomes. J Manag Care Spec Pharm. 2015; 21(12): 1134–43.

17 Barku VY A, Boye A, Quansah Q. Antioxidant and wound healing studies on the extracts of *Corchorus olitorius* leaf. World Essays J. 2013; 1:67–73.

18 Barku VYA, Boye A, Erzah F, Tsamenyi P. In-vitro antioxidant and wound healing properties of *Combretum dolichopetalum* Engl. & Diels (Combretaceae). J App Pharm Sci. 2016; 6 (5):185–92.

19 Oh SY, Cedergreen N, Yiannikouris A, Swamy HV, Karrow NA . Assessing interactions of binary mixtures of Penicillium

mycotoxins (PMs) by using a bovine macrophage cell line (BoMacs). Toxicol Appl Pharmacol. 2017; 318:33–40.
20 do Vale LR, Delbem AC, Arias LS, Fernandes RA, Vieira AP, Barbosa DB, et al. Differential effects of the combination of tyrosol with chlorhexidine gluconate on oral biofilms. Oral diseases. 2017; 23(4):537–41.
21 Trease GE, Evans WC. Pharmacognosy. USA: W.B. Saunders; 1996. 243–83p.
22 Sofowora A. Medical plants and traditional medicine in Africa. Ibadan, Nigeria: John Willey and Sons Ltd; 1984.
23 Wolfe K, Wu X, Liu RH. Antioxidant activity of apple peels. J Agric Food Chem. 2003;51:609–14.
24 Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 1999; 64:555–9
25 Afroze F, Hossain M T, Proximate analysis, phytochemical screening and antioxidant activity of *Psidium guajava* leaves growing in coastal area of Bangladesh. World J Pharm Pharma Sci. 2015; 4(5):140–51.
26 Chakraborthy GS. Free radical scavenging activity of *Costus speciosus* leaves. Indian J Pharm Educ Res. 2009; 43:96–8.
27 Brand-Williams W, Cuvelier ME, Berset C. Use of a free-radical method to evaluate antioxidant activity. LWT Food Sci Technol 1995; 28(1):25–30.
28 Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181:1199–200.
29 Belachew TF, Asrade S, Geta M, Fentahun E. In vivo evaluation of wound healing and anti-inflammatory activity of 80% methanol crude flower extract of Hagenia abyssinica (Bruce) J.F. Gmel in mice. Evid Based Complement Alternat Med. 2020; 2020:9645792.
30 Olugbami JO, Gbadegesin MA, Odunola OA. In vitro evaluation of the antioxidant potential, phenolic and flavonoid contents of the stem bark ethanol extract of *Anogeissus leiocarpus*. Afr J Med Med Sci, 2014; 43(Suppl 1):101–9.
31 Shiddhuraju P, Mohan PS, Becker K. Studies on antioxidant activity of Indian Laburnum (Cassia fistula L.): A preliminary assessment of crude extracts from stem back leaves, flower and fruit pulp. Food Chem. 2002; 79:61–7.
32 Reddy JS, Rao PR, Reddy MS. Wound healing effects of *Heliotropium indicum*, *Plumbago zeylanicum* and *Acalypha indica* in rats. J Ethnopharmaco. 2002;79(2):249–51.
33 Süntar I, Akkol EK, Nahar L, Sarker SD. Wound healing and antioxidant properties: Do they coexist in plants? Free Rad Antiox. 2012; 2(2):1–7.
34 Barku VYA, Boye A, Adinortey C, Bobie-Ansah G, Kwame-Femi EK. Preliminary phytochemical screening, antimicrobial examination and wound healing potential of the root extract of *amaranthus spinosus*. J Basic & Appl Sci. 2014; 1(2): 84–95.
35 Sasidharan S, Logeswaran S, Latha LY. Wound healing activity of *Elaeis guineensis* leaf extract ointment. Int J Mol Sci, 2012; 13:336–47.