Dynamic Analysis and Performance Assessment of Medicinal Herbs Active in Wound Healing Activities

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Abstract:This paper confers about detailed examination of the pace involved in wound healing process by disparate medicinal herbs. Wounds are the consequences of bruises to membrane of skin that distort the mushy tissues. Wound healing can be described exactly as a lively process which emerges in reconstruction of anatomical progression and purpose. In this paper, definite number of medicinal herbs available in various regions of India was investigated for its wound healing activities. The aqueous solution of extracts is extricated from selected herbs such as AllumCepa, Alternantherasessilis, Mussaendafrondosa, Aristolochiabracteata, Aspila Africana, HeliotropiumIndicum, Mimosa pudica, Tecomariacapensis, Ageratum conyzoides, Lantana camara, Moringaoleifera, Curcuma longa and MimusopsElengi. The concotion of all these herbs manifested the chief inhibition of DPPH radical at 91–93%, after 20 min of incubation at a test concentration level of 50lg/ml. For the corresponding trihydroxybenzoic acid (gallic acid) equivalents, the highest total antioxidant capacity is seen in Moringaoleifera. Relatively all the designated medicinal herbs show greater wound healing performance with better antioxidant capacity. Additionally, well-designed trials with adequate features of the contents of Curcuma longa should be carried out to determine the effectiveness of the respective herb.

Index Terms-Wound healing; Antioxidant; DPPH; Moringaoleifera; Curcuma longa.

Introduction

It is a valuable run for researchers to furnish innumerable features regarding the wound mitigating herbs and invulnerable evolution of secured and efficacious and globally acquired herbal drugs for pierces and wounds. Ayurveda - The traditional Indian medication narrates numerous herbs with anti-aging as well as wound healing properties.[1] proposes a review on medicinal plants with the potential wound healing activity also it confers the immense potential of AllumCepa Linn. (Liliaceae) and the paper proves that the particular herb is anti diabetic, Anti oxidant, anti thromboic, anti hypertensive activities. Also the paper focuses Alternantherasessilis Linn.(Amaranthaceae) which has the owner ship of chemical constituents like α & β spinosterols. The leaves used in eye disorders, laceration & wounds, antitoxin for snake bite & scorpion sting and skin diseases. [2] review the current aspects of wound healing agents from medicinal plants. The author protrudes through classification of wounds, factors affecting wound healing, phases of wound healing, and variables worned in evaluating the wound healing activity and concludes with a qualitative review about heterogeneous wound healing herbs. Traditionally used medicinal plants for Wound Healing activities in unique sites of maharastra is discussed in [3] that incorporates the medicinal stuffs and preparation techniques associated in the particular activities. [4] Tends to explore the medicinal plants of India by approaching the Ethnopharmacological approaches to wound healing. This paper depicts the ayurvedic remedies of natural herbs and corresponding Ethnopharmacological validation. A. Shuklaet. al, in [5] proposes the wound healing venture of asiaticoside secluded from a remarkable herb, Centellaasiatica. Analysis is made as delayed type wound healing process. The outcomes of corresponding wound healing process specify that asiaticoside reveals significant wound healing activity in habitual as well as delayed healing models. Medicines and preparation of herb extraction and wound healing assessment were performed in order to quantify the performance of the asiaticoside. Antimicrobial activity of opted Peruvian medicinal plants were discussed in [6] by expressing their organic and biologically active compounds by associated studies related to Phyto-chemistry. Antimicrobial study of those herbs has segregated various extricate that are robust against several pathogenic micro-organisms through ethno medical use. The clinical applications of chemotherapy drugs (taxol and etoposide) have assisted to revive an interest in higher plants as origin of brand new drugs. Despite the belief that majority of clinical drugs are synthetic in origin [7]. By this review the author quits the discussion with a strong pattern of belief that massive arsenal of clinical agents evolved by pharmaceutical industry has been tamed and herbal remedies have manifested to be favored as substitute or a complementary for related treatments. To treat infectious diseases that are screened for antibacterial activity against harmful bacteria, toxicity venture has been over viewed in [8]. The treatment was undertaken using

Ethanolicextracts of selected plant species used by Yemeni traditional healers Prashanth et.al in [9] conducts a review for antibacterial and anti fungal agents on some selected Indian medicinal herbs. In this study, screening of the test organism is performed and the extracts of same herbs exhibited significant premises that support indigenous ply in therapy of some diseases that expels its opposition over a large variety of heterogeneity as wide view of antimicrobial representatives. In [10] a review of specified range of patents on herbal products refined for wound therapy is furnished and the study intends to ignite the recent technical advancement in herbal medicines.

Assessment of Medicinal

Activity

The ethnobotanical analysis of medicinal uses of herbs led us to investigate some edible and wound- healing herbs for antioxidant activity, using variant assay methods. In[11] the author conducts a ethnobotanical review of different medicinal plants for investigating the wound healing activity using vitro DPPH_ and phosphomolybdenum assay methods. The authors investigated 19 plants in a specified region in which the aqueous ethanol extracts of Plucheaarabica, Beciumdhofarense, Pulicariacrispa, Allophylusrubifoliu, Acacia senegal,Oleaeuropaea showed the best inhibition of DPPH sweep at 89–93%, from a instant of 15 min after incubation at a test concentration of 50 lg/ml.

The highest total antioxidant capacity as Gallic acid equivalents of 1790, 910, 810, 890 mg/g of ethanol extracts were obtained for Peperomiacrispa, Oleaeuropaea and is active against Pseudomonas aeruginosa and Staphylococcus aureus.

A. Material stuffs and routines II. A.1 Selection of Chemicals

Analytical grade stuffs were used in order to acquire the optimized result in extracts. A phenolic antioxidant tert-butylhydroxyanisole (BHA) and tertbutylhydroquinone (TBHA) an aromatic organic compound which is a phenol type additive were opted initially. It is an imitated derivate of hydroquinone, substituted with a tert-butyl group and a- tocopherol [12].An organic Chemical compound such as an 2,2-diphenyl-1-picrylhydrazylradical (DPPH) [13], trihydroxybenzoic acid (a type of phenolic acid) , n-propyl gallate (ester formed by the condensation of gallic acid and propanol) [14], All these chemical stuffs were lay hold for bearing the performance enquiry over the medicinal herbs. Optical densities were noted with the aid of atomic absorption visible spectrophotometer and solvents for dissolving those extract were also re-distilled before conducting the test. In the next stage of testing process Wound-healing herbs were screened out for antimicrobial activities in opposition to fight against Escherichia coli, Candida albicans, Staphylococcus aureus and Pseudomonas aeruginosa.

A.2. Plant Materials Preparation

Plant collections were made during appropraite time periods. Different parts of the plants were air-dried at well-ventilated hot room at a temperature ranging from 45°C ~ 55°C. After the thermal treatment the dried plant specimen is pulverized to very finest particle ranging at size of 10µm. One hundred grams of each pulverized plant material was formally extracted by maceration in which, is the guiding force, with higher temperatures encouraging more extraction of phenols from the herbal specimen materials. During macreation the apted plant specimen powder is soaked with 1.95 litre of chloroform solution integrately containing 20% of acetone, 15% of dichloromethane and 10% of methanol for more than three weeks (apprx. 25days) and extracted further with the identical quantity of 20% aqueous ethanol for additional duratin of threeweeks. The respective extracts were concentrated and the solvents were quantified based on the solution concentraor and the level of concentration is deduced. The chloroform and the acetone residues and ethanol extracts were weighed and cached in sealed vials and stowed in freezer till the end of experiment.

B Medicinal Activity

B.1. DPPH Scavenging Activity

The antioxidant activity of the plant extract was estimated using a slightly altered DPPH radical scavenging protocol as reported by [15] For a typical reaction, 2 ml of 100 lM DPPH solution is hold and blended in ethanol and accompanied with 2 ml of 100 lg/ml of herbal extract. The efficacious test concentrations of DPPH and the herbal extract were therefore 50lM and 50lg/ml, respectively. The blended reaction mixure was incubated to certain warmer temperature by keeping the concotion in the dark for more than 25 min. After completing this span of instant the optical density of the respective concotion was recorded at 491 nm against the blank. Again 2 ml of DPPH solution is holded and blended in association with 2ml of ethanol and the optical density of the respective concotion was recorded after 20 min. The assay was carried out and has been sparingly ordered and further it can be duplicated. The diminishing optical density of DPPH solution on inclusion of the test specimens in association to control was handed-down to compute the antioxidant activity, as percentage inhibition (%IP). Extracts whose %IP values were above 70% at 50 lg/ml were serially diluted to give concentrations between 0 and 30 lg/ml. The DPPH values for ethanol extracts of opted chemicals is depicted in table 1

Table 1. DPPH values for ethanol extracts of opted chemicals

Chemical Stuffs	Antioxidant activity (DPPH assay)
Tert-butylhydroxyanisole (BHA)	5.4±0.7
Tertbutylhydroquinone (TBHA)	2.0±0.7
Trihydroxybenzoic acid	0.9±3.1
n-propyl gallate	0.9±3.9

B.2. Antimicrobial Activity

Antimicrobial evaluation trial was executed by means of the agar and broth dilution method by . Through which the lowest concentration of assayed antimicrobial extract under defined test conditions were determined [16]. The test specimen of 20 mg is quantified in the plates and it is dissolved in 0.2 ml of dimethyl sulfoxide. An pinch of the dissolved solution (apprx. 0.3 ml) or its heterogeneous mixtre suspension was fetched into a Petridish and assored with 50 ml of germ-free agar to test extract at 500 lg/ml. The existinf 0.1 ml solution was thinned with an aid of similar quantity of dimethyl sulfoxide and 0.15 ml of emerging solution was disparated with 30ml of germ-free agar in a Petridish to test at 200lg/ml. Using 2x serial dilutions a Lower test concentrations of the solutions were obtained. The salty impregnate solution was separately used to streak the test samples, negative and positive controls by using solutions such as gentamycin and ketoconazole as test standards. The concentration of test specimen that inhibited the growth of each organism after incubation at 41°C for 72hours was recorded.

Wound Healing Analysis ofHerbs

The antioxidant activity of the plant extract was estimated using DPPH radical scavenging protocol. For a similar reaction, 2 ml of 100 lM DPPH solution is hold and blended in ethanol and combined with 2 ml of 100lg/ml of herbal extract. The efficacious test concentrations of DPPH and the herbal extract were therefore 50lM and 50lg/ml, respectively. The reaction mixture was incubated to temperature range of 45°C by keeping the mixed herbal concotion in the dark for more than 25 min. After completing the duration of incubation the optical density of the respective concotion was recorded at 500 nm (apprx.) against the blank. Again 2 ml of DPPH solution is hold and blended in association with 2ml of ethanol and the optical density of the respective herbal concotion was recorded after 30 min. The assay was carried out and has been sparingly ordered and further it can be duplicated. The diminishing optical density of DPPH solution on inclusion of the test specimens in association to control was handed-down to compute the antioxidant activity. The DPPH Scavenging and antimicrobial activity of various plant extracts opted for scrutinization is listed in the table 2.

Table 2. Scavenging and Antimicrobial activity of opted plant extracts

Herbal Plant extracts	Antioxidant activity (DPPH assay)		Escherichia coli	Candida albicans	Staphylococcus aureus	Pseudomonas aeruginosa
Allum Cepa	13.56±0.5	a	+++	-	+++	+++
		b	+++	+++	+++	+++
Alternanthera sessilis	14.56± 0.4	a	+++	+++	+++	-
		b	+++	+++	+++	+++
Mussaenda frondosa	16.63± 0.3	a	+++	+++	+++	-
		b	+++	-	+++	+++
Aristolochia bracteata	11.56± 0.4	a	+++	+++	+++	+++
		b	+++	-	+++	+++
Aspila Africana	13.26± 0.4	a	+++	+++	-	+++
		b	+++	+++	+++	-
Heliotropium Indicum	15.54± 0.6	a	+++	-	+++	+++
Mimosa pudica	17.56± 0.1	b	+++	+++	-	+++
Tecomaria capensis	15.63± 0.2	a	+++	+++	+++	+++
		b	+++	+++	+++	+++
Ageratum conyzoides	12.13± 0.3	a	+++	-	+++	+++
		b	+++	+++	+++	+++
Lantana camara	17.24± 0.5	a	+++	+++	+++	-
		b	+++	+++	-	+++
Moringa oleifera	19.89± 0.6	a	+++	+++	+++	+++
		b	+++	+++	+++	+++
Curcuma longa	19.87± 0.6	a	+++	+++	+++	+++
		b	+++	+++	+++	+++
Mimusops Elengi	16.51± 0.5	a	+++	+++	-	+++
		b	+++	+++	+++	-

For the opted Gallic acid equivalents, Moringaoleifera shows the highest total antioxidant capacity in the group. Relatively all the picked medicinal herbs show greater wound healing performance with better antioxidant capacity. Additionally, there is an equated member of the group with adequate features of antioxidant and antimicrobial characteristics, Curcuma longa. The performance assessment of Curcuma longa should be carried out to determine the effectiveness of the respective herb.

Results and Discussions

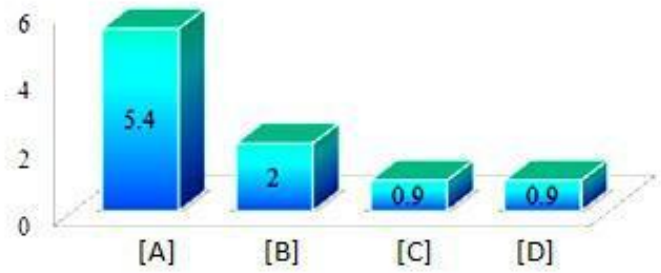

Figure 1. DPPH IC50 Worth for selected Chemical Stuffs

[A] - Tert-butylhydroxyanisole (BHA)
[B] - Tertbutylhydroquinone (TBHA)
[C] - Trihydroxybenzoic acid
[D] - n-propyl gallate

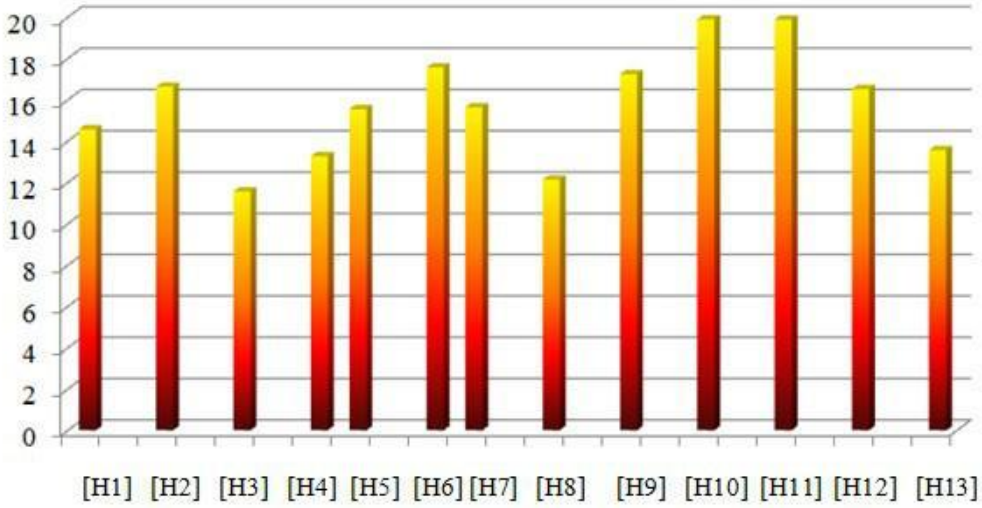

Figure 2. DPPH Values for of opted plant extracts

[H1] - Alternantherasessilis
[H2] - Mussaendafrondosa
[H3] - Aristolochiabracteata
[H4] - Aspila Africana
[H5] - HeliotropiumIndicum
[H6] - Mimosa pudica
[H7] - Tecomariacapensis
[H8] - Ageratum conyzoides
[H9] - Lantana camara
[H10] - Moringaoleifera
[H11] - Curcuma longa
[H12] - MimusopsElengi
[H13] - AllumCepa

Conclusion

Prevailing results of the selected herbs demonstrates that all of them were used in a heterogeneity dosage forms, that reflects a potent inhigher sprinting of wound healing process and tend toelevate the rate ofsuccessful accomplishment ofhealing process. Asfar as we know, this initiated study concerning the free radical scavenging capacity and antimicrobial activity of the marginal knownplants that are endemic to India. In the experiments conducted over variant medicinal herbs investigated for wound healing purpose, Curcuma longa appears to be the most promising because it combines optimal antioxidant properties with remarkable antimicrobial activities. At the end of conclusion, our resultsrecommends that the extracts and concoction of some edible or wound-healing medicinal herbs investigated have radical scavenging capacity.

References

[1] Chah, K. F., et al. "Antibacterial and wound healing properties of methanolic extracts of some Nigerian medicinal plants." Journal of Ethnopharmacology104.1 (2006): 164-167.

[2] Shukla, A., et al. "In vitro and in vivo wound healing activity of asiaticoside isolated from Centellaasiatica." Journal of ethnopharmacology 65.1 (1999): 1-11.

[3] Rojas, Rosario, et al. "Antimicrobial activity of selected Peruvian medicinal plants." Journal of ethnopharmacology 88.2 (2003): 199-204.

[4] Phillipson, J. David. "Phytochemistry and medicinal plants." Phytochemistry56.3 (2001): 237-243.

[5] Ali, NA Awadh, et al. "Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities." Journal of Ethnopharmacology 74.2 (2001): 173-179.

[6] Khalil, Enam A., Fatma U. Afifi, and Maysa Al-Hussaini. "Evaluation of the wound healing effect of some Jordanian traditional medicinal plants formulated in Pluronic F127 using mice (Musmusculus)." Journal of Ethnopharmacology109.1 (2007): 104-112.

[7] Kumar, V. Prashanth, et al. "Search for antibacterial and antifungal agents from selected Indian medicinal plants." Journal of Ethnopharmacology 107.2 (2006): 182-188.

[8] Nagori, BadriPrakash, and RenuSolanki. "Role of medicinal plants in wound healing." Research Journal of Medicinal Plant 5.4 (2011): 392-405.

[9] Okwu, D. E., and C. Josiah. "Evaluation of the chemical composition of two Nigerian medicinal plants." (2006).

[10]Di Stasi, L. C., et al. "Medicinal plants popularly used in the Brazilian Tropical Atlantic Forest." Fitoterapia 73.1 (2002): 69-91.

[11]Marwah, Ruchi G., et al. "Antioxidant capacity of some edible and wound healing plants in Oman." Food chemistry 101.2 (2007): 465-470.

[12]Okubo, T., et al. "Cell death induced by the phenolic antioxidant tert-butylhydroquinone and its metabolite tert-butylquinone in human monocytic leukemia U937 cells." Food and chemical toxicology 41.5 (2003): 679-688.

[13]Sharma, Om P., and Tej K. Bhat. "DPPH antioxidant assay revisited." Food chemistry 113.4 (2009): 1202-1205.

[14]Becker, Lillian. "Final report on the amended safety assessment of propyl gallate." International Journal of Toxicology 26 (2007): 89-118

[15]Lu, Yinrong, and L. Yeap Foo. "Antioxidant and radical scavenging activities of polyphenols from apple pomace." Food chemistry 68.1 (2000): 81-85.

[16]Wiegand, Irith, Kai Hilpert, and Robert EW Hancock. "Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances." Nature protocols 3.2 (2008): 163-175.