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ANTIOXIDANT ACTIVITY OF *CASSIA AURICULATA* AND *CASSIA FISTULA* EXTRACT ALONG WITH WOUND HEALING ACTIVITY OF ITS POLYHERBAL FORMULATION

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ABSTRACT: Due to emergent concerns about unhealthy consequences of chemicals in the health industry, the interest towards natural and herbal substances has been growing every day. In this study, the antioxidant effect of Cassia *fistula* [CFF] and *Cassia auriculata* [CAF] extract was evaluated. Also discover wound healing activity of polyherbal formulation (CFF and CAF). The antioxidant activity of the extract was evaluated by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity. Total phenolic content (TPC) was determined to screen the prepared extracts by using the Folin-Ciocalteu phenol reagent method. The polyherbal formulation (CFF and CAF) using the excision wound model. The CFF and CAF extracts showed variable degrees of antioxidant activity. The formulated gel accelerates the wound healing process which may be due to enhancing the cellular defense mechanisms, proliferation, suppression of inflammation, and contraction of the collagen tissue and could be delayed by reactive oxygen species or microbial infection. The results suggest that extracts have antioxidant properties, which may be a potentially promising agent and favorable for wound healing, and this plant extract used in polyherbal formulation may be useful in the management of abnormal healing.

INTRODUCTION: Wound healing is the natural process of repair that follows injury to the skin and other soft tissues. It is an interaction of the complex cascade of cellular and biochemical actions healing to the restoration of structural and functional integrity with the recovery of the strength of injured tissues [1].

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Healing involves continuous cell-cell interaction and cell-matrix interactions that allow the process to continue in different overlapping phases, which include inflammation, wound contraction, re-epithelialization, tissue remodeling, and formation of granulation tissue with angiogenesis [2]. These events are controlled by several mediators, including platelets, inflammatory cells, cytokines, growth factors, and matrix metalloproteinases and their inhibitors [3].

Numerous factors such as microbial infection, necrotic tissue, and interference with blood supply, lymphatic blockage, oxidative stress and disease condition such as diabetes delay the wound healing

process. The reactive oxygen species (ROS) are harmful to the wound healing process due to the destructive effects on cells and tissues. Free-radical-scavenging enzymes (FRSE) are a cytoprotective enzyme group that has a vital role in the reduction, deactivation, and removal of ROS as well as regulating the wound healing process [4]. However, if the above factors may be altered by any agent, an augmented healing rate could be achieved [5]. Nevertheless, wound healing can have severe complications that invoke high costs for therapy. Therefore, it is necessary to develop more efficient methods for improving wound healing and reduce the cost involved [6].

Plants are an abundant source of phytochemicals, which could have wound healing and antioxidant properties. Several indigenous drugs have been described in Indian folklore medicine for the management of cuts, bruises, burns, and wounds. Moreover, the phytomedicines for wound healing are cheap, well-tolerated and affordable and supposedly effective and nontoxic as hypersensitive reactions are rarely encountered with the use of these agents [7].

Cassia is a native medicinal plant genus, in which CFF has large biodiversity in north India and CAF in south India. CFF is a rapidly medium-sized, deciduous tree that is now widely cultivated worldwide as an ornamental tree for its beautiful, attractive yellow flowers. CFF also is known as a golden shower, and Amaltash belongs to the family Fabaceae. These plants are consisting of the various active principles of therapeutic value and possessing wide biological activity. The root is prescribed as a tonic, febrifuge, astringent, and strong purgative [8].

CAF, commonly known as Tanners Cassia, also known as 'Taroda' in Hindi is a shrub that belongs to the Fabaceae family is import to tanner as well as workers in iron and well known for its contribution in Ayurveda as Avarai Panchaga Chooranam [9] and Kalpa Herbal tea. The plant has been accounted to possess antipyretic [10], hepatoprotective, antidiabetic, antiperoxidative and antihyperglycaemic [11,] and microbicidal activity [12]. The present study was aimed to assess the antioxidant and wound healing activity of extract CFF and CAF used in traditional Indian medicine.

MATERIALS AND METHODS:

Plant Material: The roots of *Cassia auriculata* Linn and *Cassia fistula* Linn were collected from the fields of Walgaon Road, Amravati (Maharashtra), and interiors of Bhopal (Madhya Pradesh), respectively. Both the plant have been authenticated by Safia College of Science, Bhopal, (Madhya Pradesh), and were given the voucher specimen number 159/Bot/Safia/2010 (*Cassia auriculata* Linn.) and 160/Bot/Safia/2010 (*Cassia fistula* Linn.).

Extraction of Drugs: The authenticated plant material (root) was dried in the shade, powdered, and used for extraction. The extraction was carried out with the help of water by decoction method at 40 ºC ± 5 ºC. Then this aqueous extract was filtered, and ethanol was added slowly into this aqueous liquid extract to precipitate out polysaccharides. Then the filtrate was evaporated to $1/4^{th}$ of the total volume. Further, it was successively extracted with ethyl acetate. Then the ethyl acetate extract was acidified with 0.1 N HCl to increase the yield of the extract. Then this fraction was evaporated to get precipitate which was then dissolved in methanol and evaporated slowly to get crystalline powder. The obtained powder was purified using column chromatography by solvent by their polarity.

Evaluation Parameters for the Extract:

Determination of Extractive Value: A total of ten gm. of the powdered root was extracted with 100ml solvent using Soxhlet extraction apparatus. The percentage yield of each extract was determined.

***In-vitro* Antioxidant Assay Method:** The phenolic compounds might be the essential plant material and could, therefore, be a natural source of antioxidants [13]. The high scavenging property of plant extract may be due to hydroxyl groups present in the phenolic compounds. Henceforth total phenolic content of the prepared extracts was determined to screen the bioactive extract.

Determination of Total Phenolic Content (TPC): The total phenolics in extracts were determined according to Folin- Ciocalteu procedure of Singleton and Rossi [14]. Gallic acid was used as a standard, and the total phenolic contents were expressed as mg/g gallic acid equivalent (GAE).

Test mixture consists of one ml of extract solution (1mg/ml), 0.5ml of Folin-Ciocalteau reagent, and five ml of distilled water. The mixture incubated at room temperature for ten min. Then 1.5 ml of anhydrous sodium carbonate solution (10% w/v) was added, and the final volume made up to ten ml. The final mixture was allowed to stand at room temperature for 30 min. The absorbance measured at 760 nm using a UV-Vis spectrophotometer. The experiment was carried out in triplicate.

***In-vitro* Antioxidants Activity:**

DPPH Free Radical Scavenging Activity: The antioxidant activity of the plant extract was estimated using a slight modification of the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) radical scavenging protocol given by Chen *et al*. [15] For DPPH radical scavenging activity, different concentrations of plant extract and different concentration of Ascorbic acid were prepared. Afterward, these dilutions were mixed with 0.5 ml DPPH solution (4mg in 100 ml methanol) and incubated at room temperature for 30 mins in dark conditions. After incubation, the absorbance was noted at 517 nm using methanol as a blank. Percentage inhibition was calculated using the following formulae:

% inhibition = Absorbance (control) - Absorbance (test) × 100 / Absorbance (control)

Polyherbal Gel Formulation: Carbopol 934 was dispersed in 50 ml of distilled water with constant stirring. The required quantity of plant extracts was mixed in 20 ml, and components were mixed properly to the Carbopol 934 gel with constant stirring and volume was made up to 100 ml, and triethanolamine was added dropwise to the formulation for adjustment of required skin pH (6.8-7) and obtained the gel at required consistency. The same method was followed for the preparation of the control sample without adding any extract [16].

TABLE 1: FORMULAE FOR GEL PREPARATION

S. no.	Ingredient	Formulation (weight)		
		F1	F2	F3
1	Carbopol-934	3 gm	3 gm	3 gm
2	CAF	10 %	--	--
3	CFF	--	10%	--
4	CAF+CFF (1+1)	--	--	10%
5	Purified water	100 mL	100 mL	100 mL
6	Triethonolamine	QS to neutralize gel base	QS to neutralize gel base	QS to neutralize gel base

Evaluation Parameters for the Polyherbal Formulation: [17, 18]

Measurement of pH: The pH of the gel was measured by using a pH meter.

Determination of Homogeneity: All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container. They were tested for their appearance and presence of any aggregates.

Determination of Viscosity: The viscosity of the gel was measured by using Brookfield viscometer with the spindle.

Determination of Color: It was done with naked eyes against a white background.

***In-vivo* Pharmacological Activity for Polyherbal Gel Formulation:**

Animals: Healthy Wistar albino rats of either sex weighing 150-250 gm. were used for the study. All experimental and housing conditions for animals were maintained as per CPCSEA guidelines. Animals were provided standard feeding pellets (Golden feeds, New Delhi) and water *ad libitum*. The temperature was maintained at 22±2 °C, with a light and dark cycle of 12:12 h. The animals have been transferred to the laboratory for at least one hr. before the experiment for proper acclimatization. The experiments were performed during the day (08:00-16:00 h). All animal experiments were conducted with the prior permission of the Institutional animal ethical committee (IAEC) of PBRI (Regd No. 1283/c/09/CPCSEA).

Wound Healing Activity:

Excision Wound Model: The back of each animal was shaved and prepared after washing with spirit. An area of two sq.cm as described by Bhat *et al*., [19] was defined with a marker on the shaven back of the animals. The marked circular area was excised with its full thickness using a surgical sterile blade and scissors under phenobarbitone anesthesia. The Control/ formulations were applied to the wounded rats of the respective groups three times a day. The wounded rats of the first group were used as the baseline control for all the formulations. The application was repeated for 16 days post-operatively. The wound contractions were measured as the percentage of wound reduction in

the wound area for every four days. The progressive wound area reduction was monitored periodically by tracing the wound margin on paper, and the area was measured using graph paper. The wound size reduction was calculated by the formula:-

Wound contraction% = (difference in the area of the wound in mm^2 between the initial and on a particular post-operative day) × 100 / area of the wound in mm^2 immediately after the wound excision.

Statistical Analysis: The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by the Holm-Sidak test and Bonferroni t-test. The values were expressed as mean ± SD, and $P<0.05$ was considered significant.

RESULTS AND DISCUSSION:

Total Phenolic Content: In a flavonoid-rich extract of both plants, total phenol content was also estimated. For total phenol content estimation (TPC) standard curve of Gallic acid was used and estimated as Gallic acid equivalent (GAE). Total Phenol Content in *Cassia auriculata* and *Cassia fistula* was found to be 67.32 and 63.84 µg/mg GAE, respectively.

TABLE 2: STANDARD CURVE OF GALLIC ACID

S. no.	Conc. (µg/mL)	Absorbance	Line of regression and R_2
1	10	0.1098	
2	20	0.1763	
3	30	0.2468	Y=0.005x + 0.065
4	40	0.2981	and R_2 = 0.976
5	50	0.3258	

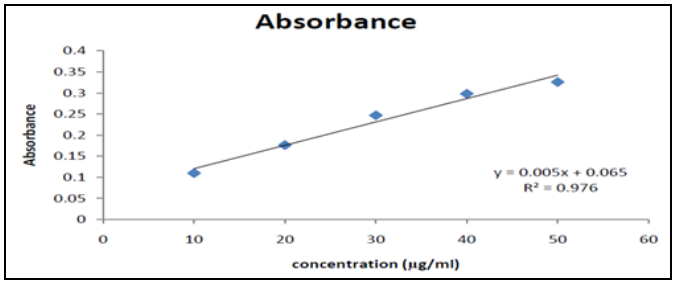

FIG. 1: STANDARD CURVE OF GALLIC ACID

TABLE 3: TOTAL PHENOLIC CONTENT

S. no.	Test sample	Absorbance	Total phenol content (GAE) µg/mL
1	CAF	0.4016	67.32
2	CFF	0.3842	63.84

DPPH Free Radical Scavenging Activity: Antioxidant potential of the flavonoid-rich extract was ascertained by an effect on percentage inhibition of DPPH free radicals. DPPH assay was done by the concentration required for 50% inhibition (IC_{50}). Flavonoids-rich extract of *Cassia auriculata* showed a significant effect on DPPH free radicals. In the concentration range of 10 µg/ml to 100 µg/ml, the goodness of fit for a line of regression was good with R_2 = 0.976. IC_{50} was found to be 60.97µg/ml. In the DPPH assay effect of flavonoids, a rich extract of *Cassia fistula* was not significantly different as compared to flavonoids rich extract of *Cassia auriculata*. IC_{50} in DPPH assay was 62.19µg/ml for extract.

TABLE 4: EFFECT OF CAF IN DPPH FREE RADICAL SCAVENGING ASSAY

S. no.	Conc. (µg/mL)	% inhibition	IC_{50}
1	10	21.46	
2	20	28.67	
3	40	32.25	60.977
4	60	48.56	
5	80	64.24	
6	100	72.5	

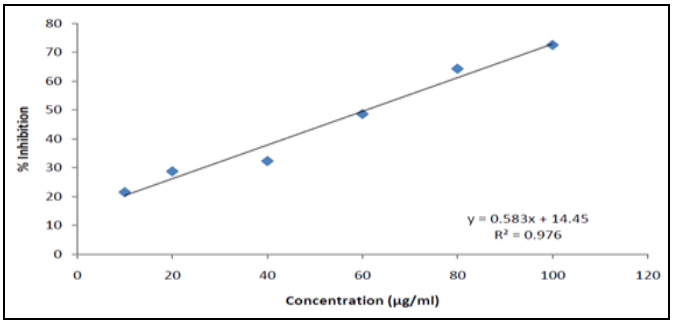

FIG. 2: EFFECT OF CAF IN DPPH FREE RADICAL SCAVENGING ASSAY

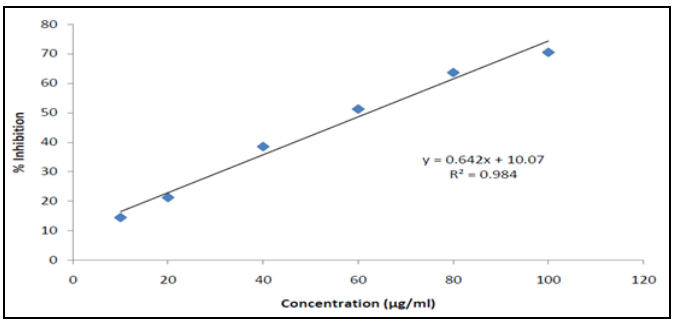

FIG. 3: EFFECT OF CFF IN DPPH FREE RADICAL SCAVENGING ASSAY

TABLE 5: EFFECT OF CFF IN DPPH FREE RADICAL SCAVENGING ASSAY

S. no.	Conc. (µg/mL)	% inhibition	IC_{50}
1	10	14.5	
2	20	21.26	
3	40	38.55	62.196
4	60	51.26	
5	80	63.62	
6	100	70.5	

Evaluation of Polyherbal Gel Formulations:

TABLE 6: EVALUATION OF POLYHERBAL GEL FORMULATIONS

S. no.	Treatment	% Wound Contraction (Day)			
		4	8	12	16
1	Control (gel base)	11.00±2.10	23.67±2.88	45.83±2.64	59.17±5.19
2	F1	14.33±2.25	32.17±2.14[ab]	60.67±4.89[a]	77.83±5.78[b]
3	F2	13.83±1.60	30.17±3.43[ab]	58.17±3.19 [ab]	70.00±5.73[ac]
4	F3	15.17±1.33	32.83±2.64[ab]	61.00±1.90 [ab]	79.17±3.31[b]
5	Standard Cream	16.17±2.04	40.00±4.05	68.33±2.16[b]	86.33±5.32[b]

All Data presented in mean ± SD, [a] P<0.05 as compared to Standard cream treated a group, [b] P<0.05 as compared to gel base treated a group, [c] P<0.05 as compared to F3 treated a group

The flavonoid-rich extract had antimicrobial and antioxidant property they were incorporated in a gel base for topical use. Basic consideration was used in cancerous wounds. Carbopo l 934 was used as a gel base and Triethanolamine as a neutralizing agent for pH changes due to Carbopol. Ten % extract formulations were prepared in which F1 consisted of CAF (flavonoids rich extract of CAF*)*, F2 of CFF (flavonoids rich extract of CFF*)*, and F3 of CAF and CFF in equal proportion. The formulated gel accelerates the wound healing process which may be due to enhancing the cellular defense mechanisms, proliferation, suppression of inflammation, and contraction of the collagen tissue and could be delayed by reactive oxygen species or microbial infection [16].

The wound healing property of extract was ascertained by percentage contraction of the wound. The observation was done on the fourth, eighth, 12th, and 16th day. It was observed that on fourth -day formulation one and two were not having any significant effect on wound contraction as compared to the control group, but different formulation which was having an equal proportion of both extracts showed a significant effect (P<0.05) as compared to control group. Thus it could be considered that components present in both extracts, when combined in equal proportion, would be giving a synergistic effect when combined in equal proportion. Afterward, it was observed that formulation F1, F2, and F3 showed a better effect as a wound healing activity. On the eighth, 12^{th,} and 16th day, all formulations showed a significant effect (P<0.05) as compared to the control group. The study results demonstrated that F3 was better than F2 and F1, and F2 was better than F1. Flavonoids have been possessing the antioxidant potential and free radical scavenging effect, which is believed to be one of the important components of wound healing. Bioflavonoids are thought to benefit connective tissue by binding to elastin, preventing its degradation by elastases [20]. Many studies have shown that antimicrobial activities of plants can also be attributed to their flavonoids content [21-22]; hence, they are helpful in the prevention of wound infection. Most of the delay in wound healing is due to insufficient or excessive fibroblast activity. Thus, inhibition of fibroblast growth by flavonoids such as apigenin could be beneficial for the treatment of any skin injury.

CONCLUSION: The present study concludes that the polyherbal gel of the extracts of CFF and CAF has significant wound healing activity. The better activity of polyherbal formulation could be due to the synergistic action of the plant's constituents present in the formulation. The phytoconstituents like flavonoids are known to promote the wound healing process due to their anti-oxidant properties. The investigation reveals that the wound healing activity of polyherbal formulation may be due to the combined action and presence of phytoconstituents.

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