Int. J. Pharm. Investigation, 2025; 15(1):228-236.
https://www.jpionline.org

Original Article

Determination of Antioxidant and Anti-inflammatory Potentials of Selected Therapeutic Plants Aqueous Extract and their Formulation for Wound Healing

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ABSTRACT

Background: Since ancient times, medicinal plants with antioxidant and anti-inflammatory abilities have been utilized for relieving a variety of health problems. Individual herbs, however, are inadequate to provide the necessary therapeutic effect. It will have a greater therapeutic impact with less toxicity when tailored as a multiple herb mixture in a specific ratio. In Ayurveda, this combination of herbs is known as polyherbalism. **Aim:** The current research aimed to create a polyherbal combination from extracts of *Anogeissus latifolia* (AL), *Centella asiatica* (CA), *Curcuma longa* (CL), *Glinus oppositifolius* (GO) and *Soymida febrifuga* (SF) in order to produce such a treatment. The locals in Chhattisgarh state use these chosen medicinal herbs to cure wounds. **Materials and Methods:** Spectrophotometric techniques were used in this work to assess the wound-healing capability of the extracts and their polyherbal combination. The total polyphenols were optimized using Folin's ciocalteu and aluminium chloride techniques. The antioxidant capacity was assessed using the DPPH and FRAP tests. Anti-inflammatory activity was determined using protein denaturation tests, whereas wound healing potential was determined using phenol-protein precipitation assays. **Results:** All experiments yielded significant ($p<0.05$) findings from the extracts and their combinations, however aqueous extracts and their polyherbal mixes were shown to be superior to petroleum ether and methanol extracts and their polyherbal mixtures. **Conclusion:** In conclusion, the studied polyherbal mixture is being employed for the different formulation processes as well as for further *in vitro* and *in vivo* research.

Keywords: Indian traditional medicine, Polyherbalism, Polyphenols, Antioxidants, Anti-inflammatory activity, Wound healing.

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Received: 08-07-2024;
Revised: 15-07-2024;
Accepted: 21-08-2024.

INTRODUCTION

Traditional herbal remedies are significant practices in a variety of countries and continue to be utilized across the globe as alternative and supplementary therapies for a wide variety of diseases. As a result of the secondary metabolites that plants produce, which are responsible for inducing biological changes within the body they are of tremendous significance for the health of both individuals and communities.[1-3] At the moment, there is a significant amount of interest in the investigation of medicinal plants with the goal of obtaining molecules that have a variety of pharmacological effects.[4,5] Since ancient times, India's healthcare has been based on the usage of medicinal plants and these plants are still considered the most important source of treatment. In this context, it is difficult to express the concept of polyherbalism in terms of modern standards, yet Ayurveda has its own unique approach to herbal medicine that is known as polyherbalism. The "Sarangdhar Samhita" is a piece of Ayurvedic literature that was written centuries ago and stresses the principle of synergism behind polyherbal compositions. These formulations offer a broad therapeutic spectrum, less side effects, are friendlier to the environment, are more affordable and are easily accessible.[6-8]

The potential for polyherbal formulations to speed wound healing is the one use of these medicines that has received the greatest attention. Since ancient times, traditional healing substances have played an important role in the treatment of wounds. The integration of these conventional treatments with contemporary therapeutic approaches has facilitated the creation of a variety of wound healing solutions that are more effective than their predecessors.[9,10] The active component in polyherbal compositions comes from one of a number of different medicinal plants.[8] Plants like *Arctium lappa* increased dermal ECM

DOI: 10.5530/ijpi.20251847

metabolism, which in turn reduced the appearance of wrinkles in human skin *in vivo* while the healing properties of *Calendula officinalis* have led to its inclusion in a wide variety of polyherbal preparations.[11,12]

There have been a lot of researches that have proposed different targets for these active chemicals that ultimately improve the healing process. These are mediated by numerous cascades, which include mitogenic pathways,[13] extracellular matrix synthesis routes,[14] free radical scavenging pathways,[6] atherosclerosis pathways,[15] and anti-inflammatory mechanisms.[16] Because of this, it should come as no surprise that research has focused mostly on the route that is responsible for scavenging free radicals. In light of this, preparations that have a strong antioxidant capacity make for effective healing agents.[17] This may be due to the fact that antioxidants are able to scavenge free radicals, hence reducing oxidative stress and speeding up the healing process.[18]

In the framework of polyherbalism, the present study focuses on the evaluation of the wound healing property of medicinal plants and their polyherbal mixture. These medicinal plants were selected from an ethnobotanical survey carried out in the local area of Chhattisgarh state in India. The selected medicinal plants are used by the inhabitants in the area to cure wounds and other diseases, among other conditions. A brief description of selected medicinal plants is given in the Table 1. In this study, the antioxidant activity was measured using the DPPH and FRAP assays, while the anti-inflammatory activity was measured using the Bovine Serum Albumin (BSA) and egg albumin assays. Both activities are very important for the wound healing purpose.

MATERIALS AND METHODS

Plant sample selection, collection and identification

The identification, collection and selection of medicinal plants were all done on the basis of the documented traditional uses of the plants and the demand for treating wounds in the area that was studied. Additionally, the plant parts are collected on the basis of how the inhabitants of the area utilize them. The specimens were sent to the department of botany at Guru Ghasidas Vishwavidyalaya, which is located in Bilaspur, Chhattisgarh, India. This was done so that the identification voucher could be created. Table 1 includes the voucher number among its entries.

Chemical and Reagents

Petroleum ether, Methanol, Folin-ciocalteu reagent, Sodium carbonate, Gallic acid, Aluminum chloride, Sodium hydroxide, Quercetin, DPPH, Potassium ferrocyanide, Tri-chloro acetic acid, Ferric chloride, Phosphate buffer saline (pH 6.5, 6.3), Diclofenac sodium, Bovine serum albumin, Acetate buffer. All chemicals were purchased from the Hi-media at analytical grade.

Plant material preparation

After the procedure of identifying the plant has been completed, each plant specimen is given a thorough washing in running tap water and, as a last step, in distilled water. Following the rinsing process, the samples are placed inside the shed for a week so that they can dry. The materials are ground into a coarse consistency with the use of a grinder and then homogenized. The extraction was carried out with Soxhlet apparatus using a ratio of 1:10, which included 1 g of dry sample and 10 mL of each solvent (petroleum ether, methanol and distilled water). The total amount of each sample that was processed was 250 g. After the extraction process is complete, the liquid extracts are concentrated in the water bath, the yield of the extraction is determined by applying the formula that is provided below and the extracts are then placed in the refrigerator until they are needed for subsequent steps.

Polyherbal mixture preparation

For the preparation of polyherbal mixture petroleum ether extracts, methanol extracts and aqueous extracts (0.1 g) were mixed in the equal ratio of 1:1:1:1:1 separately. To complete dissolve the combination of extracts was mixed in the 5 mL of extracted solvent separately and utilized to evaluate the aforementioned activities. The ratio of the present polyherbal mixture was prepared on the basis of studies of Soujanya *et al.* and Ganga *et al.*[19,20]

Determination of total amount of antioxidant compounds

Determination of the total phenolic content

The folin-ciocalteu assay was used to determine the Total Phenolic Content (TPC), with some minor adjustments made to the methodology established by Singleton *et al.*[21] After 1 mL of the sample was poured into the test tube, half a ml of the folin-ciocalteu reagent was added. After waiting for 5 min, 1.5 mL of 20% sodium carbonate was added to the combination and the remaining volume was brought up to 10 mL with deionized water. The mixture was then left to incubate at room temperature for another 30 min. The samples that were analyzed revealed a significant concentration of phenolic chemical as evidenced by the dark blue color. The absorbance was determined by employing a UV-visible spectrophotometer (Elico Double beam SL-210, India) and measuring it at a wavelength of 750 nm. TPC were expressed as Gallic Acid Equivalents (GAE), which were determined as µg of GAE/gm of dry weight using the following equation:

$$C = \frac{c \times V}{m}$$

Where, C=total content of phenolic compounds, c=the concentration of Gallic acid established from the calibration curve, V=the volume of extract and m=the weight of crude plant extract.

Table 1: A brief detail of the selected medicinal plants for the study.

Local name	Botanical name	Family	Part used	Plant habit	Voucher no.
Gotu kola	*Centella asiatica* (CA)	Apiaceae	Leaves	Herb	GGV/BOT/APIA/056
Dhaura	*Anogeissus latifolia* (AL)	Combretaceae	Bark	Tree	GGV/BOT/COMB/154
Hardi	*Curcuma longa* (CL)	Zingiberaceae	Rhizome	Rhizome	GGV/BOT/H/ZIN/DKS/431
Rohina	*Soymida febrifuga* (SF)	Meliaceae	Bark	Tree	GGV/BOT/MELIA/352
Kadu bhaji	*Glinus oppositifolius* (GO)	Molluginaceae	Arial part	Herb	GGV/BOT/MOLL/010

Determination of the Total Flavonoid Content (TFC)

For the determination of Flavonoid Content (TFC) aluminum chloride colorimetric method was used as standard protocol determined by the Chang *et al.*[22] Each plant extract (0.5 mL) was diluted with 4 mL of deionized water and added 0.3 mL of sodium nitrate (5%). After 5 min 0.3 mL of aluminum chloride (10%) was added and incubated for 6 min. After incubation 2 mL sodium hydroxide (1 M) was added in the mixture and final volume make with 10 mL deionized water and shaken well. Reaction mixture was incubated at room temperature for 30 min. Orange yellowish colour was observed the positive indicator for the presence of flavonoid content. Absorbance was measured with the help of the UV-visible, spectrophotometer (Elico Double beam SL-210, India) at 510 nm wavelengths. TFC expressed as Quercetin Equivalents (QUE) in µg QCE/gm dry weight and calculated by the following formula:

$$C = \frac{c \times V}{m}$$

Where, C=Total Flavonoid Content (TFC), c=the concentration of quercetin established from the calibration curve, V=the volume of extract and m=the weight of crude plant extract.

In vitro determination of free radical scavenging activity

DPPH Assay

DPPH scavenging activity was measured by standard method established by Dinis *et al.*[23] Plant extract (1 mL) mixed with 3 mL of DPPH (0.1 mM) and allowed reacting at room temperature for 30 min in the dark. Absorbance was measured at 517 nm after 30 min and converted into the percentage of antioxidant activity using the below-mentioned formula. The concentration of ascorbic acid served as the standard.

$$Inhibition\ \% = \frac{A_0 - A_1}{A_0} \times 100$$

Where, $A_{0=}$Absorbance of the blank, A_1=absorbance of extracts. However, the extract concentration provided 50% Inhibition (IC_{50}) which calculated through interpretation of data as a linear regression analysis of ascorbic acid.

Ferric Reducing Antioxidant Power assay (FRAP assay)

The FRAP assay was analyzed using a method similar to the one published by Thaipong *et al.*,[24] with a few minor adjustments. In this scenario, 1 mL of each extract was placed in a separate test tube and then 2.5 mL of phosphate buffer was added to each concentration. The concentrations tested were 10, 20, 30, 40 and 50 µg/mL. The reaction mixture was placed in a water bath at a temperature of 50°C for 20 min after having 1 mL of potassium ferrocyanide added to it. After the mixture had been heated, 2.5 mL of Tri-Chloro Acetic acid (TCA) was added and then it was centrifuged at 3000 rpm for 5 min. 2.5 mL of this reaction mixture was transferred to a clean test tube and then 2.5 mL of distilled water was added to bring the volume up to 5 mL. Following this, 0.5 mL of $FeCl_3$ was added to the mixture. The emergence of a bluish tint confirmed the test. A measurement of absorbance was taken at 700 nm (Elico Double beam SL-210, India). The concentration of quercitin served as the standard. The following formula was used to convert the results into a weight of $FeSO_4$ that was stated in mg/gm of dry weight. After plotting absorbance against the corresponding concentration, we were able to get the IC_{50} value.

$$[\text{FRAP value}=(A1 - A0)/(Ac - A0)] \times 2$$

Where Ac is the absorbance of the positive control, A1 is the absorbance of the sample and A0 is the absorbance of the blank.

In vitro Anti-inflammatory activity

Egg albumin denaturation assay

The estimation of the inhibition of egg protein denaturation was done by the method of the Mizushima and Kobayashi with some minor changes.[25] After adding egg albumin (0.2 mL), phosphate buffered saline (2.8 mL, pH 6.5) and a test sample (2 mL) with varying concentrations (50, 100, 200, 300 µg/mL) were added

in a test tube and created a reaction mixture of 5 mL. After incubating the mixture for 20 min at 40°C, it was heated at 75°C for 5 min and then it was cooled. This process was repeated three times. 660 was the value that was reported for the absorbance of both the reaction mixture and the standard with the help of spectrophotometer (Elico Double beam SL-210, India). The amount of diclofenac sodium that was employed served as the standard, while phosphate buffer solution was the variable that served as the control. Percent inhibition of this assay is calculated by the below mentioned formula as well as IC_{50} was calculated by the linear regression curve equation.

$$Inhibition\ \% = \frac{A_c - A_t}{Ac} \times 100$$

Where, A_c=Absorbance of the control, A_t=absorbance of treated.

Bovine Serum Albumin (BSA) assay

The procedure for the analysis was carried out in accordance with the guidelines provided by Sakat *et al.* but with some slight modifications.[26] Reaction mixture (0.5 mL) that was created by mixing bovine serum albumin (0.45 mL) and test samples (0.05 mL) of varied concentrations (50, 100, 200 and 300 µg/mL). The reaction mixture was heated to 40°C for 25 min, the tubes were then filled with phosphate buffer saline (2.5 mL, pH 6.3), the tubes were allowed to cool and a reading of the turbidity was obtained using a spectrophotometer (Elico Double beam SL-210, India) set at 660 nanometers. The phosphate buffer solution (0.05 mL), was given to the control and diclofenac sodium was given to the standard rather than the experimental extracts. Percent inhibition of this assay is calculated by the below mentioned formula as well as IC_{50} was calculated by the linear regression curve equation.

$$Inhibition\ \% = \frac{A_c - A_t}{Ac} \times 100$$

Where, A_c=Absorbance of the control, A_t=absorbance of treated.

Statistical Analysis

The data was collected in triplicate and illustrated as the mean±SEM. The SPSS version 16.0 program was implemented to carry out a *post hoc* ANOVA Tukey test at $p<0.05$. The software GraphPad Prism performed graphical analysis.

RESULTS

Determination of total amount of antioxidant compounds

Determination of the total phenolic and flavonoid content

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) were calculated using calibration curves for gallic acid (y=0.4777x+0.0848, R^2=0.9244, Figure 1) and quercetin (y=0.0047x+0.039, R^2=0.9849, Figure 2). Table 2 shows the total

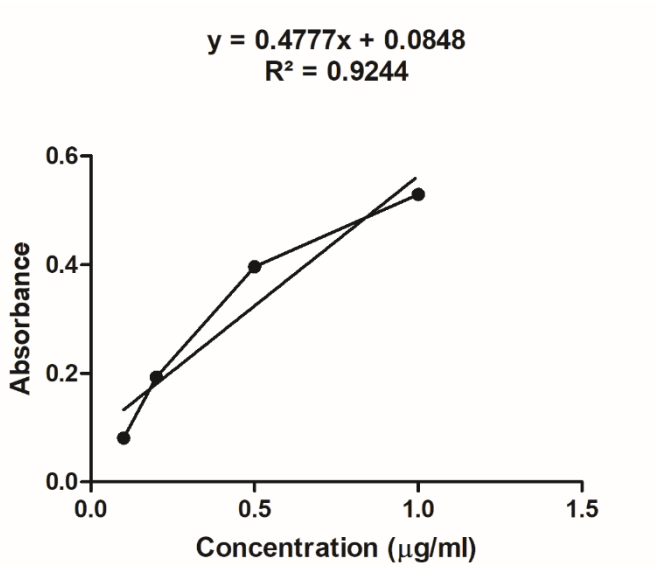

Figure 1: Gallic acid standard calibration curve for Total phenolic content estimation.

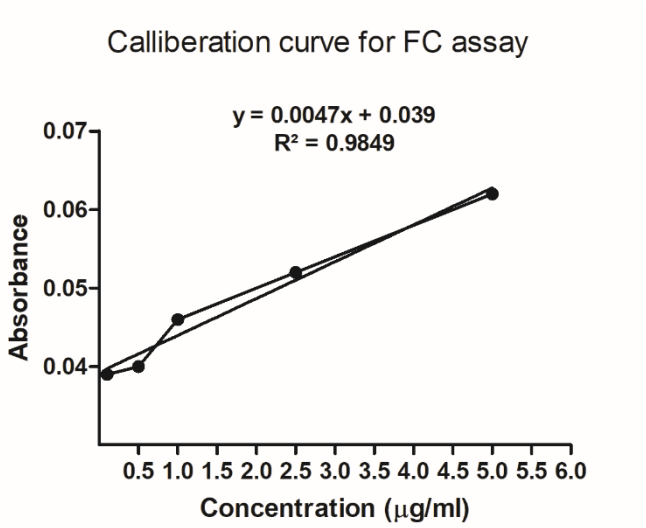

Figure 2: Quercetin standard calibration curve for Total flavonoid content estimation.

phenolic and total flavonoid contents of the various extracts, which are statistically significant at $p<0.05$. TPC data was represented in µg of GAE/gm dry weight, while TFC data was expressed in µg of QE/gm dry weight. When compared to petroleum ether and methanol extracts, all aqueous extracts had the greatest phenolic concentration. In the case of TPC, all the chosen plants followed the same trends: SF (618.52±0.09)>CA (485.39±0.99)>AL (297.06±0.00)>GO (236.89±1.71)>CL (174.20±0.17). Plants exhibit the following tendencies in terms of TFC content: SF (168.24±0.19)>AL (120.26±0.28)>GO (115.96±0.03)>CL (104.08±0.08)>CA (101.32±1.08). In polyherbal mixtures, the aqueous extract combination had the highest levels of TPC (886.65±0.29) and TFC (208.66±0.16). It was followed by the petroleum ether and methanol extracts.

Antioxidant Assay

DPPH radical scavenging activity

The DPPH scavenging experiment demonstrated that the polyherbal formulation had the highest scavenging activity (87.33±2.16) when compared to individual aqueous extracts, which were ordered as follows: CL (77.65±1.46)>CA (73.10±1.79)=AL (73.58±1.23)>SF (67.46±2.29)>GO (56.85±1.12). Standard (Gallic acid) was shown to have the best scavenging activity (96.46±1.65) of all. There were also significant (p<0.05) variations in the IC_{50} mean values of the extracts which calculated with the help of linear regression curve of ascorbic acid (Table 3, Figure 3). Both the standard and polyherbal formulations showed 50% inhibition at 3.33±2.05 µg/mL and 19.52±4.05 µg/mL, respectively, which was the lowest and most significant concentration of both when compared to all individual aqueous extracts.

FRAP assay

The FRAP test findings showed that all samples had a substantial variation in the reduction of ferric ion (Fe^{3+}) into ferrous ion (Fe^{2+}). In this scenario, ascorbic acid (used as a control) showed 85.28±1.15% inhibition, followed by the polyherbal combination with 78.53±1.25% inhibition. Individual aqueous extracts prepared in the following order: GO (56.57±1.25)>CL (43.99±0.93)>SF (36.18±1.05)>AL (22.96±0.84)>CA (17.29±0.91). There were also significant (p<0.05) variations in the IC_{50} mean values of the extracts which calculated with the help of linear regression curve of ascorbic acid (Table 3, Figure 4). Ascorbic acid and polyherbal mixes were shown to have 50% inhibition at 1.81±0.10 µg/mL and 1.22±0.11 µg/mL, respectively, which was the smallest and most significant concentration of both compared to all other aqueous extracts.

In vitro Anti-inflammatory activity

Egg albumin denaturation assay (EAD assay)

Table 3 shows the results of the egg albumin denaturation test, which were determined to be significant at p<0.05. The standard medicine diclofenac sodium had the highest inhibition percent

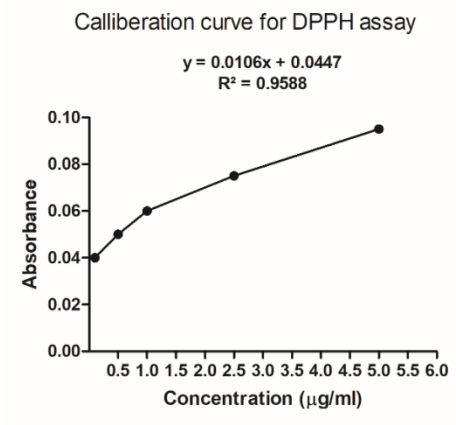

Figure 3: Ascorbic acid standard calibration curve for DPPH free radical scavenging activity estimation.

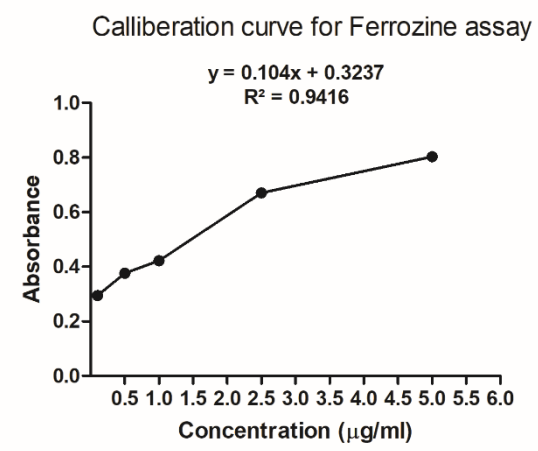

Figure 4: Ascorbic acid standard calibration curve for ferric ion reducing potential estimation.

Table 2: Effect of extracting solvent on the Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of the selected medicinal plants.

Plant name	Total Phenolic content (µg GAE/gm dry wt.)			Total Flavonoid content (µg QCE/gm dry wt.)		
	Extracts					
	Aqueous	Methanol	Pet ether	Aqueous	Methanol	Pet ether
Soymida febrifuga	$618.52\pm0.09^{a}_{a}$	$341.77\pm0.75^{b}_{a}$	$42.11\pm0.98^{c}_{a}$	$168.24\pm0.19^{a}_{a}$	$142.61\pm0.18^{b}_{a}$	$30.58\pm0.16^{c}_{a}$
Curcuma longa	$174.20\pm0.17^{a}_{b}$	$99.78\pm1.88^{b}_{b}$	$24.34\pm0.11^{c}_{b}$	$104.08\pm0.08^{a}_{b}$	$86.41\pm0.12^{b}_{b}$	$34.51\pm0.31^{c}_{a}$
Centella asiatica	485.39 ± 0.99^{a}c	$408.97\pm0.05^{b}_{c}$	$110.43\pm1.05^{c}_{c}$	$101.32\pm1.08^{a}_{b}$	$40.64\pm0.43^{b}_{c}$	$11.50\pm0.18^{c}_{b}$
Glinus oppositifolius	$236.89\pm1.71^{a}_{d}$	$160.81\pm1.32^{b}_{d}$	$33.56\pm0.95^{c}_{d}$	$115.96\pm0.03^{a}_{c}$	$60.81\pm1.32^{b}_{d}$	$28.28\pm0.12^{c}_{a}$
Anogeissus latifolia	$297.06\pm0.00^{a}_{e}$	$221.04\pm0.52^{b}_{e}$	87.45 ± 0.28^{c}e	$120.26\pm0.28^{a}_{c}$	$37.71\pm0.99^{b}_{c}$	$22.63\pm0.19^{c}_{a}$
Polyherbal Mixture	$886.65\pm0.29^{a}_{f}$	$811.23\pm0.70^{b}_{f}$	$11.03\pm0.39^{c}_{f}$	$208.66\pm0.16^{a}_{d}$	$126.31\pm0.10^{b}_{d}$	$12.40\pm0.11^{c}_{b}$

GAE: Gallic Acid Equivalent; QCE: Quercetin Equivalent; µg: Microgram; wt.: weight.Data is represented in Mean± SEM of triplicates.Significance was calculated by post hoc ANOVA Tukey test at p<0.05.Varying Superscript alphabets from left to right indicate the differences between the solvents. Significant diversity among plants is indicated by different subscript alphabets arranged from top to bottom in a different column.

of 94.27±2.61, followed by a polyherbal combination with 86.46±3.32% inhibition. Different plant extracts are ordered in this panorama as follows: GO (82.01±1.21)>AL (73.61±1.04)>SF (67.36±0.89)>CA (63.60±1.66)>CL (56.48±3.44). The IC_{50} value was also determined by the linear regression curve (Figure 5). The standard had a significant IC_{50} value of 2.14±1.33 µg/mL, whereas the polyherbal combination had a value of 4.75±2.63 µg/mL, followed by the GO (8.27±0.96 µg/mL), which was statistically insignificant. Similarly, the CA (22.84±1.31 µg/mL), CL (28.47±2.73 µg/mL), AL (14.91±0.82 µg/mL) and SF (19.86±0.70 µg/mL) revealed statistically insignificant results.

Bovine Serum Albumin (BSA) assay

The findings from the bovine serum albumin test were significant at $p<0.05$ and are provided in Table 1. The standard drug diclofenac sodium had the highest inhibition percentage of 85.27±0.87, followed by the polyherbal combination with a percentage of 56.14±2.60. Individual plant extracts are ordered in this panorama as follows: SF (47.27±2.14)>GO (37.64±1.92)>CL (40.42±1.38)>AL (29.86±2.79)>CA (16.29±1.37). The IC_{50} value was also computed by the linear regression curve equation (Figure 6). The standard had a substantial IC_{50} value of 0.06±0.00 µg/mL, whereas the polyherbal combination had an IC_{50} value of 0.04±0.00 µg/mL. CA (0.01±0.00 µg/mL) and AL (0.02±0.00 µg/mL) showed comparable patterns. However, statistically insignificant IC_{50} values were found for the CL, SF and GO.

DISCUSSION

In the present investigation, spectrophotometric models were used to assess the potential for wound healing of selected medicinal plants and their polyherbal mixture. A variety of assays were used, including the protein precipitation assay, the DPPH assay, the FRAP assay, the egg albumin denaturation assay, the bovine serum albumin assay and the quantitative antioxidant compound estimation assay. To quantify antioxidant substances, total flavonoid content (polyphenols) and total phenolic content were used. Selected medicinal plants were found to contain good amounts of polyphenols in their petroleum ether and methanol extracts, but their aqueous extracts and mixtures were said to be better because of the polar solvent (Table 2). Similar results were noted by Mathew *et al.*, Sharma *et al.*, Dubey *et al.*, Quyen *et al.* and Nawaz *et al.*[27-31] during their studies of certain Indian traditional medicinal herbs. According to Mathew *et al.* and

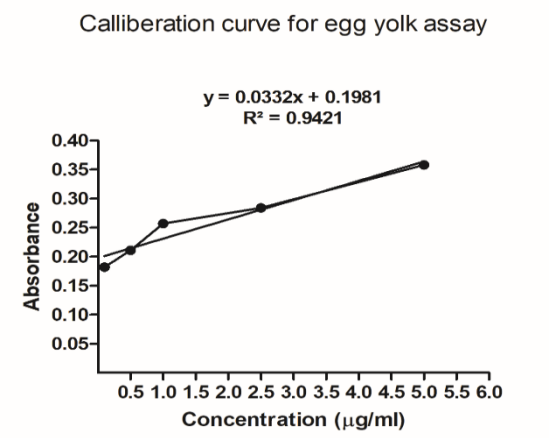

Figure 5: Diclofenac sodium standard calibration curve for egg albumin denaturation assay estimation.

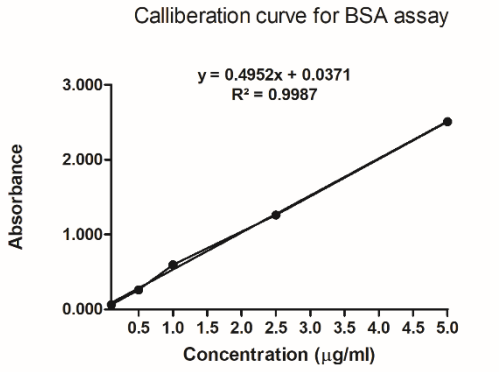

Figure 6: Diclofenac sodium standard calibration curve for BSA denaturation assay estimation.

Table 3: **Result of IC_{50} values (in µg/mL) of the aqueous extract of selected medicinal plants and their polyherbal mixture.**

Plant Samples	Antioxidant assays (IC_{50} in µg/mL)		Anti-inflammatory assays (IC_{50} in µg/mL)	
	DPPH assay	FRAP assay	BSA assay	Egg albumin assay
Standard	3.33±2.05[a] Ascorbic acid	1.81±0.10[a] Ascorbic acid	0.06±0.00[a] Diclofenac sodium	2.14±1.33[a] Diclofenac sodium
Polyherbal Mixture	19.52±4.05[b]	1.22±0.11[b]	0.04±0.00[b]	4.75±2.63[b]
Centella asiatica	46.18±3.36[c]	4.19±0.08[c]	0.01±0.00[c]	22.84±1.31[c]
Anogeissus latifolia	45.29±2.31[c]	3.69±0.07[d]	0.02±0.00[d]	14.91±0.82[d]
Curcuma longa	37.66±2.73[c]	1.83±0.08[a]	0.03±0.00[e]	28.47±2.73[c]
Soymida febrifuga	56.76±4.30[c]	2.52±0.09[e]	0.03±0.00[e]	19.86±0.70[d]
Glinus oppositifolius	76.63±2.11[d]	0.72±0.11[f]	0.03±0.00[e]	8.27±0.96[b]

Data is represented in Mean of triplicate±SEM. Significance was calculated by *post hoc* ANOVA Tukey test at $p<0.05$. Significant diversity among standard, plants and polyherbal mixture is indicated by different superscript alphabets arranged from top to bottom in a different column.

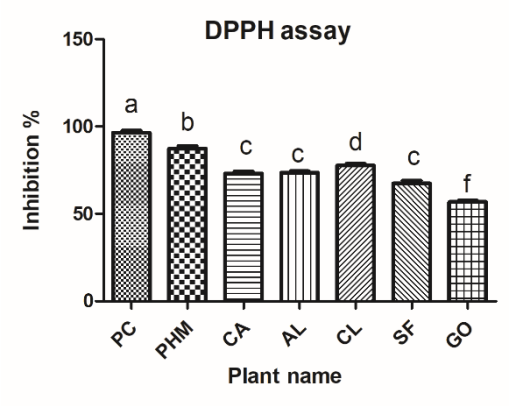

Figure 7: DPPH inhibition percent of aqueous extract of selected medicinal plants and their polyherbal mixture. The same alphabets in different bars demonstrate homogeneity and significance at $p<0.05$ using the *post hoc* ANOVA Tukey test.

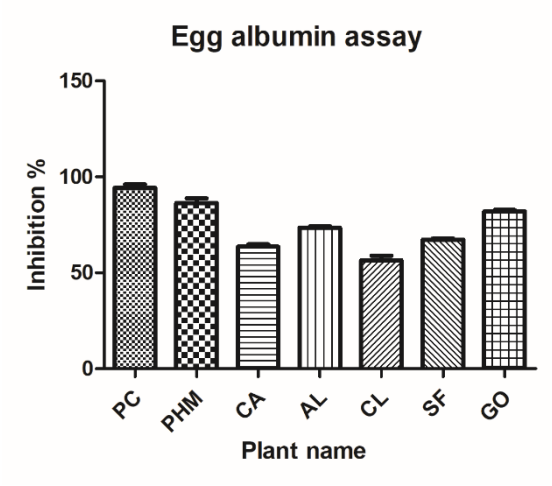

Figure 9: Egg albumin assay inhibition percent of aqueous extract of selected medicinal plants and their polyherbal mixture. The same alphabets in different bars demonstrate homogeneity and significance at $p<0.05$ using the *post hoc* ANOVA Tukey test.

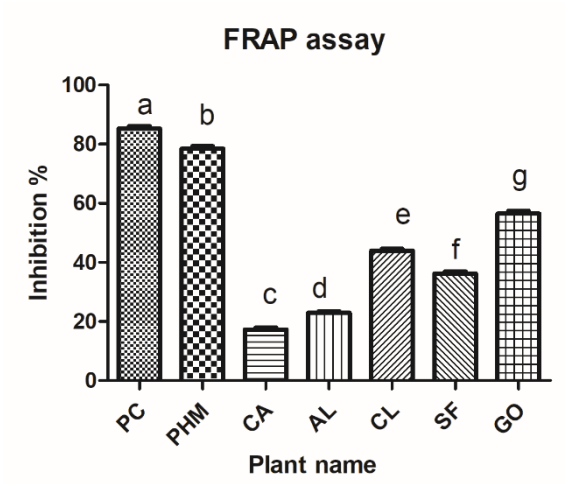

Figure 8: FRAP inhibition percent of aqueous extract of selected medicinal plants and their polyherbal mixture. The same alphabets in different bars demonstrate homogeneity and significance at $p<0.05$ using the *post hoc* ANOVA Tukey test.

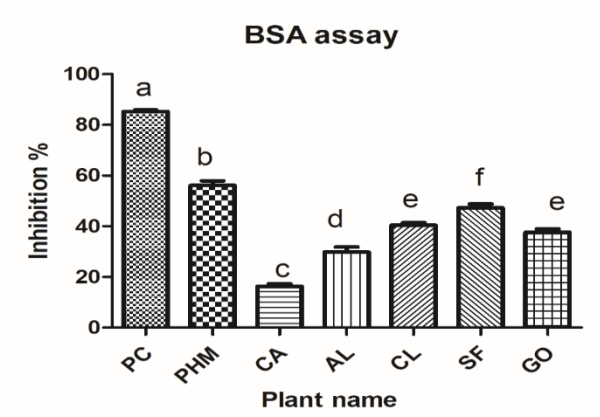

Figure 10: BSA assay inhibition percent of aqueous extract of selected medicinal plants and their polyherbal mixture. The same alphabets in different bars demonstrate homogeneity and significance at $p<0.05$ using the *post hoc* ANOVA Tukey test.

Nawaz *et al.*[27,31] the low concentration of polyphenols in the methanol and petroleum ether extracts may be attributed to both their polarity-dependent characteristics and the chemical makeup of the polyphenols in the solvents.

Based on the polyphenol findings, aqueous extracts and their mixes were selected for testing the antioxidant capacity of extracts using the widely used DPPH and FRAP tests. Among many different assays, the DPPH test is one of the most common methods for assessing plant antioxidant potential. The addition of antioxidants with hydrogen-donating groups, such as flavonoids and phenols, reduces the methanolic DPPH solution owing to the generation of nonradicals.[32,33] In our investigation, the polyherbal combination had a lower IC_{50} value (Table 3). The lower the IC_{50} value, the faster the DPPH radical was diminished and hence the stronger the antioxidant.[34] The activity varied significantly amongst plant extracts, with most plant species exhibiting the highest radical scavenging activity. The polyherbal combination had the strongest inhibitory capacity in this scenario (Figure 7).

The FRAP test measures the reducing capability of an antioxidant when it reacts with a ferric (Fe^{3+}) complex and produces a colored ferrous (Fe^{2+}). In general, the existence of diverse compounds is connected with the presence of reducing characteristics of the substances, which work by interrupting the free radical chain by donating a hydrogen atom. The FRAP test considers the antioxidants in the sample as a reductant in a redox-linked colorimetric reaction.[35,36] The trend for ferric ion-reducing activities of all aqueous extracts and their combinations in the current investigation is presented in the Figure 8. The blend of aqueous extracts of selected medicinal plants had the strongest chelating activity as well as the highest inhibition percentage in our

investigation. Rahim *et al.* and Yap *et al.* both showed comparable patterns for polyherbal formulations in this context.[37,38]

Protein denaturation is a random process that involves changes in electrostatic hydrogen, hydrophobic and disulfide bonds.[39] Protein denaturation results in the generation of autoantigens in inflammatory diseases such as rheumatoid arthritis, cancer and diabetes. As a result, reducing protein denaturation may reduce inflammatory activity.[40] In this respect, both the egg albumin denaturation assay and the bovine serum albumin assay give a low-cost alternative approach of investigating the anti-inflammatory effectiveness of herbal medicines utilizing the denaturation methodology, which should be confirmed by several investigations. Diclofenac sodium, a Nonsteroidal Anti-Inflammatory Medication (NSAID) that inhibits inflammation by inhibiting cyclooxygenase enzyme activity, was employed as a control in the current investigation. The anti-inflammatory effect of many medicinal plants has been described in multiple studies, but we are the first to report on their polyherbal combination. The polyherbal combination was shown to be superior over single extracts but inadequate to the standard in both egg albumin (Figure 9) and BSA tests (Figure 10). In this panorama, Joshi *et al.* discovered similar findings in the polyherbal combination.[41,42]

In the current research, it was shown that a polyherbal formulation made from a selection of medicinal plants had a much higher potential for protein binding when compared to individual plant extracts from the same plants. According to Dinakaran *et al*. polyherbal formulations include a large number of plant chemicals such polyphenols that act together to provide a synergistic effect.[43,44] In traditional medicine, rather than using individual components, entire plants or plant mixes are often employed as treatment. The synergistic effects of polyherbalism result in the conferral of advantages that cannot be obtained from the use of individual herbal compositions. According to Karole *et al*. polyherbal formulations shows excellent efficiency in treating a wide variety of disorders at safe doses.[45] Several different polyherbal formulations have been discovered to possess extremely excellent antioxidant, antibacterial and anti-inflammatory effects.[6,9-12,16] These findings were published in a number of different academic journals. In separate investigations, numerous plant extracts have been revealed to independently possess beneficial antioxidant and anti-inflammatory properties. In the present study, out of the five medical plants that were selected, four (CA, GO, AL and SF) exhibited noteworthy moderate activity, while the combination of these selected plants extract showed a consistent trend of high potential throughout the whole assessment.

CONCLUSION

The whole research was conceived with the intention of determining the preliminary wound healing activity of the selected medicinal plants and their polyherbal combination that they were combined with. There was a large amount of antioxidant, anti-inflammatory and wound healing activity associated with each and every medicinal plant throughout the whole trial. In addition to being employed for the different formulation processes, the polyherbal combination that was created from the chosen medicinal plants was also utilized for further *in vitro* and *in vivo* research.

ACKNOWLEDGEMENT

The authors would like to express their gratitude to the Department of Botany, Guru Ghasidas Vishwavidyalaya Bilaspur, Chhattisgarh, India, to provide basic facilities to perform the entire research work.

FINANCIAL SUPPORT AND SPONSORSHIP

Non-Net fellowship (UGC fellowship) provided by the University Grants Commission, New Delhi, India is appreciated by the authors.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AL: *Anogeissus latifolia*; **CA:** *Centella asiatica*; **CL:** *Curcuma longa*; **GO:** *Glinus oppositifolius*; **SF:** *Soymida febrifuga*; **PHM:** Polyherbal Mixture; **DPPH:** 2,2-Diphenyl-1-picrylhydrazyl; **FRAP:** Ferric Reducing Antioxidant Power; **SEM:** Standard error means; **EAD:** Egg albumin denaturation; **BSA:** Bovine Serum Albumin; **ANOVA:** Analysis of variance. **TPC:** Total Phenolic Content; **TFC:** Total Flavonoid Content; **NSAID:** Nonsteroidal anti-inflammatory medication.

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Cite this article: Dubey S, Chandrakar J, Vinodia S, Dixit AK. Determination of Antioxidant and Anti-inflammatory Potentials of Selected Therapeutic Plants Aqueous Extract and their Formulation for Wound Healing. Int. J. Pharm. Investigation. 2025;15(1):228-36.