Research Article

DEVELOPMENT AND OPTIMIZATION OF ANTIMICROBIAL HERBAL DENTAL GEL CONTAINING *TEPHROSIA PURPUREA* LINN. LEAF EXTRACT

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ABSTRACT

The objective of research work that has been undertaken is to formulate, optimize and do *in-vitro* evaluation of *Tephrosia purpurea* Linn. herbal dental gel. The ethanolic extract of *Tephrosia purpurea* Linn. was prepared by Soxhlet extraction and formulated into gel using a central composite design. Qualitative and quantitative screening, preliminary evaluation of herbal gel formulation such as pH, spreadability, extrudability, viscosity and *in-vitro* antimicrobial were followed by standard procedures. The thin layer chromatography confirmed the presence of flavonoids in the extract which shows the antimicrobial activity. ANOVA results demonstrate that the model was significant for all dependent variables. The effects of the amount of Carbopol and HPMC were found to be significant along with its quadratic and interaction terms for all the dependent variables. *In-vitro* bioactivity determination of herbal gel of *Tephrosia purpurea* leaf extract containing 4.0% w/w, HPMC-K₄M, with 3.5% w/w carbopol by zone of inhibition studies showed highly active against *Staphylococcus aureus* (11mm) and *Candida albicans* (1mm) as compared to the marketed metronidazole (1% w/v) gel 10 and 0.5 mm respectively. Stability study confirmed the *Tephrosia purpurea* Linn. extract loaded dental gel formulations to be stable after three month.

Keywords: Carbapol-934, Phytochemical screening, *Tephrosia purpurea* ethanolic extract

INTRODUCTION

Herbal medicines are great demand globally for primary healthcare due to their higher safety margins and cost effectiveness. Medicinal plants have played a key role in world health¹. The present modern therapeutic systems are not sufficiently effective for the complete removal of liver hazard, without inciting adverse drug reactions and a curative agent has not yet been found in modern medicine. The present use of corticosteroids and immunosuppressive agents just realizes symptomatic relief. However, their utilization is related with risk of relapses and risk of side effects. Disregarding of tremendous strides in modern medicine, there are not really any medications that stimulate liver function and offer protection to the liver from harm or help recovery of hepatic cell².

Oral diseases are one of the biggest general health problems of life and are expensive to treat the dental caries, gingivitis and periodontal disease in children and adults are among the most important preventable global infectious diseases in the world. Recent attention has been made to extracts and biologically active compounds isolated from plants used in herbal medicine³. In rural area of the developing countries, they continue to be used as the primary source of medicines about 80% of the people use traditional medicines for their health care⁴. Topical gel drug administration is localized drug delivery system to deliver the drug in the body through ophthalmic, rectal, vaginal and skin as topical routes. The conveyance of medications onto the skin is perceived as an effective means of therapy for dermatologic ailments by penetrating deeper into skin and hence gives better absorption⁵. The present busy life systems, even in common man dental diseases are most widespread chronic disorders.

Periodontal disease means of a group of problems that arise in the sulcus, the crevice between the gum and tooth the common causative organisms like; *Actinobacillus*, *Mycetemcomitans* as a pathogen responsible for Juvenile periodontitis, *Staphylococci subspecies*; *epidermidis* and *aureus*. The advantages of site specific therapy are decreased drug doses, increased drug concentration at the site of infection and reduced systemic side effects such as gastrointestinal distress. The aims of periodontal disease treatment with topical drug delivery system is delivering therapeutic agent at sufficient level inside the periodontal⁶.

Many medicinal plants are considered to be potential antimicrobial crude drugs as well as source of novel compounds with antimicrobial and antifungal activity, with possibly new modes of action. This expectation that some naturally occurring plant compounds can kill antibiotic resistant strains of bacteria. The indigenous systems of medicine in India have a long tradition of treating dental caries^{7,8}. *Tephrosia purpurea* (L.) Pers. (Fabaceae), commonly known in Sanskrit as 'Sarapunkha', is a highly branched, sub-erect, herbaceous perennial herb. According to Ayurvedic literature, this plant is also given the name of 'vranvishapaka' which means that it has the property of healing all types of wounds. In the Ayurvedic system of medicine, various parts of this plant are used as a remedy for impotency, asthma, diarrhea, gonorrhea, rheumatism, ulcers, and urinary disorders⁹. *Tephrosia purpurea* showed presence of glycosides such as rutin and quercetin, reteneoids like deguelin, elliptone, rotenone and tephrosin, flavonoids like purpurin, purpurenone and purpuritenin and sterols such as sitosterol. An isoflavone, 7, 4-dihydroxy- 3, 5-dimethoxyisoflavone and chalcone, (+) - tephropurpurin, are also reported to be present in *Tephrosia purpurea*. It is also used in the treatment of bronchitis, bilious

febrile attack, boils, pimples and bleeding piles³. The ethanolic extracts of *Tephrosia purpurea* possessed potential antibacterial activity¹⁰. Whole plant may be used for its rich flavonoid and polyphenol content. Though a lot of research is going on in the plants, it is used only for its traditional claim in Ayurveda. Substantially, the challenge to formulate a delivery system with increased therapeutic activity and least side effects may be overcome by perfect fabrication of novel drug delivery systems with a proper dose of ethanolic extract of *Tephrosia purpurea* Linn. leaves.

MATERIALS AND METHODS

All ingredients such as HPMC K4M, methyl paraben, propyl paraben, carbopol-934, ethanol, and triethanolamine were purchased from Research Lab Fine chem, Industries, India. All ingredients were used of analytical grade.

Collection and Authentication of Plant Material

The leaves of *Tephrosia purpurea* were collected from fields near to Amravati forest, Maharashtra State, India. The plant *Tephrosia purpurea* was authenticated by Dr. P.W. Deotare, Head of Department, Botany, Shri Shivaji Science College, Amravati. The herbarium voucher specimen number SC/BOT/109/2016 SA sample voucher specimen of plant was deposited for future reference.

Preparation of Extract of *Tephrosia purpurea* Linn.

Tephrosia purpurea dried leaves (500 g) were coarsely powdered and defatted with petroleum ether in a soxhlet extractor. The defatted crude drug was successively extracted with 3000 ml ethanol in soxhlet extractors at 60°C for 24 hours. The filtered extract was dried by evaporating the solvent till 15-20 % semisolid black and light greenish yellow a thick mass was obtained and stored at 0-4°C until further used. Percentage yield was calculated for extract after drying under a vacuum.

Physiochemical Characterization

Physiochemical parameters such as total ash, acid insoluble ash, alcohol soluble extractives, water soluble extractive, volatile matter, pH value & loss on drying methods were followed according to the Ayurvedic pharmacopeia and Indian Pharmacopoeia¹¹.

Preliminary Phytochemical Screening

The powdered plant material was subjected to preliminary phytochemical analysis to test the presence or absence of phytochemical constituents by standard method^{12, 13}.

Chromatographic Profiling of *Tephrosia purpurea* Using TLC

TLC of extract was carried out by precoated Silica gel-G plates which possess standardized adsorption layers, and activated in the oven at 105°C for 30 minutes. All the solvents systems were selected by trial and error basis. The chromatograms were developed in twin through glass chambers on (10x10 cm) plates till the mobile phase, methanol: water: formic acid (8:10:6.6) travelled up to a distance of 8 cm from starting point. After development the plates were dried at room temperature for 5-10 minutes and observed under UV- radiation and fluorescence was noted. Freshly prepared iodine spray reagents were used to detect the bands on the TLC plates and R_f values were recorded¹⁴.

Design and Development of Antimicrobial Herbal Dental Gel

An antimicrobial gel was prepared by modified cooled mechanical technique. 0.2% w/v ethanol extract of *Tephrosia purpurea* Linn. shows the satisfactory antimicrobial activity and to be used as the minimum effective concentration of extract¹⁵. Based on the literature data, 0.2%w/v ethanol extract of *Tephrosia purpurea* Linn. was used for the development of dental gel formulations. The compositions of dental gel are tabulated in Table 1. Different combinations of *Tephrosia purpurea* leaves extract (0.2%) were tried with different types of polymers (HPMC, Carbopol-934) using various formulae for formulation batch M1 to M2. The required quantity of carbopol-934 was slowly sprinkled into weighed amount of purified water with constant stirring to get the uniform dispersion and then kept overnight for hydration. The accurately weighted amounts of *Tephrosia purpurea* leaves extract (0.2%) along with other additives were poured into the fixed amount of hydrated Carbopol-934 dispersion with constant stirring until proper mixing. Finally full mixed ingredients were mixed properly to the carbopol 934 gel with continuous stirring and triethanolamine was added drop wise to the formulation for adjustment of required skin pH (6.8-7) and to obtain the gel at required consistency. The same method was followed for preparation of control sample without adding any *Tephrosia purpurea* leaves extract. All the formulations were stored in a screw capped wide mouthed beaker covered with aluminium foil in a cool and dark place.

Statistical Optimization Using RSM by Design Expert Software

Statistical analysis and optimization of gel formulations were analyzed using Design Expert software version 7.0.10 (Stat-Ease, USA) and used to generate the study design and the response surface plots¹⁶. Central composite designs are a factorial or fractional factorial design with center points, augmented with a group of axial points that help to estimate curvature. Polynomial models, including linear, interaction and quadratic terms were generated for all the response variables using the software. The polynomial equation for quadratic model or second order regression model generated by this experimental design is as follows;

$$Y_i = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 \dots\dots\dots (1)$$

Where, Y_i is the dependent variable; b₀ is the intercept; b₁ to b₂₂ are the regression coefficients; and X₁ and X₂ are the independent variables that were selected from the trial trials. The main effects of X₁ and X₂ represent the average result of changing one variable at a time from its low level to its high level. The interaction terms (X₁X₂, X₁² and X₂²) represent changes when two variables are simultaneously changed. The best fitting model was selected based on comparisons of several statistical parameters, including the coefficient of variation (CV), coefficient of determination (R²) and adjusted coefficient of determination (adjusted R²) provided by Design Expert software. In addition, analysis of variance (ANOVA) was used to identify significant effects of factors on response regression coefficients. One-way ANOVA was applied to estimate the significance of the model (*p* < 0.05) and individual response parameter. Compositions of gel formulation F1 to F9 by central composite design by considering two factors X₁ (HPMC), X₂ (Carbopol) are given in Table 2. The F test and P values were also calculated using the software. The relationship between the dependent and independent variables was further elucidated using response surface plots¹⁷. These plots are useful to study the effects of various factors on the response at a given time and to predict the responses of dependent variables at intermediate levels of independent variables.

Evaluation of Topical Gel Formulation

All the prepared gel formulations were subjected for preliminary evaluation for various parameters of gel as per standard guidelines¹⁸.

Organoleptic Evaluation

Physical parameters such as color and appearance were visually checked.

Measurement of pH

2.5g of gel was accurately weighed and dispersed in 25ml of distilled water and stored for two hours. The measurement of pH of each formulation was carried out in triplicate. The pH of dispersions was measured using pH meter¹⁹.

Evaluation of Response Variables

Spreadability

Spreadability was determined by the apparatus which consists of a wooden block, which was provided by a pulley at one end¹⁹. By this method, spreadability was measured on the basis of slip and drag characteristics of gels. An excess of gel about 2g under study was placed on this ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. A 100 g weight was placed on the top of the two slides for 5 minutes to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 20 g. With the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5 cm be noted. A shorter interval indicates better spreadability. Spreadability was calculated using the following formula;

$$S = M \times L / T \dots\dots\dots (2)$$

Where, S= Spreadability, M= Weight in the pan (tied to the upper slide), L= Length moved by the glass slide and T = Time (in sec.) taken to separate the slide completely each other.

Viscosity and Rheological Studies

Viscosities of gels were determined using Brookfield viscometer. The correct spindle was selected (spindle no. 4) for the product and the operating condition was setup. The viscosity was measured directly at 6 rpm speed by keeping the torque constant²⁰. Gels were tested for their rheological characteristics at 25°C using Brookfield viscometer. The measurement was made over the whole range of speed settings from 6 rpm to 60 rpm with 30seconds between 2 successive speeds and then in a descending orders.

Swelling Index

Swelling index studies were performed at 37°C using a thermo jacketed cell containing phosphate buffer saline pH 6.8 as swelling medium. About 2g of *Tephrosia purpurea* loaded dental gel was taken and the study was carried out for 6 h. After 6 h, the gels were scrapped from aluminum foil and the weight was determined by removing the adhered water from the surface by blotting immediately¹⁹. The swelling Index of the dental gel forming polymer was determined using formula;

$$\% St = \frac{(W_t - W_0)}{W_0} \times 100 \dots\dots\dots (3)$$

Where, 'St' represents the swelling of the dental gel at time 't'. W₀ and W_t represent the initial and final weight of gelling solution and gel respectively.

Evaluation of Statistically Optimized Herbal Dental Gel Formulation

Drug-polymer Compatibility Studies

The interaction studies were carried out to ascertain any kind of chemical interaction of drug with the excipients used in the preparation of gel formulations. Fourier-transform infrared spectra were obtained by using an FTIR spectrophotometer (DRS-8000) Shimadzu, Japan. 2 mg of the sample was mixed with 100 mg KBr and compressed to prepare a salt disc (3 mm diameter). The disc was immediately kept in the sample holder and FTIR spectra were recorded in the absorption range between 4000 and 400 cm⁻¹.

Extrudability

Extrudability study of the formulated dental gels were performed based upon the weight required to extrude minimum of 0.5 cm ribbon of gel in 10 sec in the percentage of weight from the lacquered aluminum collapsible tube. More the quantities of the weight of gel extrude, the better will be the extrudability. The extrudability of gel formulation was measured in triplicate and average values were calculated from the following formula²⁰;

$$\text{Extrudability} = \frac{\text{Applied weight to extrude gel from tube (g)}}{\text{Area (cm}^2\text{)}} \dots\dots\dots (4)$$

Gelation Temperature Measurement

Gelation temperature was determined by following tube tilting method²¹. In this method 2g of aliquots of formulated gel was transferred to a test tube covered with aluminium foil and immersed in a temperature controlled water bath at 4°C. The samples were heated by increasing the temperature of water bath by 1°C till there was stable meniscus on 90°tilting of the test tubes after equilibration for 5 min at every 10°C temperature increased. The data depicted was the average of three readings for formulation (n = 3).

Mucoadhesive Strength Determination

The sheep cheek mucosal tissue was collected from slaughter house and cleaned with distilled water followed by phosphate buffer saline pH 6.8 to stand for sometimes at the same pH for remove all soluble components. After confirmation of integrity of the treated sheep cheek mucosal tissues by microscopic investigation, 2g dental gel was made sandwiched in between the two slides using cyanoacrylate adhesive and the mucoadhesive force of the optimized dental gel was determined in terms of the detaching force in the weight of water required to separate the glass plates^{22, 23}.

Determination of Flavonoids in Gel Formulation

0.2 g of gel was extracted in 100 ml of ethanol to get 2 mg/ml solution. Required volume of the above solution was transferred into a 10 ml standard flask and color development was carried out as that for the standard by adding 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. From this 1 ml solution was taken and

diluted up to 5.5 ml with distilled water. The absorbance of the test solution was measured at 421 nm against blank. Total flavonoid content was calculated in terms of percentage of quercetin equivalent per 100g by prepared calibration curve with concentration range of 10-50 µg/ml for linear using UV-Visible spectrophotometer²⁴.

In-vitro Antimicrobial Activity

In-vitro antimicrobial study of dental gel on clinically isolated *Aspergillus niger*, *Candida albican*, *Staphylococcus aureus* and *Escherichia coli*, and comparison of antimicrobial efficacy with marketed 1% metronidazole gel was carried out. Several loopful of microorganisms were inoculated into 5 ml of sterilized saline solution and the turbidity was compared and adjusted with 0.5 McFarland Nephelometric standards. The cup-plate agar diffusion method was adopted according to assess the antibacterial activity of the prepared extracts. Potato Dextrose Agar (PDA) plate and a nutrient agar (NA) surface was inoculated and uniformly spread by using a swab impregnated with standardized inoculum^{25,26}. After 15 minutes of inoculation, wells (6 mm diameter) were made by well puncture; allow the plate to dry for 5 minutes and the wells were filled with gels and antibiotics and incubated for 24 hours at 37°C for bacteria and 5-6 days at 25°C for fungus. After incubation, use a metric ruler to measure the diameter of the zone of inhibition. Simultaneously addition of the respective solvents instead of gels was carried out as controls. After incubation the diameters of the results and growth inhibition zones were measured, averaged and the mean values were tabulated.

Stability Study

The stability study of optimized dental gel formulation was performed as per ICH guidelines. The formulated gel was filled in the collapsible tubes and stored at room, freeze and accelerated temperatures and humidity conditions for a period of three months and observed for any change in pH, viscosity, swelling index, mucoadhesive strength, spreadability and drug release profile after three month²⁰.

RESULTS

Tephrosia purpurea leaf extract was subjected to phytochemical screening and result represented in Table 3. *Tephrosia purpurea* Linn. extract contained 12.01±0.02 % w/w of total ash and 1.2±0.01% w/w acid insoluble ash. The 25.04% w/w of water soluble and 17.23% w/w of methanol soluble extractive were present in *Tephrosia purpurea* extract indicating that the drug is having good solubility in water. Saponins and flavonoids were found to be abundant while glycosides, phenolic compounds, carbohydrates, and amino acids were in trace amount. All the parameters were found to comply with the standards (Table 3). The method of adsorption chromatography is at present an important analytical tool for qualitative and quantitative analysis of a number of phytochemical. TLC fingerprint profile carried out may be used as standard to identify the plant drug and to detect the adulteration by observing the number of spots and R_f values for particular extract with respect to its mobile phase. The powdered drug extract (2g/25 ml) has been extracted with different solvents at room temperature further used to establish the TLC fingerprints by using different solvent systems on trial and error basis such as hexane: ethyl acetate (6:3.5) for ethanol extracts. In the ethanolic extract of *Tephrosia purpurea*, five spots were visualized with R_f values 0.21, 0.30, 0.41, 0.44 and 0.54. The herbal gel was dark greenish in color and translucent in appearance and had a cool and smooth feeling on application. pH was also maintained constant throughout the study which was

found to be 6.0 to 7.0. The viscosity, swelling index, spreadability is the most important parameter in the evaluation of the gels. Viscosity governs the many properties of the product such as spreadability, swelling index. The rheological behaviors of the gel formulations were studied with Brookfield viscometer. The results indicated the viscosity of gel formulations M1 was consistent neither too thick nor too thin (Table 1).

Statistical Optimization of Gel Formulation

A 2-factor, 3-level design was used to optimize the gels by studying the effect of independent variables (concentration of HPMC (X_1) and concentration of carbopol (X_2) on the dependent variable (spreadability, swelling index and viscosity). Compositions of gel formulation F1 to F9 by central composite design by considering two factors X_1 (HPMC), X_2 (Carbopol) and their corresponding responses; swelling index, spreadability and viscosity are given in Table 2. Response Surface Quadratic Model, F-value of 10.51, 11.79 and 5.22 implies the models are significant for Y_1 , Y_2 and Y_3 respectively. In Y_1 response, X_1 , X_2 , X_1^2 , X_2^2 , Y_2 response X_1 , X_2 , and Y_3 response X_1 , X_2 are significant model terms. This model can be used to navigate the design space¹⁷. Summary of ANOVA results in analyzing for the responses of gel formulations is given in the Table 4. The effects the amount of carbopol and HPMC were found to be significant along with its quadratic and interaction terms for all the dependent variables. The application of response surface methodology yielded the following regression equations:

$$Y_1 \text{ (swelling index)} = 1086.00 + 75.06X_1 + 41.94X_2 + 10.25X_1X_2 + 17.56X_1^2 - 156.44X_2^2 \text{ --- (5)}$$

$$Y_2 \text{ (spreadability)} = 4.11 + 0.74X_1 - 0.048X_2 \text{ ---- (6)}$$

$$Y_3 \text{ (viscosity)} = 51111.11 + 11815.86X_1 + 431.98X_2 \text{ ---- (7)}$$

The equation in terms of actual factors can be used to make predictions about the responses for given levels of each factor. Here, the levels should be specified in the original unit for each factor. The impact of independent factors at different levels on the response variables are shown on contour and three-dimensional response surface plots in Figure 1. Developed model was further validated based on experimental versus predicted values and their corresponding residual overlay plot. For validation of RSM results, six check point batches were prepared and evaluated for swelling index, spreadability and viscosity and responses were compared with the predicted and measured value. The experimental values of the responses were compared with that of the anticipated values, linear correlation plots drawn between the predicted and experimental values demonstrated high values of R^2 (ranging 0.982 to 0.995) indicating excellent goodness of fit ($p < 0.001$). Thus the significant values of R^2 in the present investigation prove the high prognostic ability of the RSM. The experimental values were in agreement with the predicted values confirming the predictability and validity of the model showed in Table 5. Finally, the best formulation was selected from 9 trial formulation (F1-F9) based on achieving optimum value set for the response variables. Various response variables were adjusted and comprehensive evaluation of feasibility search along with exhaustive grid search was done. Expert software from the above plots which has the highest desirability near to 1.0. The extensive grid and feasibility searches provided that the optimum formulations and the respectively desired function response overlay plot (Figure 2), where one solution was found with a highest desirability.

Evaluation of Optimized Gel Formulation

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The result of FTIR peak values was represented in Figure 3. The FTIR spectra of *T. purpurea* leaf extract showed a broad band at 3402 cm^{-1} bonded O-H and at 1604 cm^{-1} band indicated bonded carbonyl and a strong absorption peak at 3296 cm^{-1} indicates presence of carboxylic groups. The FT-IR spectra of physical mixture of gel formulation did not show the presence of any additional peaks for new functional groups. The major peaks of the drug remained unchanged in the mixture were observed in FT-IR spectra. The good extrudability and spreadability are required for the therapeutic efficiency of the formulation. The extrudability of optimized gel formulation was found to be $16.05 \pm 0.032\text{ g/cm}^2$ indicated within acceptable limit. The viscosity of gel formulations was consistent neither too thick nor too thin. Formulation F7 was found nearly highest $60000 \pm 2.03\text{ mPa-s}$ viscosity among all formulation. This may be due to the change of mechanical property of polymer. Spreadability of F7 formulation ($25.1 \pm 0.35\text{ g. cm/sec}$) and swelling index $1046 \pm 0.38\%$ was found more than other formulation. For optimized gel formulation, gelling temperature was found to be $37.04 \pm 0.51^\circ\text{C}$. The mucoadhesive strength of optimized gel formulation was found $28 \pm 0.32\text{ g}$. This can lead to a strong adhesion which may lead to the prolong drug release as more the mucoadhesive strength less would be the washout effect of the formulation. The total flavonoids content in gel formulation was found to be as 51.17 mg GAE/g . The antimicrobial by zone of inhibition studies proved that dental gel of *T. purpurea* leaf extract are highly active against *Staphylococcus aureus* and *Candida albican* with the zone of inhibition in 11mm and 1mm which was similar result obtained to marketed metronidazole (1%) gel formulation with the zone of inhibition in 10 mm and 0.5 mm. Antifungal activity against *Aspergillus niger* does not show zone of inhibition at 500 mg/ml concentration of gel. The antimicrobial study of dental gels resulted in high level of antimicrobial property than that of the standard marketed metronidazole (1% w/v) gel (Table 6). The degradation or spoilage of herbal product may be due to certain chemical reaction such as oxidation reactions. Accelerated stability testing based on single condition of elevated temperature and humidity is more appropriate and suitable for herbal products because of their very basic nature. The stability study results shows stable with no significant changes in the physio-chemical properties of the optimized gel formulation (Table 7). The gel formulation stored in the freeze was more stable as compare to accelerated $40^\circ\text{C}/75\%\text{RH}$.

DISCUSSION

The link between oral diseases and the activities of microbial species that form part of the microbiology of the oral cavity is well established and over 750 species of bacteria inhabit the oral cavity and a number of these are implicated in oral disease²⁰. Flavonoids are found to be abundant in the leaves and they protect against allergies, platelet aggregation, microbial infections and hepatotoxins¹⁰. Excess of water in drug encourage microbial growth, presence of fungi or insects and deterioration following hydrolysis. Ash values are the criteria to judge the identity and purity of crude drug, where total water soluble and acid insoluble

ashes are considered¹². The ethanolic extract showed a positive shinoda test indicating the presence of flavonoids. Co-chromatography with the standard flavonoidal compound, i.e. quercetin on the thin layer chromatographic plates (TLC) showed the spots at the same R_f as the standard compound, further clarifying the presence of flavonoidal compounds in the extract²⁷. The pH of all the formulations was in the range compatible with normal pH range of the skin²⁸. From the results, it is clearly evident that all the gel formulations showed good gelling property and homogeneity. A comparative study of viscosity and spreadability showed that with increase in viscosity of the formulation, the spreadability decreased and vice versa. The swelling index study revealed that the materials under study absorbs more or less ten times of water by its weight. The result of ANOVA demonstrates that the model was significant for all dependent variables. All the independent variables were found to be significant for three responses R_1 (swelling index), R_2 (spreadability) and R_3 (viscosity). The residuals were the difference between observed and predicated value. The polynomial equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the unit of each factor and the intercept is not at the design space²⁹. Three dimensional response surface plots depict a curvilinear relationship between the factors and the response. Higher concentration of HPMC was used, lower swelling index was obtained. This might be due to the fact that higher amount of HPMC stabilized the formulated topical gel, and prevented it from aggregation. The two-dimensional response surface plots were presented significant non linear relationships between the amount of carbopol and HPMC K₄M which are very useful to study the interaction effects of the factors on the responses. The final optimized dental gel formulations (F7) containing 4.0% w/w, HPMC-K₄M, with 3.5% w/w carbopol possesses significant along with its quadratic and interaction terms for all the dependent variables. The similar FTIR result has been reported by Srikar et al³⁰. The broad absorption band was observed between $3436\text{--}3220\text{ cm}^{-1}$ due to the O-H stretching and H-bonded alcohol and phenol groups. A weak band was observed at 1634 cm^{-1} corresponding to N-H bending primary amines. In the current research, ethanolic extract of *T. purpurea* was used which contains majority of phenolic compound for flavonoids that reflect the appearance of absorption peak for particular function groups at that wavelength. The similar results are reported by Packia lakshmi et al³¹ for extract of *T. purpurea*. At higher gelling temperature the dental gel formulations may be in solution form within the dental cavity and at lower gelling temperature the dental gel formulations will be in gel form²⁸. Dental gel formulation in solution form possesses the property of flowing out or wash out easily. So when the dental gel formulations are in solution form the residence time within the dental cavity is less and release will be less, so bioavailability is less as at 37°C , the behaviour of the formulations changed, depending on the polymer concentration. The effect like, the amount of carbopol and HPMC was found to be significant along with its quadratic and interaction terms for all the dependent variables. The optimized *Tephrosia purpurea* extract loaded dental gel formulation (F7) containing 4.0% w/w HPMC-K₄M with 3.5% w/w carbopol possesses marked antimicrobial activity as compared with Metronidazole (1% w/v) gel, found stable of the drug based on ICH guidelines and thus, it can be used in the treatment of dental carries.

Table 1: Compositions of Gel Formulation for Preliminary Batches

Ingredients	Formulation		
	M1	M2	M3
<i>Tephrosia purpurea</i> extract (%)	0.2	0.2	0.2
Carbopol-934 (%)	0.35	0.40	0.45
HPMC K ₄ M (%)	0.45	0.50	0.55
Propyl Paraben (%)	0.06	0.06	0.06
Methyl Paraben (%)	0.2	0.2	0.2
Ethanol (ml)	5	5	5
Triethanolamine	Q.S	Q.S	Q.S
Distilled water (ml)	Upto 100	Upto 100	Upto 100

Table 2: Compositions of Gel by Layout and Coded Value of Variables for Central Composite Design

Ingredients (%)	Formulation Code								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
<i>Tephrosia purpurea</i> extract	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Carbopol-934	0.35	0.21	0.49	0.35	0.25	0.25	0.45	0.45	0.35
HPMC K ₄ M	0.45	0.45	0.45	0.52	0.4	0.5	0.5	0.4	0.38
Propyl Paraben	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Methyl Paraben	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Triethanolamine	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Y ₁ (%)*	1086	1039	1072	848	800	890	1046	915	767
Y ₂ (g.cm/sec)*	3.8	3.2	4.9	3.6	3.4	3.7	5.1	5.5	3.8
Y ₃ (mPa-s)*	65000	41000	64000	57000	40000	26000	60000	68000	39000
Independent variables		Low			high				
X ₁ = Carbopol (%)		0.25			0.45				
X ₂ = HPMC K ₄ M (%)		0.40			0.50				

*Dependent variables; Y₁=Swelling index, Y₂=Spreadability and Y₃=ViscosityTable 3: Preliminary Phytochemical Screening and Physicochemical Parameters of *Tephrosia purpurea* Extract

Phytoconstituents	Inference	Parameters	<i>T. Purpurea</i> extract
Carbohydrate	+	Description	Light green coarse powder
Protein	-	Average particle size	128 μ m
Amino acids	+	Loss on drying	6.8 $\pm$ 0.2%
Glycosides	+	Foreign matter	Less than 2.0%
Flavonoids	+	pH	6.9 $\pm$ 0.2
Alkaloids	-	Total ash	12.01 $\pm$ 0.02%
Steroids and triterpenoids	+	Acid insoluble ash	1.2 $\pm$ 0.01%
Tannins and Phenolics	+	Water soluble extractive	25.04%
Gums	+	Alcohol soluble extractive	17.23%
Fixed oils	-		
Volatile oils	-		
Saponine	+		

‘+’ Indicates Present and ‘-’ Indicate Absent, (n=3, Mean $\pm$ SD)

Table 4: Statistical Response Surface Models

Statistic	Response Variables		
	Y ₁	Y ₂	Y ₃
Std. Dev.	61.37	0.43	10347.9
Mean	962.56	4.11	51111.1
C.V. %	6.38	10.48	20.25
PRESS	N/A	2.61	
R-Squared	0.946	0.7972	0.6351
Adeq Precision	10.248	8.394	5.594
F Value	10.51	11.79	5.22
p-value Prob > F	0.0406	0.0083	0.0485
Model	Significant	Significant	Significant

Table 5: Validation Model Report for Model by Central Composite Parameters

Sr. No	Carbopol	HPMC	Viscosity		Swelling index		Spreadability	
			Measured	Predicated	Measured	Predicated	Measured	Predicated
1	0.45	0.4	62495	62487	241	243	4.89	4.99
2	0.45	0.41	62575	62581	1032	1029	4.88	4.98
3	0.45	0.42	62656	63567	1084	1074	4.87	4.97
4	0.45	0.42	62673	63784	1094	1099	4.87	4.97
5	0.45	0.42	62680	63590	1097	1103	4.87	4.97
6	0.45	0.42	62912	63022	1176	1180	4.5	4.6
Mean			62665.17	63635.5	1075.33	1104.67	4.81	4.91
SD			140.27	480.84	69.59	125.01	0.15	0.51
% RSD			0.22	0.76	6.47	11.32	3.19	3.13

Table 6: In-vitro Antimicrobial Activity of Optimized Gel *Tephrosia purpurea* Extract

Formulation	Zone of inhibition (mm)			
	<i>Aspergillus Niger</i>	<i>Candida Albican</i>	<i>Staphylococcus Aureus</i>	<i>Escherichia coli</i>
Marketed Metronidazole formulation (1%)	R [#]	0.5	10	10
Optimized gel formulation (F7)	R [#]	1	11	12
Ampicillin	R [#]	R [#]	8	9

#R- Resistant (no zone of inhibition)

Table 7: Physiochemical Parameters of *Tephrosia purpurea* Gel at Stability Study

Formulation Parameter	Storage condition		
	Room temperature	40°C/75%RH (accelerated)	-20°C ± 5°C (freezer)
Color	No change in color	No change in color	No change in color
pH	6.8	6.4	6.8
Viscosity	60000 ± 2.03	58000 ± 3.3	60100 ± 2.35
Swelling index	1046 ± 0.38	964 ± 0.25	1065 ± 0.32
Spreadability	25.1 ± 0.35	23.7 ± 0.15	25.9 ± 0.09
% drug release (upto 6 h)	94.43 ± 0.46	98.13 ± 0.16	93.87 ± 0.66

Figure 1: Perturbation, Contour and 3D response graph (a) for Y_1 , (b) for Y_2 and (c) for Y_3 response variables

Figure 2: Overlay plot for optimization of gel by central composite design

Figure 3: FTIR spectra of a) *Tephrosia Purpurea* leaf extract, b) Polymer (HPMC K4M and Carbopol), c) physical mixture of polymer and extract

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