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# FORMULATION AND EVALUATION OF HERBAL ANTIOXIDANT GEL

Samruddhi R. Nale *, Varsha M. Jadhav and Vilasrao J. Kadam

Department of Quality Assurance, Bharati Vidyapeeth's College of Pharmacy, C.B.D. Belapur, Thane, Navi Mumbai - 400614, Maharashtra, India.

**Keywords:**

*Glycyrrhiza glabra*, *Punica granatum*, *Citrus sinensis*, DPPH, Herbal Gel, Antioxidant activity

**Correspondence to Author:**
**Samruddhi R. Nale**

Department of Quality Assurance, Bharati Vidyapeeth's College of Pharmacy, C.B.D. Belapur, Thane, Navi Mumbai - 400614. Maharashtra, India.

**E-mail:** samruddhinale2296@gmail.com

**ABSTRACT: Introduction:** Herbal drugs have great growth potential in the global market. Natural product research continues to explore traditional medicines to develop new novel drugs. The present study aimed to investigate antioxidant potential of gel formulated with *Punica granatum* peel extract, *Glycyrrhiza glabra* root extract and *Citrus sinensis* peel extract. **Method and Materials:** Different gels were formulated by varying the concentration of the extracts and evaluation was done. The formulated gel was compared for antioxidant activity with standard marketed preparation. **Results:** The prepared formulations showed satisfactory rheological characteristics, release behavior, appearance, pH, and spreadability. The optimized gel formulation showed excellent antioxidant activity. The IC50 value of gel was 43.74 µg/ml, and that of marketed preparation was 51.15 µg/ml, which shows good antioxidant property of gel. **Conclusion:** The formulated 5% gel formulation showed good antioxidant activity when compared to the marketed preparation. Thus, the present investigation revealed that the developed gel formulation has potential antioxidant activity.

**INTRODUCTION:** The skin, an organ in direct touch with the environment, shields the body from environmental harm and environmental deterioration, including ultraviolet (UV) rays. However, excessive exposure to UV rays on the skin of the face accelerates skin aging. UVA and UVB make up the two categories of UV light. The epidermis absorbs UVB rays, whereas the endodermis does similarly for UVA rays. In contrast to UVA radiation, which, when absorbed, can hasten the formation of free radicals, UVB rays produce sunburn, which can be avoided with photoprotection or stated as a sun protection factor number.

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Free radicals have unpaired free electrons, which makes them highly reactive substances. Antioxidants can be used to stop this. Reactive oxygen species are neutralized by antioxidants, which stop oxidative damage to cells and tissues [1].

## *Punica granatum* (Pomegranate) [2, 3]:

**FIG. 1:** ***PUNICA GRANATUM PEEL***

**Botanical Name:** *Punica granatum* Linn, Family: Puniceae

**Vernacular Name:** Sanskrit: Dadima, English: Pomegranate, Hindi: Anar, Marathi: Dalimba.

**Botanical Description:** The fruit is a spherical berry measuring 5 to 12 cm and its pericarp is leathery. Each pink-red piece of pulp-like tissue that divides the interior into compartments holds a seed grain. Globose fruits with a coriaceous, woody rind and persistent callipe.

**Chemical Constituents:** Alkaloids, Flavonoids, PolyPhenol, Tannin, Saponins.

**Pharmacological Activities:** Antioxidant, Anti-inflammatory, Antiulcer, Antidiabetic, Antipyretic and Analgesic, Diuretic, CNS Effects, Snake Bite Therapy, Anticonvulsant, Antimicrobial, Immuno-modulatory, DNA Protective, Anticancer.

**Part used:** Peels

***Glycyrrhiza glabra* L. (Licorice): [4, 5]**

**FIG. 2: *GLYCYRRHIZA GLABRA* ROOTS**

**Botanical Name:** Root of *Glycyrrhiza glabra* Linn, Family: Leguminoseae.

**Vernacular Name:** Sanskrit- Yashti-madhuh, English- Licorice, Hindi- Jothi-madh or Mulhatti, Marathi- Jeshtamadha.

**Botanical Description:** Licorice has a thick network of branching roots. The roots are long, cylindrical (round) and straight pieces of wrinkly, fibrous wood that spread out horizontally underground.

**Chemical Constituents:** Amino Acids, Triterpene Saponin, Flavonoids, Annins, Glycosides, Protein, Resins, Sterols, Volatile oils.

**Pharmacological Activities:** Antioxidant, Antiulcer, Anti-inflammatory, Anti-diabetic, antimicrobial effects. It may ease upper respiratory infections, treat ulcers, aid digestion and Skin lightening and tightening activity.

**Part used:** Roots

***Citrus sinensis* L. (Orange) [6, 7]:**

**FIG. 3: *CITRUS SINENSIS* PEELS**

**Botanical Name:** *Citrus sinesis* (L.), Family: Rutaceae, is known botanically.

**Vernacular Name:** Marathi – Santri, Hindi – Santra

**Chemical Constituents:** Flavonoids, Alkaloids, Coumarins, Vitamin C and Triterpenes.

**Pharmacological Activities:** Antioxidant, Cardio vascular health, Antianxiety, used in treatment of depression and stress and also Menstrual disorder.

**Part used:** Peels

## MATERIAL AND METHODS:

**Plant Material:** The *Punica granatum* peel, *Glycyrrhiza glabra* root and *Citrus sinensis* peel in powdered form was purchased from 'Yucca Enterprises' Wadala (e), Mumbai. Alarsin Pharmaceuticals authenticated all drugs. A/32, Street No. 3 M.I.D.C. Andheri (East). Mumbai, India. Herbal powders were stored in an airtight container. The reagents and chemicals utilized were all of LR grade.

**Preparation of Extracts:** *Punica granatum*, *Glycyrrhiza glabra*, and *Citrus sinensis* individual drugs weighing 50 g each were extracted with ethanol using the hot continuous percolation method in a Soxhlet system. The ethanolic extracts

were filtered, evaporated, and dried in an electric water bath. For the creation of the gel, dried extracts of *Punica granatum*, *Glycyrrhiza glabra* and *Citrus sinensis* were also employed.

**Development of Formulation [8, 9, 13]:** Ingredients used for gel formulation are given in **Table 1.** A water-soluble gel was prepared using the dried ethanol extract of Pomegranate peels, Licorice roots and orange peels. The gel comprised carbapol-940, propylene glycol, DMSO, methyl paraben, propyl paraben, Triethanolamine, perfume and distill water.

**Procedure:** The amount of water needed for these formulations was split into two portions. The exact amount of extract dissolved in propylene glycol and DMSO in one portion. Carbopol 940 was uniformly mixed in a different section for 30 minutes with a mechanical stirrer set to 1200 rpm. Methyl and propyl paraben were then gradually added to this solution along with the medication solution. The pH was then corrected as necessary (6.8-7) were in the narrow range of neutral pH using Triethanolamine (TEM) and swirled gently until a clear gel was created. The volume was then increased to 100 ml by adding the remaining distilled water. A few drops of fragrance liquid were added. Formulations with different extract ratios of 1%, 3% and 5% were produced. Collapsible tubes were used to hold gel.

**TABLE 1: PRELIMINARY FORMULATION DEVELOPMENT**

| Ingredients | Batches quantity taken per 100 g (in grams) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| | G1 (1%) | G2 (3%) | G3 (5%) | G4 (1%) | G5 (3%) | G6 (5%) | G7 (1%) | G8 (3%) | G9 (5%) | G10 (1%) | G11 (3%) | G12 (5%) | G13 (1%) | G14 (3%) | G15 (5%) |
| Pomogranate Extract | 0.4 | 1.2 | 2 | 0.4 | 1.2 | 2 | 0.4 | 1.2 | 2 | 0.4 | 1.2 | 2 | 0.4 | 1.2 | 2 |
| Licorice Extract | 0.2 | 0.6 | 1 | 0.2 | 0.6 | 1 | 0.2 | 0.6 | 1 | 0.2 | 0.6 | 1 | 0.2 | 0.6 | 1 |
| Orange Extract | 0.2 | 0.6 | 1 | 0.2 | 0.6 | 1 | 0.2 | 0.6 | 1 | 0.2 | 0.6 | 1 | 0.2 | 0.6 | 1 |
| HPMC | 1 | 1 | 1 | - | - | - | - | - | - | - | - | - | - | - | - |
| Sodium CMC | - | - | - | 1 | 1 | 1 | - | - | - | - | - | - | - | - | - |
| Propylene glycol 400 | 20 | 20 | 20 | 20 | 20 | 20 | 9 | 9 | 9 | 9 | 9 | 9 | 8 | 8 | 8 |
| Carbopol 940 | - | - | - | - | - | - | 1 | 1 | 1 | 0.6 | 0.6 | 0.6 | 0.8 | 0.8 | 0.8 |
| DMSO | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 5 | 5 | 5 | 5 | 5 | 5 |
| Methyl Paraben | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 |
| Propyl Paraben | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Triethanolamine | Qs. | Qs. | Qs. | Qs. | Qs. | Qs. | Qs. | Qs. | Qs. | Qs. | Qs. | Qs. | Qs. | Qs. | Qs. |
| Water | Qs. | Qs. | Qs. | Qs. | Qs. | Qs. | Qs. | Qs. | Qs. | Qs. | Qs. | Qs. | Qs. | Qs. | Qs. |
| Perfume | Qs. | Qs. | Qs. | Qs. | Qs. | Qs. | Qs. | Qs. | Qs. | Qs. | Qs. | Qs. | Qs. | Qs. | Qs. |

**Evaluation of Gel:** The topical gel formulations from each of the twelve batches were assessed. Several characteristics, including appearance, pH, spreadability, viscosity, extrudability, drug content and *in-vitro* drug diffusion tests using cellulose acetate membrane, were used to choose the optimal batch. All of these factors were compared to a commercially available preparation.

**Physical Observation:** The formulation's colour, smell, transparency and appearance were noted.

**pH Measurement:** Using a digital pH metre (MK-VI, Syntronics), the pH of the formulation was determined by fully submerging the glass electrode in the gel system. Prior to usage, the pH metre was standardised using buffers with pH values of 4.0 and 7.0.

**Extrudability:** The formulation was filled taken into collapsible aluminium tubes and after the gels were set in the container, extrudability of formulation was determined.

**Rheological Study:**

**Viscosity:** The produced compositions' viscosities were measured using a Brookfield Synchro-electric Viscometer (Model LVT). A sufficient amount of sample was put in a cylindrical tube, and it was let five minutes to equilibrate before the dial reading at 0.3 spindle speed was measured. The dial reading on the viscometer was recorded for each speed. The dial reading that corresponded to each decrease in spindle speed was recorded. The viscosity in centipoises was obtained by directly multiplying the dial reading by the coefficients listed in the Brookfield viscometer catalogue. Utilizing spindle number 4 at a spindle speed of 0.3, the viscosity of gels was measured. To determine viscosity, the average of three observations was used.

**Spreadability:** 0.5g of gel was placed on a glass plate premarked with a circle of 1cm diameter, and a second plate was placed above it. Then the weight of 50g was allowed to rest on the upper glass plate for 5 min. It was observed that the diameter increased as a result of the gel spreading. Spread circle diameters were measured in centimeters and used as benchmarks for spreadability.

**Assay (%Drug Content):** 1 gm of gel in a 100 ml volumetric flask was used to determine the drug content of the gel formulation. A sufficient amount of methanol (for drug extraction from gel) was added to a volumetric flask, and the flask was kept in a warm water bath for drug extraction with intermittent shaking.

After cooling to room temperature, the volume was increased to 100 ml with methanol. The drug's methanolic solution was filtered through a 0.45 syringe-driven filter and 1 ml of the filtrate was diluted to 10 ml with methanol before being subjected to HPTLC analysis. The drug content was calculated using the calibration curve's linear regression equation [10].

***In-vitro* Drug Diffusion Studies:** Based on the antifungal study and gel evaluation, selected batches of formulation were tested for drug diffusion using a Franz diffusion cell with a surface area of 2.09 $cm^2$ and a receptor compartment having capacity of 20 ml. The cell is divided into two chambers: the donor and the receptor. As a receptor medium, a combination of pH 6.8 phosphate buffer and methanol (70:30) was used. The addition of 30% methanol to phosphate buffer pH 6.8 was done to ensure sink conditions.

The temperature of the diffusion medium was thermostatically controlled at $37^o$ C 1o C by surrounding water in the jacket, and the medium was stirred at 500 rpm by a magnetic stirrer. One gramme of gel sample was placed on a 0.45µ cellulose acetate membrane and then fixed in the diffusion cell's donor and receptor compartments. For 30 minutes before applying formulations, the membrane was allowed to equilibrate with the receptor medium. To prevent vehicle evaporation, the donor compartment was covered with aluminium foil. At predetermined intervals, 2 mL samples were withdrawn and replaced with an equal volume of fresh fluid. The withdrawn samples were spectrophoto-metrically estimated at 250 and 265 nm wavelengths using the UV simultaneous method [11].

***In-vitro* Antioxidant Activity:**

**Materials and Methods:**

**Measurement of Antioxidant Activity:** The antioxidant activity of the ethanolic extracts was determined by their ability to scavenge the free radical 1,1-diphenyl-2-picryl hydrazyl (DPPH). DPPH is a stable free radical with an odd electron in its structure commonly used in chemical analysis to detect radical scavenging activity. 1ml of each extract solution at different concentrations (20, 40, 60, 80 and 100 g/ml) was added to 3ml of 0.5mM ethanolic DPPH free radical solution.

After 30 minutes, the absorbance of the preparations was measured at 517 nm using a UV spectrophotometer and compared to the absorbance of standard ascorbic acid concentrations (20, 40, 60, 80, and 100 g/ml). The method described was used to measure the absorbance with some modifications. Then the % inhibition was calculated by the following equation:

$$\%\ radicalscavenging\ activity = [(absorbance\ of\ blank - absorbance\ of\ sample) / absorbance\ of\ blank] \times 100$$

From calibration curves, obtained from different concentrations of the extracts, the $IC_{50}$ (Inhibitory concentration 50%) was determined. $IC_{50}$ value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals [12].

## RESULT AND DISCUSSION:

**Formulation Development and Evaluation of Gel:** Precipitation and turbidity occurred in batches of the HPMC gel-containing drug, possibly due to system incompatibility; thus, this batch was discarded. Sodium CMC batches formed an overly stiff gel system with an unappealing appearance. Batches containing carbopol 940 (G7-G15) formed a proper gel, so these nine batches were chosen for further investigation.

**Physical Appearance of Gel Formulations:** Gel formulations were examined visually for clarity, colour, homogeneity, consistency and particle presence. A microscope was used to examine homogeneity. To investigate the consistency of the

formulations, a small amount of gel was pressed between the thumb and index fingers and the gel's consistency was noted. All gel formulation batches (G7-G15) were translucent with a smooth and homogeneous texture.

**TABLE 2: EVALUATION OF BATCHES**

| Batches | Appearance | | Extrudability | Homogeneity | | |
|---|---|---|---|---|---|---|
| | Translucent | | Excellent | Good | | |
| | Spreadability* (cm) | pH* | Viscosity* (cP) | Ellagic acid | Glycyrrhizic acid | Hesperidin |
| G7 | 4.5 | 6.96 | 8654.10 | 88.16 | 91.25 | 87.96 |
| G8 | 4.2 | 6.99 | 8595.98 | 98.32 | 90.54 | 98.63 |
| G9 | 4.6 | 6.90 | 8501.20 | 97.81 | 94.55 | 95.55 |
| G10 | 5.3 | 6.98 | 8420.19 | 89.65 | 93.33 | 92.05 |
| G11 | 5.5 | 6.97 | 7562.32 | 96.2 | 94.25 | 89.62 |
| G12 | 5.4 | 6.98 | 7512.08 | 91.63 | 96.80 | 94.06 |
| G13 | 5.4 | 6.95 | 7490.42 | 95.25 | 89.60 | 91.35 |
| G14 | 5.5 | 6.97 | 7279.11 | 92.38 | 97.54 | 93.60 |
| G15 | 5.6 | 6.99 | 7020.14 | 98.57 | 99.24 | 98.99 |

*_Average of Three Determinations

## *In-vitro* Drug Release:

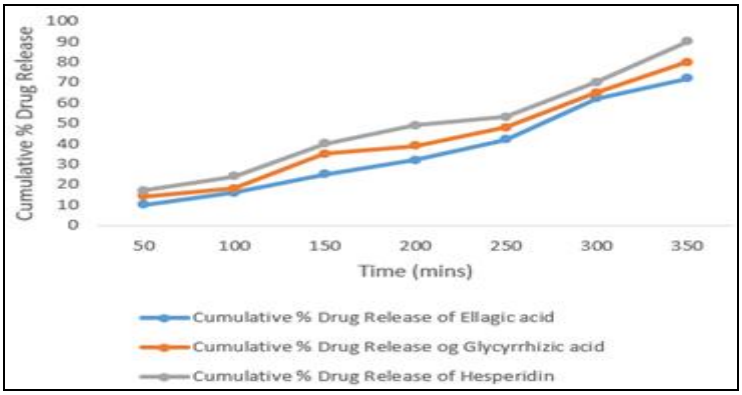


**FIG. 4: *IN-VITRO* DRUG RELEASE OF ELLAGIC ACID, GLYCYRRHIZIC ACID AND HESPERIDIN**

## *In-vitro* Antioxidant Test:

**DPPH Free Radical Scavenging Activity (*In-vitro* Antioxidant Activity):** The present findings exhibited a concentration-dependent free radical scavenging (antioxidant) activity by the extracts throughout the selected concentration ranges with reference to ascorbic acid. The $IC_{50}$ values of *Punica granatum* peel extract were found to be 50.47µg/ml, for *Glycyrrhiza glabra* root extract 52.38µg/ml and *Citrus sinensis* peel extract was found to be 54.08 µg/ml. The $IC_{50}$ value for ascorbic acid was found to be 61.9µg/ml which Indicates that the extract shows better antioxidant activity than standard ascorbic acid.

## *Punica granatum* Peel Extract:

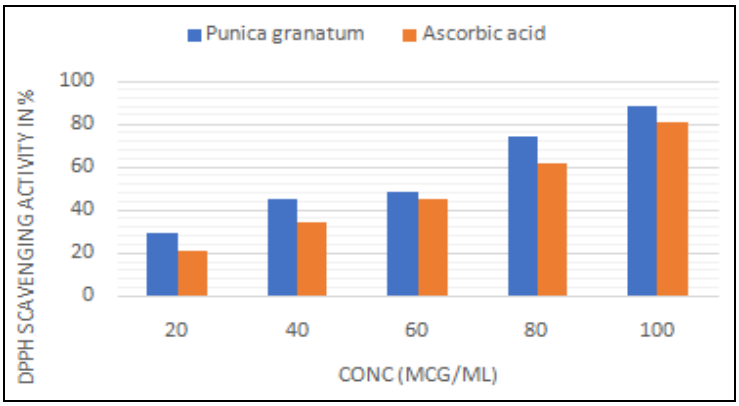


**FIG. 5: GRAPH SHOWING FREE RADICAL SCAVENGING ACTIVITY OF *PUNICA GRANATUM* EXTRACT**

**TABLE 3: ANTIOXIDANT ACTIVITY OF *PUNICA GRANATUM* EXTRACT**

| Concentration (µg/ml) | | % Scavenging activity | |
|---|---|---|---|
| ***Punica granatum* peel Extract** | **Ascorbic acid** | ***Punica granatum* peel Extract** | **Ascorbic acid** |
| 20 | 20 | 29.12 | 20.89 |
| 40 | 40 | 45.04 | 34.25 |
| 60 | 60 | 48.32 | 45.05 |
| 80 | 80 | 74.54 | 62.01 |
| 100 | 100 | 88.1 | 80.5 |

## *Glycyrrhiza glabra* Root Extract:

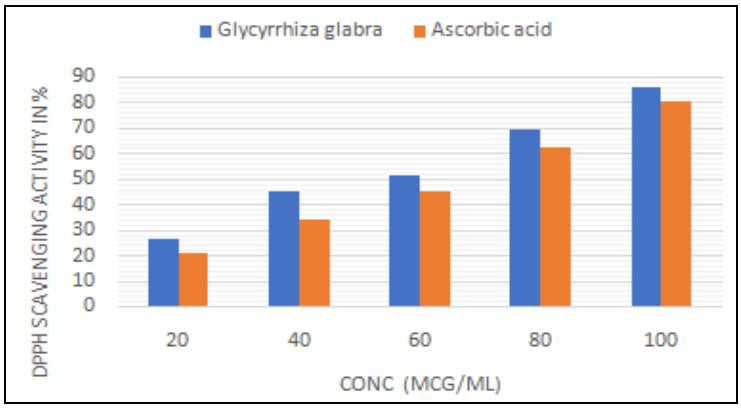


**FIG. 6: GRAPH SHOWING FREE RADICAL SCAVENGING ACTIVITY OF *GLYCYRRHIZA GLABRA* EXTRACT**

**TABLE 4: ANTIOXIDANT ACTIVITY OF *GLYCYRRHIZA GLABRA* EXTRACT**

| Concentration (µg/ml) | | % Scavenging activity | |
|---|---|---|---|
| ***Glycyrrhiza glabra* root Extract** | **Ascorbic acid** | ***Glycyrrhiza glabra* root Extract** | **Ascorbic acid** |
| 20 | 20 | 26.52 | 20.89 |
| 40 | 40 | 44.71 | 34.25 |
| 60 | 60 | 51.26 | 45.05 |
| 80 | 80 | 68.99 | 62.01 |
| 100 | 100 | 85.68 | 80.5 |

## *Citrus sinensis* Peel Extract:

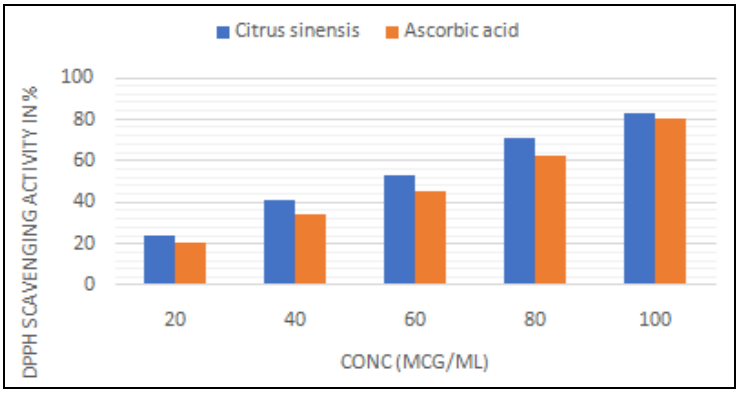


**FIG. 7: GRAPH SHOWING FREE RADICAL SCAVENGING ACTIVITY OF *CITRUS SINENSIS* EXTRACT**

**TABLE 5: ANTIOXIDANT ACTIVITY OF *CITRUS SINENSIS* EXTRACT**

| Concentration (µg/ml) | | % Scavenging activity | |
|---|---|---|---|
| ***Citrus sinensis* peel Extract** | **Ascorbic acid** | ***Citrus sinensis* peel Extract** | **Ascorbic acid** |
| 20 | 20 | 24.16 | 20.89 |
| 40 | 40 | 40.88 | 34.25 |
| 60 | 60 | 53.14 | 45.05 |
| 80 | 80 | 70.57 | 62.01 |
| 100 | 100 | 83.04 | 80.5 |

The antioxidant activity of prepared gel was then compared with marketed formulation which is Patanjali Soundarya gel.

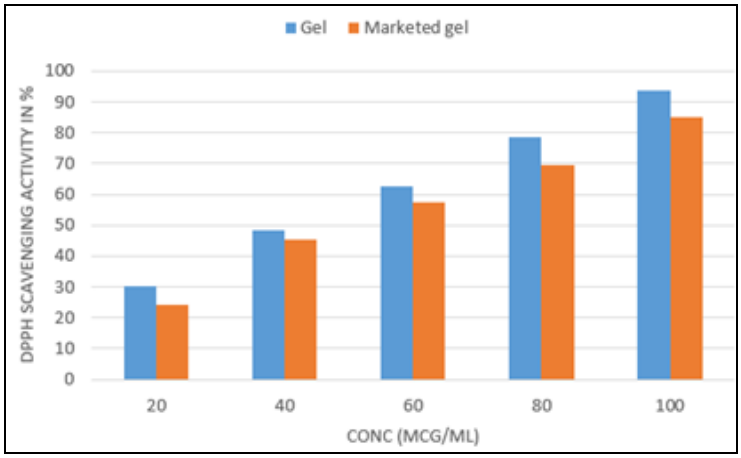


**FIG. 8: GRAPH SHOWING FREE RADICAL SCAVENGING ACTIVITY OF GEL FORMULATION AND MARKETED GEL EXTRACT**

**TABLE 6: ANTIOXIDANT ACTIVITY OF GEL AND MARKETED GEL FORMULATION**

| Concentration (µg/ml) | | % Scavenging activity | |
|---|---|---|---|
| **Gel** | **Marketed gel** | **Gel** | **Marketed gel** |
| 20 | 20 | 30.30 | 24.4 |
| 40 | 40 | 48.5 | 45.5 |
| 60 | 60 | 62.6 | 57.6 |
| 80 | 80 | 78.8 | 69.7 |
| 100 | 100 | 93.9 | 84.9 |

$IC_{50}$ value of prepared gel was found to be 43.74 µg/ml and that of marketed gel was found to be 51.15 µg/ml, which show that the prepared gel has good antioxidant property.

**CONCLUSION:** In the present work, an attempt has been made to formulate and evaluate the combination of herbal drugs and incorporation in gel formulation. Gel containing crude extracts of *Punica granatum* peel, *Glycyrrhiza glabra* root, and *Citrus sinensis* peel extract with different concentrations was prepared.

Which is having good antioxidant. It can be summarized that the prepared formulations showed satisfactory rheological characteristics, release behaviour, appearance, pH, spreadability and antioxidant activity.

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