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Research Article

Formulation And Evaluation of Herbal Gel of *Syzygium cumini* of Antioxidant Activity

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ABSTRACT

Syzygium cumini (L.) Skeels, commonly known as Jamun or Indian blackberry, is a medicinal plant widely recognized for its diverse pharmacological properties, particularly its antioxidant potential. This project investigates the antioxidant activity of bark extract of *Syzygium cumini* using standard in vitro method such as Superoxide Dismutase Assay (SOD). The phytochemical screening revealed the presence of phenolic compounds, flavonoids, and tannins, which contribute significantly to its antioxidant activity. The results demonstrated that the ethanolic seed extract showed the highest free radical scavenging activity, indicating its potential as a natural antioxidant source. The present study focuses on the formulation and evaluation of an antioxidant herbal gel using *Syzygium cumini* (L.) Skeels, a medicinal plant known for its rich phytochemical profile and potent antioxidant properties. The antioxidant herbal gel demonstrated significant free radical scavenging activity, indicating its potential to protect the skin from oxidative stress and environmental damage. The findings suggest that *Syzygium cumini*-based herbal gel could be a promising natural alternative for skincare applications and further studies may explore its therapeutic potential in dermatological conditions.

INTRODUCTION

The project mainly aims in preparing pharmaceutical gel dosage form which is the "Formulation and evaluation of herbal antioxidant gel" it has antioxidant activity, which prevents free radicals from the skin and helps as moisturizing and hydrating medication.

Plant profile:

Binomial nomenclature:

- Kingdom: Plantae
- Order: Myrtales
- Family: Myrtaceae
- Genus: Syzygium
- Species: Cumini

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- Binomial name: *Syzygium cumini* (L) Skeels.^[1] **(2) Preparation of ethanolic extract:**



Fig 1: *Syzygium cumini* bark

Syzygium cumini (syn. *Eugenia Jambolana*), a member of family Myrtaceae is commonly known as Jamun or Jambul in Hindi and Black Plum or Indian Blackberry in English. *S. Cumini* is an evergreen tree distributed in the Indian sub-continent, south-east Asian countries and eastern Africa. *S. cumini*, is widely used in different countries including India for the treatment of many disorders including diabetes. Various parts of this plant have been recognized to possess several medicinal properties in the traditional system of medicine. The bark of the plant is carminative, digestive, anti-oxidant, anti-helminthic and antibacterial.^[2]

MATERIALS AND METHODS:

Materials:

The bark of *Syzygium cumini* was collected from local source.

Method:

(1) Preparation of crude drug powder:

Bark of *Syzygium cumini* is collected from village and cleaned through washing. Then shade dried for 15 days under room temperature and grinded well. Fine powdered drug is obtained and used for extraction process.^[4]

The powdered bark of *Syzygium cumini* were used for extraction. The bark of *Syzygium cumini* were carefully cleaned/washed and then using a soxhlet apparatus with ethanol. The extraction process was carried out for 5 hours, 7 cycle of ethanolic extract were runned repeatively. The resulting filtrate was used for phytochemical test and preparation of formulation. The product is stored in desiccator for further studies.^[4]



Fig 2: Soxhlet apparatus



Fig 3: Ethanolic extract of *Syzygium cumini*

Phytochemical screening:

The preliminary phytochemical screening of *Syzygium cumini* bark was done by the following tests;

Test for Carbohydrates:

- Molisch's test: To 2-3ml aq extract, add few drops of alpha-naphthol solution in alcohol, shake

add conc. H_2SO_4 from side test tube. violet ring is formed at junction of two liquid.

Test for Alkaloids

1. Dragendorff's test: To 2-3 ml filtrate extract, add few drops Dragendorff's reagent. Orange brown ppt. is formed.
2. Hager's test: 2-3 ml filtrate with Hager's reagent gives yellow ppt.

Test for Tannins:

- a) Ferric chloride test: Add a few magnesium turnings and concentrated hydrochloric acid to the extract. Appearance of pink or red color indicates flavonoids.
- b) Potassium Dichromate: To 1-2 ml extract, add pinch of Potassium Dichromate appearance red ppt.

Test for Steroids:

- a) Salkowski reaction: To 2 ml of extract, add 2ml chloroform and 2ml conc. H_2SO_4 . Shake well chloroform layer appears red and acid layer shows greenish yellow fluorescence.
- b) Liebermann-Burchard Reaction: Mix 2 ml extract with chloroform, Add 1-2 ml acetic anhydride and 2 drops conc. H_2SO_4 from the side of test tube, First red, then blue and finally green colour appear.

Test for Flavonoids:

- a) Lead acetate test: to small quantity of residue, add lead acetate, yellow coloured precipitate.
- b) NaOH and HCL test: Addition of increasing amount of sodium hydroxide to the residue shows colouration which decolourises after addition on acid.

Test for Saponin:

Foam test: About 2 ml of the extract was shaken with 5ml of distilled water Foam shows the presence of Saponins^[3]

Physico-chemical Test:

(a) Total ash:

The method is designed to measure the total amount of material remaining after ignition. This includes both 'physiological ash', which is derived from the plant tissue itself, and 'non-physiological ash' which is the residue of the extraneous matter (e. g. sand and soil) adhering to the plant surface.

Procedure:

1. Weigh about 2gm of powdered drug and ignite flat thin porcelain dish or a tared silica crucible.
2. Support the dish on a pipe clay triangle placed on a ring of retort stand.
3. Heat with burner, using a flame about 2 cm high and support the dish about 7cm above the flame, heat with vapour almost ceased to be evolved: then lower the dish and heat more strongly until all the carbon is burnt off. Cool in desiccator.
4. Weigh the ash and calculate the percentage of the total ash with reference to the air-dried sample of the drug.

$$\text{Total ash percentage} = \frac{\text{Pw} - \text{Fw}}{\text{W}} \times 100$$

Where, Pw = Pre weight of the crucible

Fw = Final weight of the crucible

W = Total weight of powdered plant material.
^[3]

(b) Determination of alcohol-soluble extractive value:

Alcohol-soluble extractive value is applied for the drug which contain alcohol soluble constituents such as tannins, resin, and alkaloids.



Procedure:

1. Weigh about 4 gm of coarsely powdered drug in a weighing bottle and transfer it to a dry conical flask.
2. Fill a 100 ml graduated flask to the delivery marks with the solvent (90% alcohol).
3. Wash out the weighing bottle and pour the washing, together with the remainder of the solvent into the conical flask.
4. Cork the flask and set aside for 24 hours, shaking frequently.
5. Filter into a 50 ml cylinder.
6. When sufficient filtrate has collected, transfer 25 ml of the filtrate to a weighed, thin porcelain disk as used for the ash values determination.
7. Evaporate to dryness on a water bath and complete the drying in a oven at 105 degree Celsius for 6 hrs
8. Cool in a desiccator for 30 min and weigh immediately. [3]

(c) Determination of water-soluble extractive value:

Water soluble extractive value is applied for the drug which contain water soluble constituents such as tannins, sugars, plant acid.

Procedure:

1. About 4 gm of coarsely powdered drug in a weighing bottle and transfer it to a dry 250 ml conical flask.
2. Fill a 100ml graduated flask to a delivery mark with the water.
3. Wash out the weighing bottle and pour the washing, together with the remainder of the solvent into the conical flask.
4. Cork the flask and set aside for 24 hours, shaking frequently.
5. Filter into a 50 ml cylinder.

6. When sufficient filtrate has collected, transfer 25 ml of the filtrate to a weighed, thin porcelain dish, as used for the ash values determination.
7. Evaporate to dryness on a water bath and complete the drying in an oven at 105degree Celsius for 6 hours.
8. Cool in a desiccator for 30 min and weight immediately.
9. Calculate the percentage w/w of extractive with reference to the air dried drug. [3]

Formulation:

(a) Preparation of Gel formulation:

1 g of carbapol-940 was dispersed in 50 ml of distilled water kept the beaker a side to swell the carbapol-940 to form gel. Take 5ml of distilled water and required quantity of propyl paraben were dissolved by heating on water bath solution was cooled and propylene glycol 400 and CMC added. Further required quantity of extract was mixed to the above mixture and add this solution into the carbapol 940 gel with continuous stirring and add triethanolamine was added to the formulation to adjust pH required to skin.[4]



Gelling



Gel

Fig 4:Gel Formulation

Chemicals: *Syzygium cumini* extract, Carbopol940, Propylene Glycol, Triethanolamine, Methyl paraben, Glycerine, Carboxy methyl cellulose

Apparatus: Beaker, stirrer, tripod stand, burner

Instruments: Brookfield viscometer, pH meter.

(d) Methylparaben: Preservative

Excipient functions:

(a) Carbopol 940: Gelling Agent

- Forms a clear, stable gel with high viscosity, enhancing gel consistency.
- Provides muco-adhesion, ensuring prolonged contact time with the skin.
- Requires neutralization with Triethanolamine to form a smooth gel.¹³

(b) Propylene Glycol: Penetration Enhancer & Humectant

- Increases drug penetration through the skin by disrupting stratum corneum lipids.
- Acts as a humectant, preventing drying of the gel.
- Enhances the solubility of Clitoriaternatea extract in the gel.

(c) Triethanolamine: pH Adjuster

- Neutralizes Carbopol 940, converting it into a stable gel.
- Maintains the gel's pH within the skin-compatible range (5.5-6.5).
- Ensures better consistency and uniform dispersion of ingredients.

- Prevents microbial contamination, extending shelf life.
- Effective against bacteria and fungi, ensuring product stability.
- Widely used in pharmaceutical and cosmetic formulations due to its safety and effectiveness at low concentrations.¹⁸

(e) Glycerine: Moisturising agent

- Glycerin attracts water from the air and binds it to the skin, helping to retain moisture and prevent dryness.
- It's a natural humectant, meaning it helps the skin hold onto moisture.
- It can help soften and heal dry, cracked, or irritated skin.

(f) Carboxy methyl cellulose: Thickening agent

- CMC is used as a thickening and stabilizing agent in food products like salad dressings, sauces, and dairy products.
- It can improve the texture and consistency of foods, especially in low-fat or diet products.
- It helps prevent ingredient separation and settling.

Formulation table:

Table no.1: Formulation table

Ingredient	Quantity						Roles of Ingredient
	F1	F2	F3	F4	F5	F6	
Drug Extract	0.5g	1g	1.5g	0.5g	1g	1.5g	Antioxidant Activity
Carbopol940	1g	1g	1g	1.5 g	1.5 g	1.5 g	Gelling Agent
Propylene Glycol	2.5ml	2.5 ml	2.5 ml	2.5 ml	2.5 ml	2.5 ml	Moisturizer
Methylparaben	0.1g	0.1 g	0.1 g	0.1 g	0.1 g	0.1 g	Preservative
Triethanolamine	0.1ml	0.1ml	0.1ml	0.1ml	0.1ml	0.1ml	Surfactant, Neutralizer
Glycerine	1ml	1ml	1ml	1ml	1ml	1ml	Moisturising agent
Carboxy methyl cellulose	0.5g	0.5g	0.5g	0.5g	0.5g	0.5g	Thickening agent
Distilled water	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	vehicle

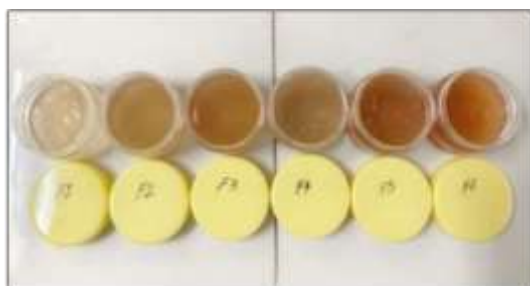


Fig 5 :Antioxidant Herbal Gel formulation of different Concentration

(3) Evaluation parameters of gel formulation:

- a) **Visual inspection:** The gel were examined for their physical properties by visual inspection of colour, clarity, homogeneity, odour etc. [4]
- b) **Appearance:** All the formulation of antioxidant gel was pale yellow/yellow in colour. [4]
- c) **Consistency:** The consistency was checked by applying on the skin. [4]
- d) **Greasiness:** The greasiness was assisted by the application on the skin and the slide [4]
- e) **Irritancy test (patch test):** Skin irritation test was performed for the selected gels on human volunteers to find out any irritation problems which could make it unsuitable for topical use. Skin irritation test was performed, for each gel on three volunteers. Approximately 1gm of gel was topically applied to the hand near the wrist over 2 square inch area and observed for any lesions, irritation, allergy or edema etc. [4]
- f) **Washability:** Gel Formulation was applied on hand and was observed continuously under running water [4]
- g) **Determination of pH:** The pH of gel was determined using digital pH meter by dipping the glass electrode completely into the gel and by using pH paper [4]

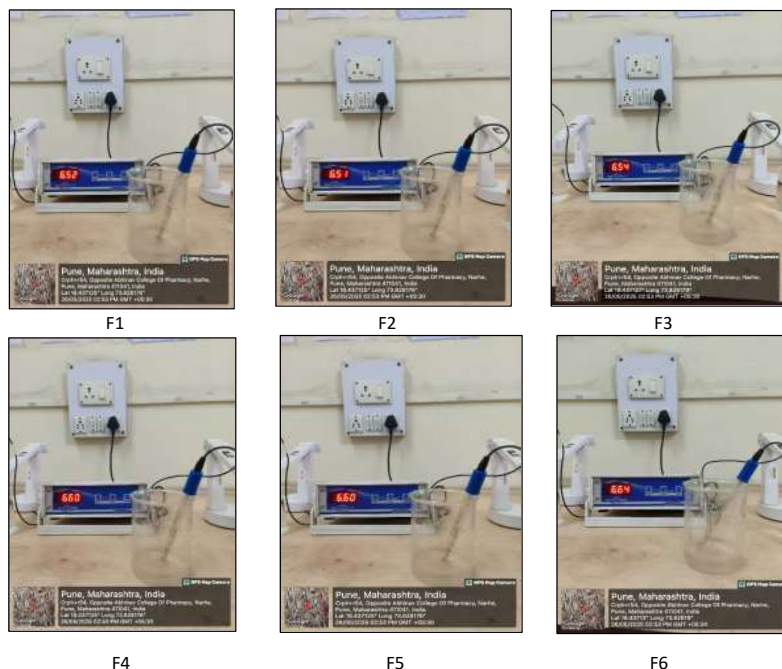


Fig 6: pH test of different batches

- h) **Determination of spreadability:** Spreadability was measured on the basis of “slip” and “drag” characteristics of the gels ground glass slide was fixed on wooden block. an weight is provided by pulley ground slide was fixed on this block. 2gm of gel placed on the slide make sandwich weight of 100 gm. placed on the top of the two slides for 5 minutes excess of gel (about 2 gm.) was scrapped out from edges top plate subjected to pull of 20 gm. weight with help of stirring attached hook. Time in seconds required by the top slide cover a distance 7.5 was noted. Spreadability was determined using following

formula, $S=M.L/T$ where, S is the spreadability in grams.cm/sec, M is the mass in grams, T is time in seconds^[4]

viscometer (speed 60 rpm. At 250 c. was used for gels, corresponding dial reading on the meter was noted. ^[4]

- i) **Determination of viscosity:** Viscosities of the formulated gels was determined using

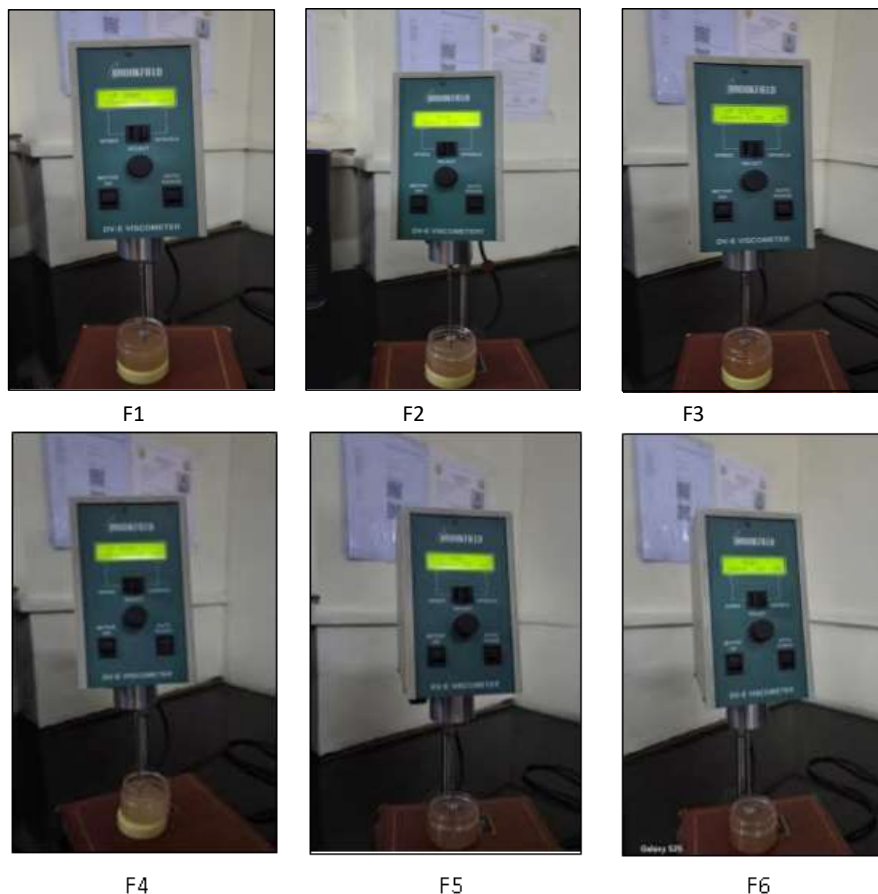


Fig 7: Viscosity test of different batches

- j) **Clarity:** The prepared gel formulation was evaluated in glass container and observed under the glass. ^[4]

Result and discussion:

As we performed all evaluation parameters tests, the results are also found for every single tests of the gel formulation. Evaluation of herbal gel has been performed with parameters given for the evaluation purpose of the gel. It is evaluated by all

factors like physicochemical as well as phytochemical factors.

Table no.2: Preliminary Characteristics of Gel

Sr.no.	Parameter	Observation
1	Colour	Light Green
2	Odour	Slight
3	Acid value	2.45
4	Ester value	36.75
5	Solubility in ethanol	Freely soluble
6	Solubility in chloroform	Soluble

Table no.3: Test for carbohydrates, Alkaloids, flavonoids, tannins, steroids and saponin

Sr.No	Test	Observation	Inference
1	Test of Carbohydrate		

	a) Molish's test	Violet ring observed	Carbohydrate Present
2	Test of Alkaloids		
	a) Dragendroff's test b) Hager's test	Orange brown precipitate Yellow precipitate	Alkaloids present
3	Test of Tannins		
	a) Ferric chloride b) Pot.dichromate	Dark green solution Red precipitate	Tannins present
4	Test of steroids a) Salkowski reaction b) Lieberman-Burchaid Reaction	Chloroform layer red and acid layer green precipitate Green colour appeared	Steroid Present
5	Test of Flavonoids a) Lead acetate b) NaOH and HCl	Yellow precipitate, colourless liquid	Flavonoids present
6	Test of Saponin a) Foam test	Foam is observed	Saponin present



Fig 8: Phytochemical screening

Table no.4: Physico-chemical test results

Sr.no.	Parameter	Observation
1	Ash value	8 % w/w
2	Alcohol-soluble extractive value	20 g
3	Water-soluble extractive value	

Calculation:**(a) Ash value:**

Weight of empty dish (x) = 22.50g

Weight of powder (y) = 2 g

Weight of dish with powder = 24.50 g

After incineration weight of dish (z) = 22.34 g

 Weight of ash (z-x) = 22.34- 22.50
 = 0.16

Total Ash = (100 × Weight of Ash) / Weight of Powder

$$= (100 \times 0.16) / 2$$

$$= 8 \% \text{ w/w}$$



Fig 9: Ash value

(b) Alcohol-soluble extractive value:

Weight of porcelain with dried extract = 66.62 g

Weight of dried extract = Weight of porcelain with

dried extract - Weight of porcelain

$$= 66.62 - 66.42$$

$$= 0.2\text{g}$$

25 ml of alcoholic extract gives = 0.2 g of dried extract

$$100 \text{ ml of alcoholic extract gives} = 0.2/25 \times 100$$

$$= 0.8\text{g}$$

4 g of air-dried drug gives = 0.8g of alcoholic
soluble extractives
100g of air-dried drug gives = $0.8/4 \times 100$
= 20 g of alcoholic
soluble extractives



Fig 10: Alcohol-soluble extractive value

(c) Water-soluble extractive value:

Weight of porcelain = 65.14g
Weight of porcelain with dried extract = 65.25g
Weight of dried extract = Weight of porcelain with
dried extract - Weight of porcelain
= $65.25 - 65.14$

= 0.09 g
25 ml of aqueous extract gives = 0.09 g of dried
extract
100 ml of aqueous extract gives = $0.09/25 \times 100$
= 0.36 g
4 g of air -dried drug gives = 0.36 g of water -
soluble extractives
100 g of air -dried drug gives = $0.36/4 \times 100$
= 9 g of water- soluble
extractives



Fig 11: Water-soluble extractive value

Table no.5: Some basic parameters like; pH, spreadability, viscosity, homogeneity and clarity is well measured as per IP

Formulation	pH	Spreadability	Viscosity	Homogeneity	Clarity
F1	6.52	5.32	2000	Average	Not clear
F2	6.51	6.08	3124	Good	Not clear
F3	6.54	6.04	3327	Very Good	Clear
F4	6.60	7.24	3749	Good	Clear
F5	6.60	7.60	6249	Good	Partially Clear
F6	6.64	7.60	7998	Good	Not clear

Result of activity determination:

In-vitro Antioxidant activity test:

Superoxide Dismutase (SOD):

Procedure:

1. Prepare the assay solutions and add to tubes in the order shown in Table 1. The following recipe is for one sample (20 tubes of the assay system).
2. Turn on the visible light wavelengths on the spectrophotometer. For measuring total SOD activity, prepare a blank solution by adding

200 µl of PB to 800 µl of total assay solution. Place the solution in a 1 ml disposable cuvette and blank the machine at 560 nm using the appropriate blank.

3. The XO concentration of the assay system should be adjusted to an absorbance/min result between 0.02-0.025. For the total SOD assay, add 100 µl of PB, 800 µl of total SOD assay solution, and 100 µl of total SOD assay XO solution. Measure the absorbance of the sample immediately. If the reading is < 0.02, adjust with XO stock. If the reading is > 0.025, adjust with DETAPAC. Repeat until the proper absorbance is measured.

4. Using a stock solution of 1,000 ng/ μ l SOD, prepare three dilutions as stated below (Table 2). Measure the absorbance of the dilutions at 560 nm and prepare a standard curve. Calculate the K_m of SOD. If the assay is working properly, the K_m should be near 7-15 ng. This standard is utilized only to ensure that the assay is working correctly.
5. To prepare and run the samples, label tubes (10 $\times$ 75 or 12 $\times$ 75 mm glass tubes) for the total SOD assay. The tubes required are (13 tubes total): blank, control 1, control 2, sample dilution 1, 2, 3, 4, 5, 6, 7, 8, 9, 10. The blank is the background subtraction, and controls are XO added only. XO sample results represent NBT-associated reduction only, where the XO control is equal to 100% NBT reduction.
6. Using the original sample lysate, prepare 500 μ l of a 1 μ g/ μ l sample. Using the diluted sample, prepare total SOD assay tubes to contain 2, 5, 10, 15, 25, 50, and 100 μ g of protein. Next, using the original lysate, prepare total SOD assay tubes containing 200, 300, and 500 μ g protein. Add the necessary amount of PB to each tube for a final volume of 100 μ l. Add 200 μ l of PB to the blank tube and 100 μ l of PB to the control XO tubes. vii. Add 800 μ l of the appropriate assay solution to the tubes. Mix by tapping the racks gently on the laboratory bench. Incubate the total SOD assay tubes for 10 min at room temperature.
7. Transfer the solutions to 1 ml disposable cuvettes. Use the total assay blank tube to calibrate the spectrophotometer. If using a spectrophotometer with a 6-well chamber, arrange the samples as Part A: XO control, dilution 1, 2, 3, 4, 5. Add 100 μ l of the total SOD XO solution to the cuvettes and immediately read the samples. Run the

kinetics assay for 5 min. Record the rates for each dilution. Repeat with the second dilution series, Part B: XO control, dilution 6, 7, 8, 9, 10.

8. To calculate SOD activity, determine the % inhibition using the following formula:

a) Critical Step: Add BSA first to prevent BCS precipitation

Table no.6: Solutions prepared for Superoxide Dismutase System

Solutions	Final Concentrations	Volume required for SOD System
PB with DETAPA C and BSA	0.05 M PB, 1 mM DETAPAC, 0.13 mg BSA	12.9 ml
Catalase 40 U ml ⁻¹	1 U	0.5 ml
Xanthine 1.18 mM	100 μ M	1.7 ml
NBT 2.24	56 μ M	0.5 ml
PB	0.05 M	0.3 ml
BCS 10 mM	50 μ M	0.1 ml
Total volume		16.0 ml

b) Preparation of three dilutions

Table no.6: Dilution Preparation

Dilution	Volume of SOD	Volume of PB	Final Concentration (ng/tube)
Dilution #1 (1:1000)	1 μ l	999 μ l	2-10
Dilution #2 (1:100)	3 μ l	297 μ l	25-50
Dilution #3 (1:10)	5 μ l	45 μ l	500

Calculation:

$$\text{Inhibition(\%)} = \frac{\text{Control XO rate} - \text{Sample rate}}{\text{Control XO rate}} \times 100$$



Table no.7: Results observed for F3 batch

Sr.no	Sample Type	Dilution Factor	Protein Concentration (µg/ml)	Control XO Rate (Abs/min)	Sample Rate (Abs/min)	Percent (100)	% Inhibition
	Control XO	-	-				
1	Sample Dilution 1	1.10	20	0.52	0.25	100	51.92
2	Sample Dilution 2	1.08	50	0.36	0.11	100	69.44
3	Sample Dilution 3	1.06	100	0.41	0.22	100	46.34
4	Sample Dilution 4	1.04	150	0.96	0.36	100	62.50
5	Sample Dilution 5	1.03	250	0.56	0.32	100	42.86
6	Sample Dilution 6	1.02.05	500	0.37	0.21	100	43.24
7	Sample Dilution 7	1.02	1000	0.45	0.11	100	75.56
8	Sample Dilution 8	1.01.05	2000	0.51	0.24	100	52.94
9	Sample Dilution 9	1.01.02	3000	0.78	0.54	100	30.77
10	Sample Dilution 9	1.01.02	3000	0.82	0.26	100	68.29
11	Sample Dilution 10	1.01	5000	0.47	0.11	100	76.60

CONCLUSION:

This explains about overall information of herbal antioxidant gel formulation, its physicochemical, phytochemical parameters and evaluation of gel formulation. Antioxidant gel formulation is an effective medication for restoring moisture and hydration to the skin. Antioxidant activity gel inhibits damage to the skin cells by reducing free radicals.

FUTURE PERSPECTIVE:

Herbal gel formulation possessing antioxidant activity is helpful and effective to deliver moisturizing and hydrating effect on topical application onto the skin. *Syzygium cumini* bark

having more flavonoid and phenolic content essential to show antioxidant activity. This herbal gel formulation can reduce damage of skin cell from environmental or other free radicals.

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