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FORMULATION AND EVALUATION OF A POLY-HERBAL GEL CONTAINING *OXALIS CORNICULATA* AND *PORTULACA OLERACEA* LEAF EXTRACTS: A TOPICAL APPROACH

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Keywords:

Oxalis corniculata, Portulaca oleracea, phytochemical screening, Antimicrobial assay, Antioxidant activity, Toxicity study on L929 cell lines

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ABSTRACT: Introduction: *Oxalis corniculata* (Family: Oxalidaceae) is a delicate therapeutic herb found in warm temperate tropical areas of India and Pakistan, while *Portulaca oleracea* (Family: Portulacaceae) thrives in sultry, equatorial regions and has origins in South America and Africa. This study aims to develop and assess a topical poly-herbal gel containing leaf extracts of *Oxalis corniculata* and *Portulaca oleracea*. **Materials and Methods:** The plant material was collected, and shade dried before being powdered. The powder was soaked and macerated in methanol, followed by extraction using Soxhlet method. Phytochemical tests were conducted qualitatively. Gel formulations were prepared and the appearance, homogeneity, pH, and viscosity of the formulations were determined. Antimicrobial activity was evaluated. Antioxidant potential was analyzed through the DPPH scavenging assay. Additionally, in-vitro toxicity of formulations was assessed using the MTT assay to determine their compatibility with normal cells. **Results:** Both plants showed presence of alkaloids, tannins, phenols, flavonoids, and steroids. The gel formulations exhibited good appearance which were tested for antimicrobial activity against *S. aureus* and *K. pneumoniae*, which demonstrated their bactericidal activity. Antioxidant potential was increased with higher concentration of extracts. The in-vitro toxicity assessment using MTT assay showed no toxic effects on L929 cell line. There were no morphological alterations or cell shrinkage, confirming plants were non-toxic to normal cells. **Conclusion:** This study revealed *Oxalis corniculata* and *Portulaca oleracea* contains a wide range of secondary metabolites with antioxidant and antibacterial properties, suggesting their potential use in treatment of various skin ailments.

INTRODUCTION: Nature offers an enormous source of medicinal composites from plant life and notable quantity of modern medications identified. Reason behind investigation were drugs known for its traditional remedial uses [1]. Since, centuries old, medicinal plants aids as prime basis of preserve for human maladies. It's not surprising that one-fourth of world's residents, rely on traditional medicines for treatment of innumerable sicknesses.

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Currently, there has been lot of focus on implementing sustainable and bio-friendly plant-based medications for prevention and treatment of many human ailments [2]. Plant species have been by tradition exploited as basis of medicine for eras and are indispensable module of Indian health-care system [3], as recounted to be nontoxic with trifling ramification linked with imitation remedies [4].

Verdant medicines are thought to be risk-free and non-hazardous to body than synthetic pharmaceuticals on global scale. As an outcome, research facilities are examining plants for latent medicinal biological processes [5]. Lavish medicinal plants are extensively utilized in treatment of epidermis infirmities, additionally acknowledged to

retain antibacterial action [6]. Inimitable herb, *Oxalis corniculata* (*OC*), **Fig. 1** ordinarily stated as Changeri, member of family *Oxalidaceae* found throughout world's tropical and subtropical regions. It is herbaceous plant that grows on greenswards, in dappled areas, beside waysides and in all of India's warmer regions. The leaves are virtuous source of Oxalate, Calcium, Phosphorus, Iron, niacin, Vitamin-C, water, fat and carbohydrate. It helps in treating variety of skin ailments, amputation of moles and corns [7]. Due to lack of acquaintance about medical properties, it's not often consumed. For skin outbursts, acne, burns there have been countless medical aspects comprising antibacterial assets. In view of that, herb is acclaimed for its uplifting qualities [8].

It is origin of India having varied range of biological actions, remarkable plant with composition of all essential modules for individual's virtuous well-being. *O. corniculata* employed as antioxidant, antifungal, anthelmintic, anti-inflammatory, analgesic, astringent, anticancer, depurative, diuretic and antibacterial properties [9]. Tiptoeing wood sorrel, tip-toe *Oxalis*, slumbering beauty and procumbent yellow sorrel are common appellations of *OC*. It's very active herb for treating stomach and liver problems in ayurvedic medication system. Plant leaves are eatable, with flavorful taste just like flavor of lemons [10].

FIG. 1: *OXALIS CORNICULATA* (OC)

Portulaca oleracea (*PO*), **Fig. 2** habitually acknowledged as Purslane, prominently from *Portulacaceae* family. Have global scattering, nurtures primarily in sultry and equatorial regions, origins in South America and Africa. *PO* used as vegetable, considered one of most far and wide disseminated, malicious weeds in world, ample research has focused on its nutritive value [11].

Traditional medicine has faith in purslane for relieving comprehensive scale of ailments, including Abdominal issues, Breathing disorders, Liver irritation, Kidney-Bladder boils, Malaises, Sleeplessness, Severe soreness, Migraines [12]. It is sneaking, prostrate groundcover shrub with fleshy ovoid leaves used in herbal treatments. This plant demonstrates wide range of pharmacological actions, anti-fungal, anticancer, anti-inflammatory, antioxidant, anti-diabetic, antibacterial, wound healing properties. Number of reserves, encompassing Magnesium, Potassium, Nitrate, as well as riboflavin, niacin, pyridoxine is existing. Influential antioxidants comprise β-carotene, vitamins C and E, flavonoids, alkaloids, polysaccharides and momentous extent of Omega-3 fatty acid [13].

Used to cure critical eczema, herpes zoster, warts, diarrhea, mammary abscess in clinical application. Up-to-date researches put forward purslane as lipid-lowering, antitumor and hypoglycemic [14].Utilization of gels at irrational spots on surface of bodyput accelerative remarkable compensations in quicker drug permeation associated with emulsion, cream [15]. Gel formulations composed of additives, gelling agents and active constituents. Carbopol is often active gelling agents. An acrylic polymer, safe for reliable usage, doesn't infuriate skin, making it apt for gel compositions [16].

FIG. 2: *PORTULACA OLERACEA* (PO)

Carbopol polymers have admirable water absorption capabilities. In water, they swell to produce gel having 1000-fold and 10-fold increase in volume and diameter respectively. Topical gel equipped via carbopol, methyl paraben, propyl paraben, propylene glycol, tri-ethanolamine [17]. As a consequence, in present study methanolic extracts of *OC* and *PO* were incorporated into poly-herbal

carbopol base gel and assessed. Antimicrobial screening, cytotoxicity study on L929 cell lines by MTT assay and antioxidant potential of *OC*, *PO* and their combination was done to substantiate its long span expenditure. Compared to additional semisolid preparations, gels are more favorable to utilize and have superior immersion through skin.

MATERIALS AND METHOD:

Chemicals and Reagents: Methanol (HPLC Grade), Dragendorff's reagent, Mayer's reagent, Hager's reagent, Wagner's reagent, Nitric acid, 5% $FeCl_3$ solution, Lead acetate, Gelatin solution, α-naphthol, Sulphuric acid, Molisch's reagent, distilled water, Chloroform, Carbopol-934, Propyl paraben, Triethanolamine, Propylene glycol, Methyl paraben, Muller-Hinton agar and broth (Himedia), DMEM, MTT (Himedia), *etc*. were used. Each chemical and reagents were analytical grade.

Collection of Plant Material and Authentication: *OC* and *PO,* were collected from local areas of Belagavi city. The plant was authenticated by KLE's Shri B.M. Kankanawadi Ayurveda Mahavidyalaya, Department of Central Research Facility, Deemed-to-be- University, Belagavi, Karnataka, India. The aerial plant parts including leaves were collected in October and November 2022.

Sample Preparation: The aerial parts of the both plants were shade dried for 3-4 weeks at a room temperature. The dried aerial parts of *OC* and *PO* was coarsely powdered using grinding machine. About 125g powder of *OC* and 150g powder of *PO* were soaked and macerated at room temperature with 750mL and 500mL methanol respectively, for 2-3 days. Thereafter, it was filtered using muslin cloth in conical flask [18].

Soxhlet Extraction: Powdered leaves of *OC* and *PO* were packed in Soxhlet. Methanol used for Soxhletion. Extraction sustained at temperature of 20°C until pure solvent was detected in thimble. Extract was concentrated in water bath at 80°C. Concentrated extract was collected in air-tight container and stored in refrigerator for supplementary use [19].

Phyto-chemical Screening: Crude extracts of *OC*, *PO* used with diverse organic-solvents and water to check solubility confirming polar, non-polar elements. Qualitatively tried to check presence of different phyto-chemical elements such as alkaloid, tannin, phenolic compound, flavonoid, carbohydrate and steroid in the mentioned *OC*, *PO* plant extract by several standard tests used for phytochemical studies [20].

Preparation of Topical Gel: Gel prepared via methanolic extracts of *OC*, *PO* and their combination. The carbopol 934 was dispersed in 20mL of distilled water, kept to swell, nonstop mixing on magnetic stirrer forms gel. Propyl-paraben, Methyl-paraben and Triethanolamine dissolved in Propylene-glycol. Triethanolamine added with continuous stirring. Further, vital amount of extract mixed to above combination [21]. The composition of herbal gel of *OC*, *PO* and their combination prepared is tabulated in the **Table 1.**

TABLE 1: COMPOSITION OF GEL FORMULATION

Sr. no.	Ingredient	Formulation		
		1% gel	1.5% gel	2% gel
1	Extract (mg)	200	200	200
2	Carbopol-934 (mg)	200	300	400
3	Propyl-paraben (mg)	2	2	2
4	Triethanolamine	QS	QS	QS
5	Propylene-glycol	QS	QS	QS
6	Methyl-paraben (mg)	700	700	700
7	Distilled water (mL)	20	20	20

Evaluation of Herbal Gel: Evaluation parameters of the formulated gel are as follows [22].

Homogeneity and Appearance: Formulated gels were evaluated for physical appearance and homogeneity by visual observation.

pH: pH measurement of gel measured using digital pH meter by plunging glass electrode into gel container to dip the electrode. Readings calculated in triplicates, average of three readings was noted.

Viscosity: Formulated gel was subjected to check viscosity by means of Viscometer (Brookfield) at 25°C having spindle speed of 100 rpm.

Antioxidant Activity:

DPPH Antioxidant Free Radical Scavenging Assay: With modifications to procedure identified by Brand-Williams *et al.,* [23] DPPH activity of *OC*, *PO*and their combination was determined. Concentrations (50µL to 250µL) of *OC*, *PO*, their combination, and a 0.004% DPPH solution were developed in methanol. 1 mL of solution combined with methanolic *OC*, *PO*, their combination extracts and a separate solution of standard ascorbic acid. At 517 nm, the mixture's absorbance was measured after 30 minutes of dark incubation. The level of DPPH-purple decolorization to DPPH-yellow specified the extract's scavenging capability. A reaction mixture with lower absorbance had higher capacity to scavenge free radicals. The free-radical scavenging action against DPPH calculated using equation:

DPPH assay (%) = A control - A sample / A control x 100

Where, A control = absorbance of control (1mL methanol + 1mL DPPH solution), A sample = absorbance of test sample (*OC*, *PO*, their combination), Outcomes were examined in triplicates [24].

Antimicrobial Activity:

Microbial Culture: The *OC*, *PO* and their combination were inspected for anti-microbial action against gram-positive and gram-negative microbial strains. Microbial strains procured-MTCC, Chandigarh, India. The gram-positive microbial strain was *Staphylococcus aureus* (MTCC-96), gram-negative microbial strainwas *Klebsiella pneumoniae* (MTCC-109). Strains preserved in Muller-Hinton Broth at 4°C, sub-cultured whenever needed.

MIC Determination: MIC is lowest concentration of anti-microbial inhibiting growth of bacteria after 24 h. The *OC*, *PO* and their combination subjected to serial broth-dilution procedure for defining MIC. Muller-Hinton broth used to make stock solutions for two-fold serial-dilution of concentrations (100mg/mL to 1.56mg/mL). Standard Gentamicin added as control. 10µL of 0.5 Mc Farland bacterial culture added to solutions, incubation for 24 h. MIC values obtained by visual observation taking into consideration no noticeable growth as MIC [25].

MBC Determination: MBC, the lowest possible concentration of the substance needed to kill microorganisms. MBC is calculated by streaking test dilutions from MIC tubes in a loop-full suspension onto fresh Muller-Hinton agar plates. Plates were incubated for 24 h. The highest dilution that resulted in microbial colonies on plates that were half of the negative control was noted as MBC [25].

Toxicity Assessment - *In-vitro* Assay: The bio-compatibility of *OC*, *PO* and their combination checked using Standard assay- "MTT [3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide]" on L929 cell lines. MTT assay based on alteration of MTT to formazan crystals by active cells, inferring its mitochondrial activity.

Culturing of Cell Line: The cell line L929 procured from NCCS, Pune, India. Data sheet with sixteen STR loci proved to be 100% matching with ATCC STR profile. After procuring cell lines, cells maintained, subcultured in DMEM (89mL) (Himedia, ref: AL250A), added with 10mL FBS (Himedia), 1mL antibiotics in T-25 flasks, maintained, incubated in 5% CO_2incubator with 95% humidity atmosphere.

All process of cell culture accomplished in Class II cabinet considering all aseptic conditions. On cells reaching 85% confluency, trypsinization performed using trypsin, subculturing done, cells observed under inverted microscope. Cell count done, cell viability verified by trypan blue with hemocytometer.

Treatment Groups: L929 cell lines treated with *OC*, *PO*, their combination (1mg/mL). Test compounds preparedin DMSO earlier to experimentation. Reactant mixes diluted with media, cells treated with concentration ranges of *OC*, *PO*, their combination (1000 µg/mL to 62.5 µg/mL), incubated 24 h. Following treatment groups for L929 normal cell line study.

Negative control: normal cells, Test groups: cells + *OC*; cells + *PO*; and cells + their combination [24].

MTT Cell Viability Assay: During cells in log phase of growth, MTT-assay performed in 96-well plate. Initially, on 96-well plate markings done considering negative and positive control in triplicate wells. Trypan blue performed, approximately 5 x 10^3 viable cells seeded in 96-well plate. Later, complete media added to make volume of 150µL, incubated in CO_2 incubator overnight.

After 24 h 100µL of *OC*, *PO* and their combination added with different concentrations (1000µg/mL to 62.5µg/mL), incubated. After 24 h, 20µL of MTT dye added, plate wrapped in silver foil (MTT is photosensitive), incubated at 37°C for 4 h, supernatant removed, formazan obtained solubilized by 100µL DMSO addition. Absorbance recorded at 570nm using ELISA reader, results analyzed in triplicates, percentage calculated [24].

RESULTS: Plants collected from native areas of Belagavi city were identified as *Oxalis corniculata* (Changeri) and *Portulaca oleracea* (Purslane) relying on their taxonomic appearances, and the presence of secondary metabolites and preliminary phyto-chemicals in selected plant extracts were identified.

Total Extractive Value of Crude Leaves Extracts: Complete extractive value of crude methanolic leaf extracts of *OC*, *PO* by solvent methanol were 20g and 18g (w/w) respectively. Properties of *OC*, *PO* extracts are depicted in **Table 2.**

TABLE 2: EXTRACT PROPERTIES

Parameter	Observation	Parameter
	OC	
Colour	Dark green	Colour
Odour	Characteristic	Odour
Appearance	Sticky	Appearance
Extractive value (w/w)	20g	Extractive value (w/w)

Phyto-chemical Screening: Preliminary screening of *OC*, *PO* methanolic leaf extracts revealed presence of phyto-chemical elements. *OC*, *PO* leaf extracts highly soluble in methanol when tested for solubility indicated alkaloid, tannin and phenols, flavonoid, steroid considerably present although carbohydrates were absent mentioned in **Table 3.**

TABLE 3: PHYTOCHEMICAL SCREENING OF OC (*OXALIS CORNICULATA*) AND PO (*PORTULACA OLERACEA)* LEAF EXTRACT

Preliminary phytochemical screening		*OC*	*PO*
Constituents	**Tests**		
Alkaloids	Dragendorff's Test	+	+
	Mayer's Test	-	+
	Hager's Test	+	-
	Wagner's Test	+	+
Tannin and	$FeCl_3$ Test	+	+
Phenolic compounds	Lead (II) acetate Test	+	+
	Gelatin Test	+	+
	Dil. HNO_3 Test	-	+
Flavonoids	H_2SO_4 Test	+	+
	Lead (II) acetate Test	+	+
Carbohydrates	Molisch's Test	-	-
Steroids	Salkowski's Test	+	+

(+) Present; (-) Absent

Topical Gel Preparation: The poly-herbal gels of methanolic extracts of *OC*, *PO* and their combination prepared using Carbopol-934. Physio-chemical properties of formulated gels shown in **Table 4.** From findings concluded that all formulated gels presented good appearance and homogeneity. Among all gels, 1% gel showed good results and selected for further studies. Colour of gel was green and translucent appearance. 6.9 to 7.2 was the pH range of formulated gel. To confirm even application of gel, viscosity was measured.

TABLE 4: EVALUATION OF FORMULATED GEL

Formulation of gel	Colour	Appearance	Homogeneity	pH	Viscosity
OC	Light green	Smooth, translucent	Good	6.87	662cP
PO	Greenish	Smooth, translucent	Good	6.94	769cP
Their combination	Light green	Smooth, translucent	Good	6.8	729cP

Antioxidant Activity: In current investigation, DPPH-free radical scavenging experiment was performed on various doses of methanolic leaves extract from *OC*, *PO*, their combination. The antioxidant capability of *OC*, *PO*, their combination was compared to ascorbic acid **Fig. 3**. With respect to increase in concentration (50µg/mL to 250µg/mL), *OC*, *PO*, their combination showed increased percentage inhibitions (antioxidant activity) **Table 5.**

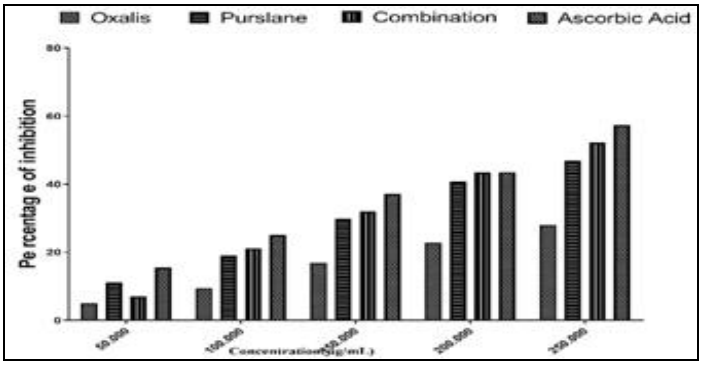

FIG. 3: DPPH FREE RADICAL SCAVENGING ASSAY FOR *OC*, *PO*, THEIR COMBINATION, ASCORBIC ACID

TABLE 5: DPPH ACTIVITY (% INHIBITION) AND IC_{50} VALUES OF *OC*, *PO*, THEIR COMBINATION, ASCORBIC ACID

Concentration (µg/mL)	Percentage of Inhibition			
	OC	***PO***	**Their combination**	**Ascorbic Acid**
50	5.05	11.04	7.02	15.48
100	9.49	19.05	21.16	25.03
150	16.77	29.83	31.95	37.11
200	22.81	40.78	43.41	43.57
250	27.92	46.92	52.24	57.35
IC_{50} Value	470.66	250.33	259.32	202.12

Antimicrobial Activity: Anti-bacterial activity of *OC*, *PO* and their combination assayed by MIC method against two bacterial strains, *viz*., *S. aureus* and *K. pneumoniae*. *OC*, *PO* and their combination found to be operative in inhibiting bacterial growth of gram-positive strain *S. aureus* with MIC ranges of 100mg/mL respectively and gram-negative strain *K. pneumoniae* with MIC ranges of 50mg/mL and 100mg/mL for *OC* and *PO* respectively. *OC* alone revealed bactericidal action by MBC ranging from 100mg/mL against *K. pneumoniae*. The *PO* and combination were not bactericidal at tested range against *K. pneumonia.*

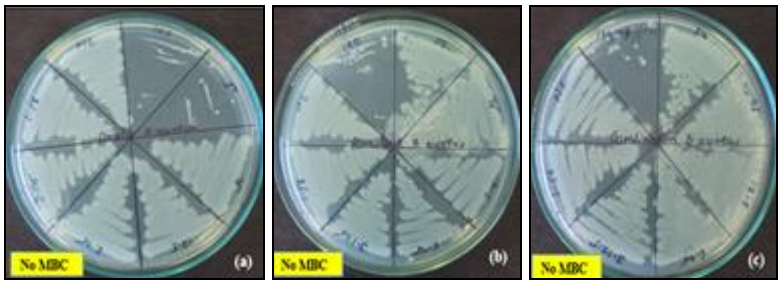

FIG. 4(A): ANTIBACTERIAL ACTIVITY OF (A) OC, (B) PO AND (C) THEIR COMBINATION AGAINST *S. AUREUS*

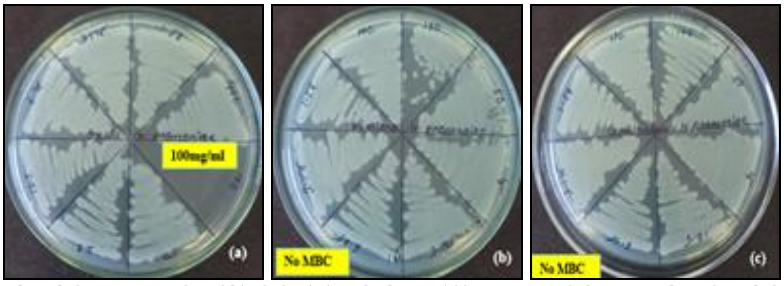

FIG. 4(B): ANTIBACTERIAL ACTIVITY OF (A) OC, (B) PO AND (C) THEIR COMBINATION AGAINST *K. PNEUMONIAE*

TABLE 6: ANTIBACTERIAL ACTIVITY OF OC, PO, THEIR COMBINATION BY MIC METHOD

Plant extract	MIC (mg mL^{-1})		MBC (mg mL^{-1})	
	Staphylococcus aureus	***Klebsiella pneumoniae***	***Staphylococcus aureus***	***Klebsiella pneumoniae***
OC	100	50	-	100
PO	100	100	-	-
Their combination	100	-	-	-

***In-vitro* Toxicity Assay:** The MTT method is used to test the toxicity of both isolated drugs and crude extracts. It is based on the reduction of MTT by mitochondrial dehydrogenase, resulting in the formation of purple formazan. Using L929, an *in-vitro* cytotoxicity test was carried out in the current study to check the biocompatibility of *OC*, *PO*, their combinations. All the three compounds *OC*, *PO* and their combination revealed no cytotoxic effect towards non-cancerous L929 cell line. Results revealed no morphological alterations and no shrinkage of cells by *OC*, *PO*, their combination in normal cell lines. **Table 7** and **Fig. 5** depicts the % cell viability of *OC*, *PO*, their combination at various concentrations (1000, 500, 250, 125, 62.5 µg/mL). **Fig. 6** depicts the influence of *OC*, *PO*, their combination on L929 cell lines.

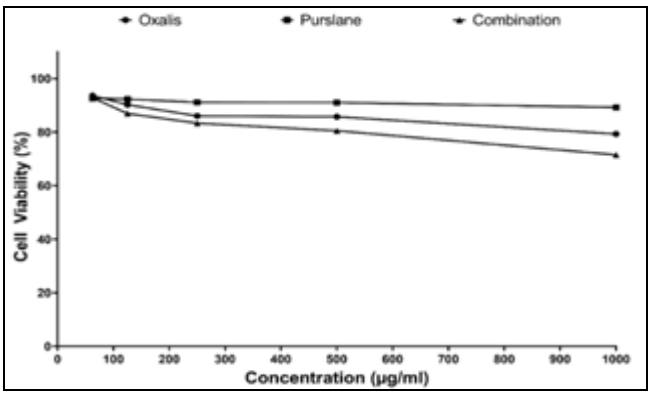

FIG. 5: % CELL VIABILITY OF *OC*, *PO*, THEIR COMBINATION ON L929

TABLE 7: PERCENTAGE OF VIABLE CELLS

Concentration (µg/mL)	% cell-viability		
	OC	***PO***	**Their combination**
1000	79.3	89.2	71.5
500	85.7	91	80.5
250	86	91.1	83.3
125	90.2	92.3	86.9
62.5	93.6	92.6	92.8

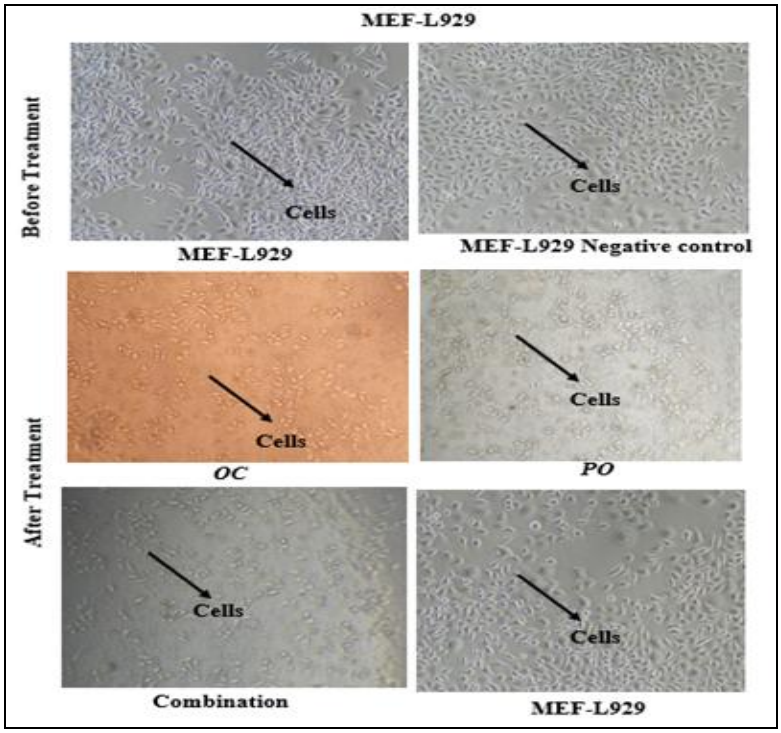

FIG. 6: INFLUENCE OF OC, PO, THEIR COMBINATION ON MEF-L929 CELL LINE

DISCUSSION: Plants collected from native areas of Belagavi city were identified as *Oxalis corniculata* (Changeri) and *Portulaca oleracea* (Purslane) relying on their taxonomic appearances, and the presence of secondary metabolites and preliminary phyto-chemicals in selected plant extracts were identified. Both *OC* and *PO* methanolic extracts were highly soluble in methanol when tested for solubility implementing organic solvents and water, then employed for phytochemical investigation. Alkaloid, Tannin, Phenols, Flavonoid and Steroid were notably present in plants, based on several biochemical analyses, whereas carbohydrates were absent. The formulation of the herbal gel was evaluated for viscosity, pH, and appearance. Formulated gels exhibited good uniformity and appearance. 1% gel produced positive outcomes and was chosen for study.

By using the MIC method, the antibacterial activity of *OC*, *PO* andtheir combination was evaluated against the gram-positive strain *S. aureus* and the gram-negative strain *K. pneumoniae*. *OC*, *PO*, their combination has been found to be beneficial for reducing bacterial growth of *S. aureus* and *K. pneumoniae*. In the current study, the total antioxidant test was evaluated using DPPH activity. Antioxidant activity of *OC*, *PO*, their combinations was studied in comparison with conventional ascorbic acid. The experimental data demonstrated that *OC*, *PO*, and their combination have free radical scavenging characteristics, with increased antioxidant capacity as concentration increases.

The MTT method is used to test the toxicity of both isolated drugs and crude extracts. It is based on the reduction of MTT by mitochondrial dehydrogenase, resulting in the formation of purple formazan.Using L929, an in-vitro cytotoxicity test was carried out in the current study to check the biocompatibility of *OC*, *PO*, their combinations. There was no influence on the growth of L929 cells. Concentrations of *OC*, *PO*, their combination (1000, 500, 250, 125, 62.5 µg/mL) examined in triplicates by serial dilution demonstrated increases in cell viability as concentrations gradually decreased.

CONCLUSION: In accordance to results of study conducted, it was discovered that plants *OC* and *PO* have wide ranges of secondary-metabolites with antioxidant and antibacterial properties, which can be used to treat variety of skin maladies. By increasing concentrations of *OC* and *PO*, it is recommended that further research to be carried out on the anti-microbial effects of methanolic *OC*, *PO* and their combination on microorganisms. The various characteristics of the developed gel formulations were assessed, and the findings suggest that physical parameters of gel formulations are good. Therefore, it may be concluded that the study provides a scientific basis for conducting additional research into the principal compounds found in *OC* and *PO*, evaluating their anticancer efficacy in *in-vivo* animal models, which could aid in future drug discovery and development.

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Author's Contribution: MK and PS contributed to the concept and designed the research study. MK contributed for experimental work of extracting plant material and manuscript preparation. MK performed the research. PS, VK provided help and advice on the experiments. PS and VK contributed to the analysis and interpretation of the data and finalization of manuscript.

CONFLICT OF INTEREST The authors declare no conflict of interest.

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