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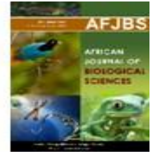

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Evaluation and Comparison of Antioxidant Activity and Phytochemical Profiles of Ouret Lanata Extracts

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Abstract

Phytochemicals can help prevent or treat numerous common illnesses. Identifying and isolating these phytochemicals would greatly benefit human civilization. This study aims to evaluate and compare the antioxidant activity and phytochemical profiles of hexane, chloroform, ethyl acetate, and water extracts of the whole plant *Ouret lanata*. The dried plant powder was sequentially extracted with ethanol for eight hours, and the solvent was evaporated under reduced pressure. Similarly, the air-dried powder was extracted with water, and the water was evaporated. This research investigates the phytochemicals and performs both qualitative and quantitative evaluations. Tannin was the only substance detected in all extracts. Tests for alkaloids, flavonoids, and tannins were positive in the chloroform extract. Flavonoids, glycosides, and tannins were significantly more abundant in all three extracts. The aqueous extract tested positive for tannins and flavonoids. According to the results of TLC for phenol and flavonoid analysis, the *O. lanata* extract contains additional classes of phenols and flavonoids compared to the standard used for comparison. The methanolic extract of *O. lanata* was found to have a total phenolic content of 282.86 ± 0.52 mg/g, while the hexane, chloroform, and ethyl acetate extracts had phenolic contents of 81.25 ± 0.81, 219.12 ± 0.17, and 161.42 ± 0.34 mg/g, respectively. The total flavonoid content was found to be 145.3 ± 2.13, 163.4 ± 1.42, 88.2 ± 2.61, and 123.7 ± 2.41 mg quercetin/g, indicating a high flavonoid content. The *O. lanata* extract showed high antioxidant and antimicrobial potential. The inhibition of cytotoxic effects was determined using the Human Keratinocyte Cell Line, HaCaT.

Keywords: *Ouretlanata*, DPPH, antimicrobial, cytotoxic, HaCaT, Phytochemicals, Polyphenols, Flavonoids

Introduction

Herbal remedies for physical and mental ailments are the focus of traditional medicine, which encompasses information, techniques, and abilities derived from previous trials and beliefs. Medicinal plants have been used for thousands of years in herbalism and medicine. According to historical documents dating back about 5000 years, around 250 different plant species were utilized medicinally. Some notable examples are the use of mandrake for medicinal purposes, henbane for drowsiness, and poppies for pain relief; these uses are still acknowledged for their therapeutic merits [1,2].
With increased public concern about the hazardous consequences of synthetic and chemical additives, particularly those used for antibacterial, antioxidant, and flavoring qualities, researchers are looking for natural alternatives [3]. Natural plant extracts, which are high in flavorful, antibacterial, antioxidant, and anticancer properties, have emerged as a possible alternative [4].
In this regard, Ouretlanata (synonym Aervalanata), also known as mountain knotgrass, has been chosen for study. This woody, prostrate, or succulent perennial herb from the Amaranthaceae family is native to the tropics of Africa and Asia, and it is widely distributed in India [5]. It has traditionally been used for its diuretic qualities, as well as to cure lithiasis and strangury. The roots are known for their demulcent and headache-relieving effects, and they are also used to cure coughs in Ceylon as well as a vermifuge for children. The Meenatribals of Rajasthan also utilize the root juice to cure liver congestion, jaundice, biliousness, and dyspepsia, and a decoction of the entire plant to treat pneumonia, typhoid, and persistent fevers [6,7].
Ouretlanata, also known as Pashanabheda, is widely used in Ayurvedic medicine for a variety of therapeutic effects, including diuretic, anthelmintic, anti-diabetic, expectorant, hepatoprotective, antibacterial, cytotoxic, antiurolithiatic, and anti-inflammatory properties. Various studies have shown that the plant is useful in treating boils, cephalalgia, cough, and urolithiasis, as well as nephroprotective, cytotoxic, antioxidant, immunomodulatory, diuretic, anti-inflammatory, antibacterial, hepatoprotective, and antihyperglycemic properties [8,9].
Given these traits, ethnobotanical surveys led to the selection of Ouret lanata(L.) for this study. The aim of the study is to assess the antioxidant, antibacterial, and cytotoxic Potential on Promoting Cell Proliferation in Human Keratinocyte Cell Line, HaCaT properties of whole plant samples, with a focus on their potential use in skin care treatment, as well as to perform a qualitative analysis of phytochemicals in different extracts of the chosen plant.

Methodology

Chemicals, Microorganisms, and Culture Media

DPPH (2,2-diphenyl-1-picrylhydrazyl), Folin-Ciocalteu reagent, gallic acid, quercetin, aluminum chloride, L-ascorbic acid, potassium persulfate, dimethyl sulfoxide (DMSO), hexane, chloroform, and ethyl acetate were all obtained from Merck Co. (Mumbai, India). Pseudomonas aeruginosa, Staphylococcus aureus, and Klebsiellapneumoniae strains were obtained from the American Type Culture Collection in India. Mueller Hinton broth (MHB), Mueller Hinton agar (MHA), nutrient agar (NA), and tryptic soy broth (TSB) were all acquired from Hi Media Laboratories in Mumbai, India.

Preparation of Sample:

The entire Ouretlanata plant was collected and properly cleansed in distilled water. The plants were cleaned and then shade dried. After drying, the samples were then crushed into a fine powder with a mechanical grinder. The samples in powder form were then placed in sealed containers to avoid contamination.

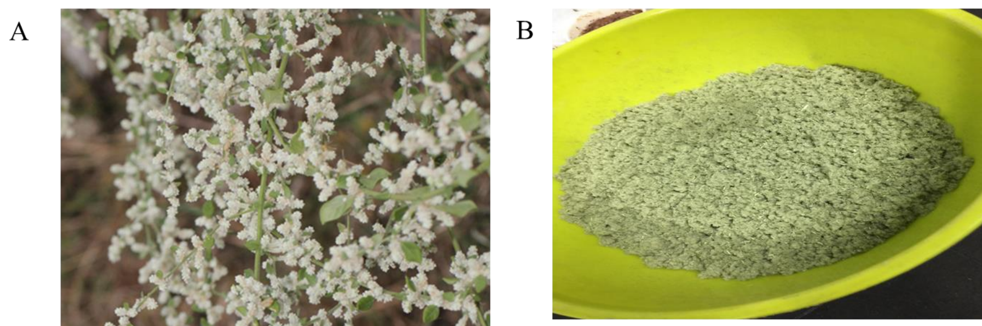

Fig 1: A. Plant of *Ouret lanata*; B. Whole plant dried powder

Sequential Extraction

Weigh some of the powdered Ouretlanata material. Hexane should be added to the plant material at a 1:10 ratio, ensuring that the solvent totally immerses it. Put the entire mixture in a conical flask and seal it. Shake or agitate the mixture with a magnetic stirrer for several hours at room temperature. Filter the mixture through filter paper and a funnel to remove any solid plant debris. Collect the filtrate containing the initial extract in a clean container.

Fig 2: Sequential Extraction using different Solvents

The marc resulting from the hexane extract was extracted and dried at room temperature. It was subsequently soaked in chloroform at a 1:10 ratio (10 g of powder per 100 mL of chloroform). After extraction, the marc from the chloroform extract was collected, dried at room temperature, and soaked in ethyl acetate at a 1:10 ratio. The marc from the ethyl acetate extract was collected, dried at room temperature, and submerged in methanol at a 1:10 ratio (10 g powder in 100 mL). Finally, the marc from the methanol extract was collected, dried at room temperature, and immersed in distilled water at a 1:10 ratio (10 g powder per 100 ml).

Determination of Total Phenolic Content

The total phenolic compounds in IPC extracts were measured with the Folin-Ciocalteu reagent, as previously described [11]. To begin, add 4.5 µL of 1 mg/mL extracts and 126 µL of deionized water to 96-well plates. Mix with 90 µL of 2% Na_2CO_3 for 3 minutes before adding 4.5 µL of 50% Folin-Ciocalteu reagent. After 30 minutes of incubation, the resultant blue molybdenum-tungsten complex was detected at 750 nm with a microplate reader (Biochrom EZ Read 2000, Cambridge, UK). The total phenolic content of IPC extracts was determined using a gallic acid standard and expressed as milligrams of tannic acid per one gram of extract (mg/GAE/g extract).

Determination of Flavonoid Content

The flavonoid components in IPC extracts were identified using the aluminium chloride test, as previously described [12]. In 96-well plates, 100 µL of extracts at 1 mg/mL were combined with 100 µL of 2% $AlCl_3$. The samples were then incubated for 30 minutes, and the absorbance at 415 nm was measured using a plate reader. The total flavonoid content of samples was determined by comparing them to the quercetin standard and interpreted as quercetin equivalent to one gram of extract (mg Quercetin/g extract).(mcg GAE/g extract).

Thin Layer Chromatography

Thin layer chromatography (TLC) was used on crude extracts derived from various solvents. 50 µL of each crude fraction was added to the TLC plate. Chromatography was performed with solvent systems containing hexane and ethyl acetate in ratios of 1:1, 3:7, and 7:3 (v/v). The dots were made visible by spraying the plates with p-anisaldehyde. After spraying, the eluted plates were thoroughly dried. The chromatograms were then observed using UV light at both long (365 nm) and short (254 nm) wavelengths. Calculating the Rf values allowed us to determine how the phytochemicals moved with the solvent [13].

Phytochemical Screening of Selected Plant Materials

The confirmatory qualitative phytochemical screening of the plant extracts was performed to identify the main classes of compounds, including carbohydrates, tannins, saponins, flavonoids, alkaloids, phenols, quinones, glycosides, cardiac glycerides, terpenoids, phenols, and coumarins. This screening followed standard techniques developed from previous investigations [14-23].

Antioxidant Activity

To make a 0.1 mM DPPH solution in methanol, add 100 µl to 300 µl of samples at varying concentrations. The preparations must be vigorously shaken before being allowed to stand for 30 minutes at room temperature. A UV-VIS spectrophotometer should then be used to measure the absorbance at 517 nm. Ascorbic acid can be used as a reference. Lower absorbance values in the reaction mixture imply increased activity in scavenging free radicals [24]. The potential of scavenging the DPPH radical can be determined using the formula below.

DPPH scavenging effect (% inhibition) = [(absorbance of control- absorbance of reaction mixture)/absorbance of control] X 100.

Antimicrobial Activity- Well diffusion Method

The crude extracts were first screened for antibacterial activity by using the agar well diffusion assay technique [25]. Plant extracts were dissolved in Dimethyl Sulphoxide (DMSO 100% v/v) to obtain working concentrations (20mg/mL). Standardized broth cultures of test bacterial isolates (*Pseudomonas aeruginosa, Staphylococcus aureus*, and *Klebsiella pneumonia*) were distributed aseptically onto the surfaces of Mueller Hinton Agar (MHA) plates with sterile cotton swabs. All culture plates were allowed to dry for about 5 minutes before agar wells were created using a sterile cork-borer (8 mm in diameter). The wells were filled with 200 µl crude extracts and controls. The plates were then maintained at room temperature for an hour to allow the agents to diffuse into the agar medium before being incubated. In the antibacterial test, positive controls included gentamycin (50 µg/mL) while negative controls included DMSO (100% v/v). The MHA plates were then incubated at 37°C for 24 hours. The inhibition zones' diameters (IZDs) were measured.

Cell Culture

The HaCaT cell line was obtained from NCCS, Pune and cultured in DMEM supplemented with 10% (v/v) fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin. The cells were maintained at 37°C in a humidified atmosphere with 5% CO2. For experiments requiring serum deprivation, the cells were cultured in serum-free media.

Cell viability Assay

Cell viability was evaluated using the MTT test [32]. H_2O_2 (50 mM) was used to stress both control and pre-incubated cells for 2 hours. Following incubation, fibroblasts were rinsed twice with PBS and treated with a salt solution of MTT at a concentration of 0.5 mg/mL for 2 hours at 37 °C. This assay works by reducing the tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenytetrazolium bromide, by intracellular dehydrogenases in viable live cells, resulting in the creation of purple formazan crystals. The medium was then withdrawn and the crystals dissolved in DMSO. The optical density was measured at 550 nm using a microplate reader (Bio-Rad PR4100). Cell viability was calculated as a percentage of living cells relative to the unexposed control. The presented data are the average values from at least three different experiments [26].

Cell Proliferation Assay

Cell Proliferation Assay To assess the effect of *O. lanata* extract on the recovery of pre-treated cells after extended oxidative stress caused by exposure to H_2O_2 (0.1, 0.5, and 1 mM) for 1 hour, cells at

time 0 were immediately examined with the MTT assay as described previously [26]. The remaining cells were cultured with new EMEM at 37°C for 24, 48, and 72 hours before being assessed for viability using the same technique.

Results and Discussion

Total phenolic contents (TPC)

The total phenolic content of the *O. lanata* extract was measured using the Folin technique with a calibration curve of various quantities of gallic acid, and the results are reported as mg tannic acid per gram of crude extract (Table 1). TPC values for *O. lanata* plant extracts extracted with hexane, chloroform, ethyl acetate, and distilled water were 81.25 ± 0.81, 219.12 ± 0.17, 161.42 ± 0.34, and 282.86 ± 0.52 mg/g, respectively. These studies demonstrated that plant distilled water and chloroform are high in polyphenolic chemicals.

The total phenol concentration of three unique plant extracts prepared using five different solvents. The methanol solvent had the highest phenol content (1.87 ± 7.26c, 2.31 ± 0.028e, and 2.42 ± 0.028e) for *Abutilon indicum, Ouret lanata*, and *Tribulsterrestris* compared to other extracts [27]. A phenolic component extracted from the ethyl acetate fraction of the leaves was shown to have a greater stone dissolving rate. It was more successful in calcium phosphate stones (67.74%) than in oxalate stones [28]. Phenolic compounds are recognized to be potent chain- breaking antioxidants with scavenging properties due to their hydroxyl groups [29].

Table 1: Total Phenolic and Flavonoids Contents, and Antioxidant activity of Ethanolic Extracts of *O. lanata*.

Extract	Concn. (µg/ml)	Total Phenolic Content (mg Tannic acid/g)	Total Flavonoids Content (mg Quercetin/g)
Hexane	100	81.25 ± 0.81	45.3 ± 2.13
Chloroform	100	219.12 ± 0.17	163.4 ± 1.42
Ethyl acetate	100	161.42 ± 0.34	88.2 ± 2.61
Distilled water	100	282.86 ± 0.52	123.7 ± 2.41

Values are expressed as the mean ± SD; statistical significance (p) calculated by one way ANOVA followed by Duncan's Range test. Data are mean ± SD from three independent experiments.

Total Flavonoid Content (TFC)

Total flavonoids in the extracts were determined using the aluminum chloride method and a calibration curve of varied catechin concentrations, with the results represented as mg Quercetin per gram of crude extract. TFC values for the plant material extracted with hexane, chloroform, ethyl acetate, and distilled water were 45.3 ± 2.13, 163.4 ± 1.42, 88.2 ± 2.61, and 123.7 ± 2.41mg quercetin/g, indicating a high flavonoid content (Table 1).

The methanol solvent contains the highest flavonoid concentration among the five solvents tested for *Abutilon indicum, Ouretlanata,* and *Tribulsterrestris* [27]. Flavonoid is one of the most common phenolic chemicals, along with flavones and flavonols. Many flavonoids and related substances are said to have substantial antioxidant properties [30]

Thin Layer Chromatography

The solvent system employed for phytochemical profiling included multiple solvents in varying concentrations, as shown in Table 2. Figure 3 depicts the phytochemical profile of the *O. lanata* extract at UV254 nm following TLC plate development. Table 2 shows the amount of phytochemicals and their retention factors (Rf).

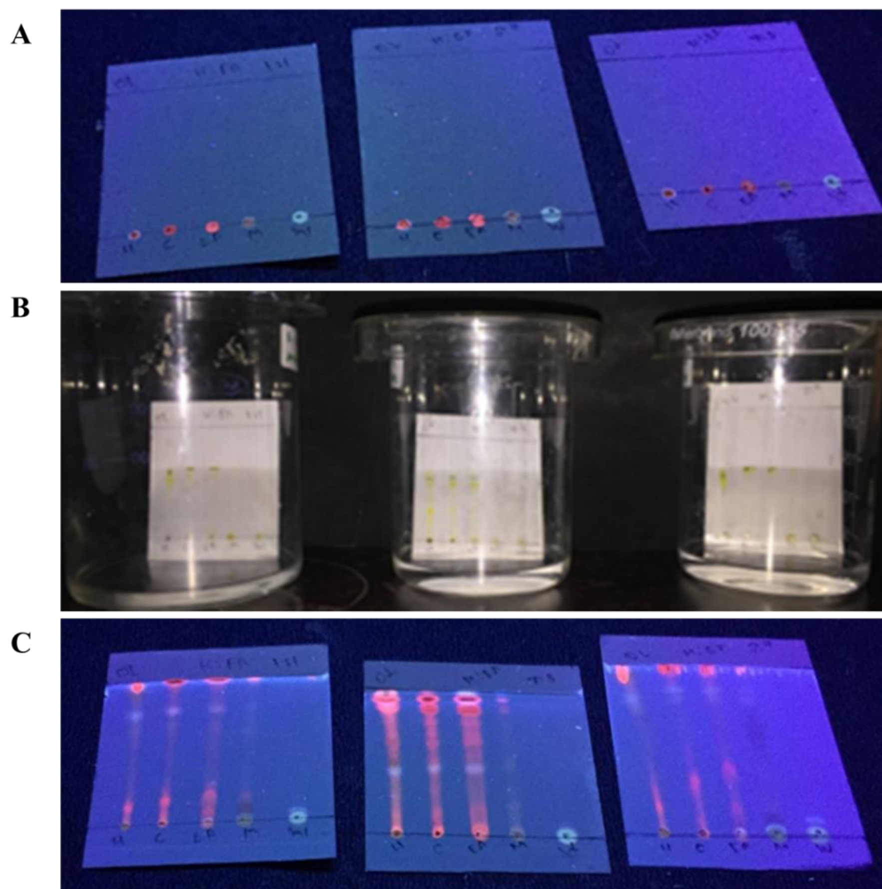

Fig 3.Phytochemicals Profiling of *O. lanata* extracts at UV_{254} nm.

Table 2. Retention Factor (Rf) Values and Number of spots at Wavelength UV_{254} nm of *O. lanata* extracts

SNO	MOBILE PHASE	SOLVENT	BANDS	RF VALUE
1.	Hexane: Ethyl acetate 1:1	Hexane	5	0.04,0.15,0.2,0.69,0.84.
		Chloroform	4	0.13,0.17,0.73,0.84.
		Ethyl Acetate	6	0.08,0.24,0.31,0.73,0.86,0.93.
		Methanol	3	0.06,0.57,0.77.
		Distilled water	2	0.04,0.95.
2.	Hexane: Ethyl acetate 3:7	Hexane	7	0.04,0.13,0.38,0.56,0.79,0.89,0.93.
		Chloroform	11	0.04,0.15,0.23,0.39,0.48,0.59,0.66,0.73,0.75,0.84,0.93.
		Ethyl Acetate	11	0.04,0.09,0.18,0.39,0.48,0.61,0.66,0.7,0.84,0.93.
		Methanol	4	0.07,0.79,0.89,0.93.

		Distilled water	1	0.04
3.	Hexane: Ethyl acetate 7:3	Hexane	6	0.13,0.18,0.70,0.78,0.89,0.94
		Chloroform	6	0.07,0.20,0.33,0.46,0.81,0.85
		Ethyl Acetate	6	0.09,0.15,0.37,0.48,0.76,0.85
		Methanol	2	0.85,0.96
		Distilled water	3	0.05,0.11,0.19

After developing the TLC plate, the *O. lanata* extract formed distinct spots in chloroform and ethyl acetate at short-wavelength UV254 nm, with different Rf values, as shown in Table 2. The TLC analysis revealed the presence of 11 distinct chemicals in the *O. lanata* extracts. Most of the Rf values observed were less than 0.5, indicating that the majority of the phytochemicals in the studied plants are non-polar molecules [31].

Phytochemical Screening of Selected Plant Materials

According to early phytochemical screening results presented in Table 2, the plants include a variety of secondary metabolites such as tannins, steroids, terpenoids, coumarins, alkaloids, flavones, anthroquinones, phenols, saponins, and quinones. Phenols were identified in all samples but were most apparent in the methanolic extracts. It was also observed that all of the plant extracts employed in this study included tannins. Tiwari et al. (2011) found that this combination of metabolites had antibacterial, antidiarrheal, and anthehelmintic properties. Surprisingly, quinone tested negative in all of the plant extracts examined [32]. One probable cause is that the solvent did not fully remove the quinone molecule. Leksawasdi et al. (2008) supported this claim [33].

S.No	Tests	Hexane	Chloroform	Ethyl Acetate
1	Test for Carbohydrate	+	++	++
2	Test for Tannins	++	++	++
3	Test for Saponins	+	+	+
4	Test for Flavonoids	++	++	++
5	Test for Alkaloids	++	++	+
6	Test for Quinones	-	-	-
7	Test for Glycosides	-	-	-
8	Test for Cardiac Glycosides	++	++	++
9	Test for Terpenoids	++	++	++

10	Test for Coumarin	++	++	++

Antioxidant activity

A substance that inhibits the consumption of oxygen is called an antioxidant. On the other hand, antioxidants function as a metal chelating agent, synergist, peroxide decomposer, electron giver, hydrogen donor, and radical scavenger [15]. Oxidants and free radicals have a dual function in the body since they can be both beneficial and detrimental. They may be created ex situ from exposure to radiation, smoke, pollution, or drugs, or in situ through regular cell metabolism. Oxidative stress is when an excess of free radicals cannot be eliminated. Numerous diseases, including cancer, aging, autoimmune disorders, cardiovascular disease, and neurological diseases, can be brought on by oxidative stress. Nonetheless, the body uses a number of exogenous (obtained from diet) or naturally occurring in situ processes to combat oxidative stress or supplements [15].

The availability of antioxidants as effective oxygen radical scavengers, such as flavonoids and phenolic compounds, is explained by their antioxidant activity. The main cause of phenolic antioxidant activity is their redox characteristics, which enable them to function as singlet oxygen quenchers, donors of hydrogen, and reduction agents. The percentage of DPPH inhibition was also computed. The findings demonstrated that with 5–54% DPPH inhibition, the DPPH radical scavenging activity rose in a concentration-dependent manner at 5–30 µg/mL (Figure 2). In contrast to other solvent extracts, this chloroform extract demonstrated possible antioxidant action.

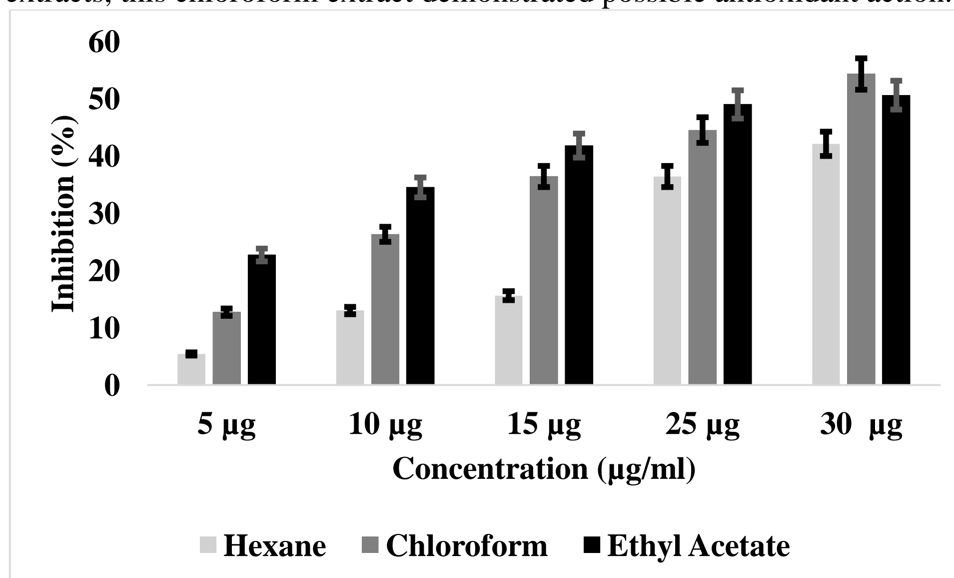

Fig. 4: Antioxidant activity of *O. lanata* extracts

The antioxidant properties of the plant *O. lanata* extracts might be connected to the chemical composition which includes bioactive phytochemicals, high levels of phenolic acids, and flavonoids. *O. lanata* could be considered as a good source of antioxidants that will help in the prevention of many diseases that are caused by oxidative stress e.g. diabetes mellitus, carcinomas, neurodegenerative disorders and cardiovascular diseases. Behera PC et al., 2018 studied in A. lanata flower extract in chloroform (68%), ethyl acetate (92%), and aqueous (65%) for 100 µg/ml produced comparable free radical scavenging activities versus standard BHT. The order of free radical scavenging of test samples (0 and 500 ppm) and standard is: BHT >methanolic extract > ethyl acetate > chloroform > aqueous. In *O. lanata* whole plant extracts got 54% in 30 µg/ml which was high compared to these studies [34].

Anti-Microbial Studies

The antimicrobial activity of plant extracts and its phytoconstituents was evaluated with antibiotic susceptible and resistant microorganisms. The antimicrobial activities of the extracts of *O. lanata* were studied in concentration of 50 µg/ml against three pathogenic bacterial strains. The antimicrobial

activities of different extracts of plants were assessed in terms of zone of inhibition of bacterial growth. The results were summarized in Table 3. The ethyl acetate and chloroform extract showed good antibacterial activity against the Gram-negative bacteria such as *P. aeruginosa, K. pneumonia* and Gram-positive bacteria such as *S. aureus*. Behera PC et al., 2018 proven that high antimicrobial activities of the flower extracts of *A. lanata* were studied in concentration of 50 µg/ml against four pathogenic bacterial strains were reported [34].

Table 4: Three Different Extracts treated with *Pseudomonas aeruginosa, Klebsiella pneumonia* and *S. aureus*

Culture	+ve	-ve	Hexane	Chloroform	Ethyl acetate
pseudomonas	17mm	_	8mm	6mm	7mm
staphylococcus	22mm	_	7mm	10mm	9mm
klebsiella	15mm	_	8mm	12mm	9mm

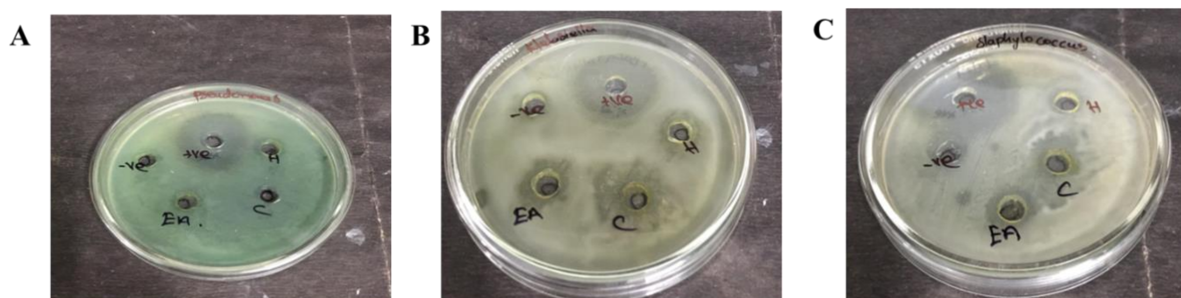

Fig. 5: A. Three different extracts treated with *Pseudomonas aeruginosa*: B. *Klebsiella pneumonia*; C. *S. aureus*

Cell viability Assay

Firstly, the possible toxic effect of the strawberry extract in relation to its increasing concentration and exposure time was studied (Figure 1).

Firstly, the possible toxic effect of the *O. lanata* extract in relation to its increasing concentration and exposure time was studied (Fig.6). Cell vitality did not vary depending on the *O. lanata* extract concentration or the exposure time, thus no cytotoxic effect was found at the chosen experimental conditions. Therefore, the extract concentration that gave the best results in terms of cell viability and reproducibility (12.5 – 250 µg/mL) was selected for all the tests. After incubation with H_2O_2 for 1 h, only *O. lanata* pre-treated cells did not show a significant decrease in their viability (Figure 2), especially at H_2O_2 concentration of 12.5 and 250 µg/ml, where cell viability remained higher than 80% ($p < 0.05$) compared to control cells.

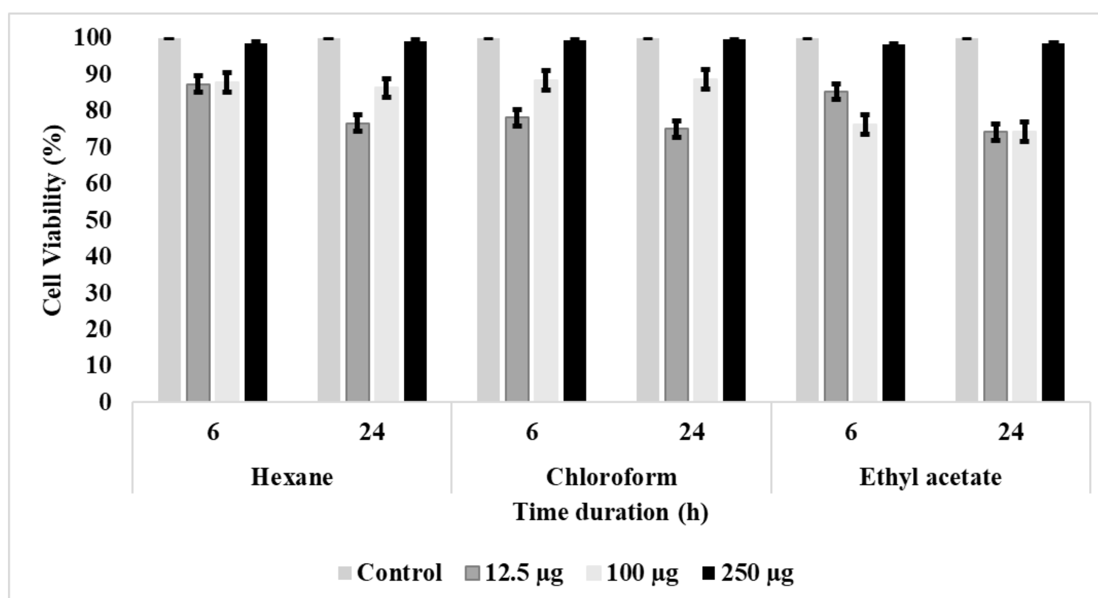

Fig. 6. Viability of Human Dermal Fibroblast (HuDe) determined by MTT Assay after Incubation with different Concentrations of *O. lanata* extracts and at different times. Data are expressed as mean ± SEM for Eight Replicas (n = 8) of three Independent Experiments

Cell Proliferation and Recovery Assay

With MTT viability assay it was possible to assess whether *O. lanata* extract was also able to influence the recovery of cell viability after prolonged oxidative stress to different H_2O_2 concentrations. As demonstrated in tests of viability for control and pre-treated cells, the curve of pretreated cell viability showed a significant difference ($p < 0.05$) with controls for concentrations of $H_2O_2 \geq 500$ µM. After 24 h (Fig,.7) the significance of recovery was recorded only for cells stressed with 500 µM H_2O_2. After a further 24 h, the difference between the two curves became highly significant ($p < 0.01$) for ≤ 50 µM H_2O_2 and this difference remained after 72 h, when the significance was recorded for all stress points analyzed. In conclusion, evidence of recovery revealed a significant restoring of vitality and cell function after 48 and 72 h from stress induced by H_2O_2.

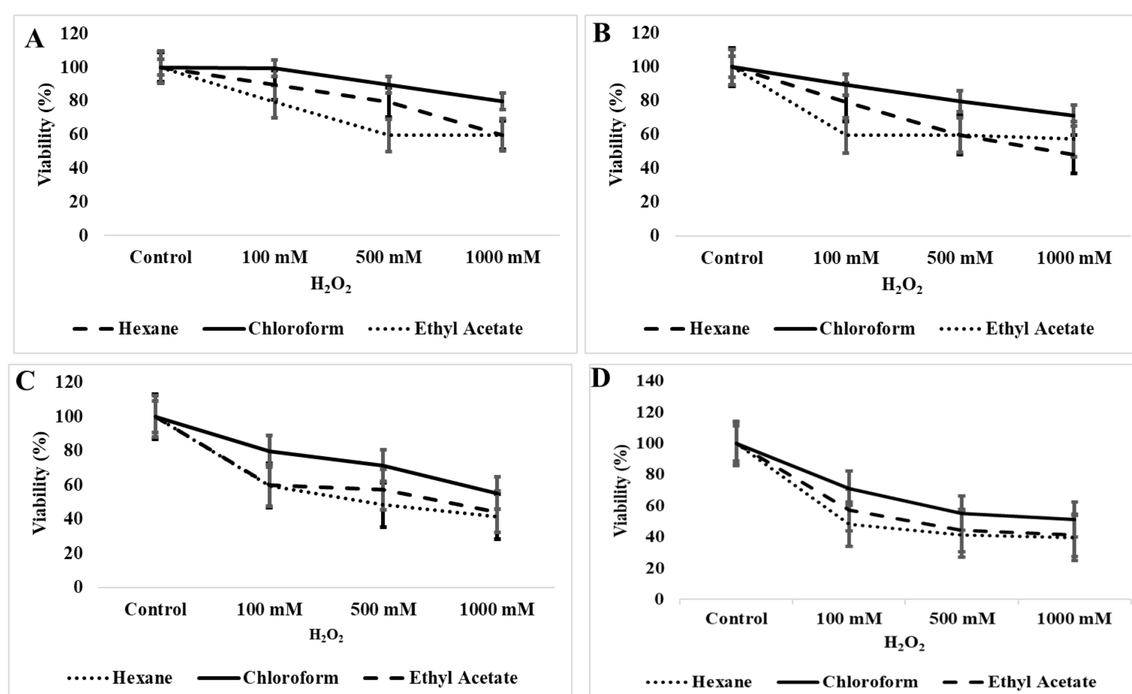

Fig.7: MTT and Recovery after Stress by H2O2. Control and Pre-incubated Cells were Stressed with different Concentrations of H2O2 (0.1, 0.5 and 1 mM) and analysed in different times to Aassess the Percentage of Vitality. A. 0 hours; B. 24 hours; C. 48 hours; D. 72 hours. Data are Expressed as mean ± SEM for Eight Replicas (n = 3) of Three Independent Experiments.

Conclusion

Our results report that the Sveva strawberry cultivar has a high antioxidant capacity, as well as an important anthocyanin and vitamin content, which results in a protective effect on skin cells against damage induced by oxidative stress. Indeed, strawberry extract was effective in decreasing intracellular ROS concentration and in protecting lipid, DNA and mitochondrial functionality from the damage induced by free radicals. Overall, the present study represents an interesting starting point for future investigations. Further studies will be of particular interest, in order to confirm our findings, to explore the direct and indirect antioxidant mechanisms underlying the beneficial effects of strawberry treatment, and to further investigate the role of specific classes of compounds in explaining the reported bioactivities.

Certainly, the authors are aware of the limitations of the study. Firstly, the absence of the key genes expression analysis, involved in antioxidant responses, is an important drawback of the study, since it could have been useful to further corroborate the role of strawberry extract on the results obtained. Another limit of the study is the lack of measurement of DNA damage and cell cycle of key markers involved in cell proliferation by western blot, since it could be essential to examine the mechanisms thorough which strawberry extract contributes to attenuate cytotoxicity induced by hydrogen peroxide. Moreover, more in-depth analysis would have added important data to define the in vitro protective effects of strawberry bioactive compounds in different subcellular districts in the recovery from oxidative insults and to verify the bioavailability of these molecules in skin cells after dietary intake or topical application, in order that strawberries can be used also as a natural antioxidant for preventing skin aging and diseases.

Our findings show that *Ouret lanata* has a strong antioxidant capacity, as well as large polyphenol and flavonoid content, which protects skin cells from oxidative stress-induced damage. The *O. lanata* extract substantially lowered intracellular ROS concentrations while protecting cells from free radical damage. Overall, this study provides a good foundation for future research. Further research is needed to confirm our findings, investigate the direct and indirect antioxidant mechanisms behind the positive effects of *O. lanata* extract therapy, and determine the significance of specific chemical classes in explaining the observed bioactivities.

One study disadvantage is the lack of data for DNA damage and major cell proliferation markers using western blot, which could be critical for understanding the processes by which *O. lanata* extract reduces hydrogen peroxide-induced cytotoxicity. Furthermore, further detailed investigations would give useful information for defining the in vitro protective effects of *O. lanata's* bioactive substances in various subcellular locations during oxidative stress recovery. It would also be advantageous to test the bioavailability of these compounds in skin cells after food or topical administration. This would assist to establish *O. lanata* as a natural antioxidant capable of reducing skin aging and related disorders.

References

1. Benzie I.F.F., Wachtel-Galor S. Herbal Medicine: Biomolecular and Clinical Aspects. CRC Press/Taylor & Francis Copyright © 2011 by Taylor and Francis Group, LLC.; Boca Raton (FL: 2011. Herbal medicine: biomolecular and clinical aspects

2. Šantić, Ž, Pravdić, N., Bevanda, M. &Galić, K. The historical use of medicinal plants in traditional and scientific medicine. Psychiatr. Danub. 29(Suppl 4), 787–792 (2017).

3. Kirtikar KP, Basu BD, Mahaskar C. 2nd ed. Allahabad: International Book Distributors; 1987. Indian Medicinal Plants; p. 2051. [Google Scholar]

4. 1A. New Delhi: CSIR Publications; 1959. Anonymous, The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products; p. 91. [Google Scholar]

5. Singh V, Pandey RP. Jodhpur: Scientific Publishers; 1998. Ethnobotany of Rajasthan; p. 38.
6. Nadkarni KM. Indian MateriaMedica. Bombay Popular Prakashan. 1999; 1: 49-50.
7. Rajesh R. In vitro Anthelmintic Activity of Aerial Parts Aervalanata Linn Juss. Int. J. of PharmaSci and Drug Res. 2010; 2(4): 269-71.
8. Dinnimath BM, Jalalpure SS. Spectral Analysis of Fractions of Aervalanata (L). Indian Journal of Natural Products 2012; 28(2): 14-8.
9. Atmani F, Slimani Y, Mimouni M, Hacht B. Prophylaxis of calcium oxalate stones by Herniariahirsuta on experimentally induced nephrolithiasis in rats. British Journal of Urology International. 2003; 92(1): 137–40.
10. https://www.quickcompany.in/patents/a-process-of-sequential-extraction-oftetrachloroethylene
11. Lu, X., Wang, J., Al-Qadiri, H.M., Ross, C.F., Powers, J.R., Tang, J. and Rasco, B.A., 2011. Determination of total phenolic content and antioxidant capacity of onion (*Allium cepa*) and shallot (*Allium oschaninii*) using infrared spectroscopy. Food Chemistry, 129(2), pp.637-644.
12. Zhishen, J., Mengcheng, T. and Jianming, W., 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food chemistry, 64(4), pp.555-559.
13. Saxena, G., Farmer, S., Towers, G.H.N. and Hancock, R.E.W., 1995. Use of specific dyes in the detection of antimicrobial compounds from crude plant extracts using a thin layer chromatography agar overlay technique. Phytochemical analysis, 6(3), pp.125-129.
14. Harborne, J.B. and Harborne, J.B., 1973. Phenolic compounds. *Phytochemical methods: A guide to modern techniques of plant analysis*, pp.33-88.
15. Dubale, S., Kebebe, D., Zeynudin, A., Abdissa, N. and Suleman, S., 2023. Phytochemical screening and antimicrobial activity evaluation of selected medicinal plants in Ethiopia. Journal of experimental pharmacology, pp.51-62.
16. Kapoor, L.D. Singh, A., Kapoor, S.L. and Srivastava, S.N. 1969. Antibacterial activity of plant extracts used externally in traditional medicine. J. Ethnopharmacal. 44, 35-40.
17. Smolenski SJ, Silinis H and Farnswoth NR. 1974. Alkaloids screening. Lloydia, 37: 506–536
18. Devika, R. and Koilpillai, J., 2012. Phytochemical screening studies of bioactive compounds of Tageteserecta. Int. J. Pharm. Bio. Sci, 3(4), pp.596-602.
19. Jana, S. and Shekhawat, G.S., 2010. Phytochemical analysis and antibacterial screening of in vivo and in vitro extracts of Indian medicinal herb: Anethumgraveolens. Research journal of medicinal Plant, 4(4), pp.206-212.
20. Ayoola, G.A., Coker, H.A., Adesegun, S.A., Adepoju-Bello, A.A., Obaweya, K., Ezennia, E.C. and Atangbayila, T.O., 2008. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. Tropical journal of pharmaceutical research, 7(3), pp.1019-1024.
21. Suresh, S.N. and Nagarajan, N., 2009. Preliminary phytochemical and antimicrobial activity analysis of Begonia malabarica Lam. *Journal of Basic & Applied Biology*, *3*(1&2), pp.59-61.
22. Bhandary, S.K., Bhat, V.S., Sharmila, K.P. and Bekal, M.P., 2012. Preliminary phytochemical screening of various extracts of Punicagranatum peel, whole fruit and seeds. Journal of Health and Allied Sciences NU, 2(04), pp.34-38.
23. Arya, V., Thakur, N. and Kashyap, C.P., 2012. Preliminary phytochemical analysis of the extracts of Psidium leaves. Journal of Pharmacognosy and Phytochemistry, 1(1), pp.01-05.
24. Mensor, L.L., Menezes, F.S., Leitão, G.G., Reis, A.S., Santos, T.C.D., Coube, C.S. and Leitão, S.G., 2001. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytotherapy research, 15(2), pp.127-130.
25. Bakht, J., Islam, A. and Shafi, M., 2011. Antimicrobial potential of Ecliptaalba by well diffusion method. Pak. J. Bot, 43, pp.161-166.
26. Giampieri, F., Alvarez-Suarez, J.M., Mazzoni, L., Forbes-Hernandez, T.Y., Gasparrini, M., Gonzàlez-Paramàs, A.M., Santos-Buelga, C., Quiles, J.L., Bompadre, S., Mezzetti, B. and Battino, M., 2014. Polyphenol-rich strawberry extract protects human dermal fibroblasts

against hydrogen peroxide oxidative damage and improves mitochondrial functionality. Molecules, 19(6), pp.7798-7816.

27. Pauline Jenifer. S and S. Beulah Jerlin. Comparative Study On The Phytochemical Analysis And Antioxidant Assessment Of Medicinal Plants Used To Treat Urolithiasis. International Journal of Food and Nutritional Sciences. 2021; 10 (6): 258-273.
28. Vivek, V., Byahatti, K., Vassantkumarpai, & Souza, G. D. (2010). Effect of phenolic compounds from BergeniaGillata (Haw) sternts, leaves on experimental kidney stones. Ancient Science of Life, 30(1), 14-17.
29. Hatano, T., Edamatzu, R., Mori, A., Fujita, Y., Yasuhara, T., Yoshida, T., & Okuda, T. (1989). Effect of interaction of tannins with co-existing substances. Chemical and Pharmaceutical Bulletin, 37, 2016-2021.
30. Dziedzic, S. Z., & Hudson, B. J. F. (1983). Hydroxyl isoflavones as antioxidants for edible oils. Food Chemistry, 11, 161-166.
31. Gomathi, D., Kalaiselvi, M., Ravikumar, G., Sophia, D., Gopalakrishnan, V.K. and Uma, C., 2012. Secondary metabolite credentials of Evolvulusalsinoides by high performance thin layer chromatography (HPTLC). Journal of Biomedical Research, 26(4), pp.295-302.
32. Tiwari, P., Kumar, B., Kaur, M., Kaur, G., &Kaur, H. (2011). Phytochemical screening and extraction: A review. International Pharmaceutical Sciences, 1(1), 99-106.
33. Leksawasdi, N., Temiyaputra, W., Suebsanga, T., Yajom, K., &Piyaworanon, S. (2008). Influences of anthraquinone extraction techniques from Morinda sp. on extraction efficiency. Kasetsart Journal (Natural Science), 42, 118-126.
34. Behera PC, Ghosh M. Evaluation of Antioxidant, Antimicrobial, and Antiurolithiatic Potential of Different Solvent Extracts of Aervalanata Linn Flowers. Pharmacogn Mag. 2018 Jan-Mar;14(53):53-57.