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Original Article

Application as cosmetic bioactives of phytochemicals extracted from the post distillation biomass of geranium

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Abstract

Pelargonium graveolens commonly known as geranium crop has received attention by essential oil manufacturers. Geranium plant is propagated using plant tissue culture followed by its cultivation in the fields. Geranium oil extraction creates a large amount of post distilled geranium biomass (PDGB). The present study utilized this unexplored PDGB for extraction of antioxidant, anti-tyrosinase, and antimicrobial constituents for cosmetic applications and determining the extract's safety. The PDGB of methanol extract and ethyl acetate extract exhibited good qualitative phytochemical profiles. Antimicrobial activity was found to be absent in both extracts. Ethyl acetate extract of PDGB exhibited the highest antioxidant activity in terms of DPPH free radical scavenging and tyrosinase inhibition. The IC_{50} for ethyl acetate extract and methanol extract were found to be 0.188 and 0.201 mg/ml, whereas for GO it was found to be 77.49 mg/ml. The tyrosinase inhibition was found to be significantly higher ($p<0.01$) for ethyl acetate (0.97mg/ml) and methanol (1.0 mg/ml) than for GO (7.44 mg/ml). Similarly, both the extracts and GO exhibited less toxicity or maximum cell viability on 3T3 fibroblast cell lines ATCC 1658 indicating suitability of PDGB extracts for cosmetics application and an additional revenue model for oil extracting farmers.

Keywords: *Pelargonium graveolens*, post distillation geranium biomass, antioxidant activity, DPPH, tyrosinase inhibition, cytotoxicity, antimicrobial activity

1. Introduction

Geranium, (family Geraniaceae) is a rose scented aromatic plant of high value and is endemic to South Africa, Madagascar, Egypt and Morocco. Out of the 700 species available only 10 are utilized in production and extraction of geranium oil, rich in geraniol or citronellol, and hence used for oil distillation world-wide (Rajeswara, Kaul, Mallavarapu, & Ramesh, 1996). Clonal propagation of geranium via tissue culture techniques and multiplication by cutting are the most common ways of maintaining varieties of oil producing geranium crops (Sastry, Rao, Rao, Ram, & Kumar, 2021). For of agricultural sustainability, these clonal varieties are maintained in nurseries and cultivated from winter to summer with soil pH of 4.9-8.9, owing to the fact that the oil content is higher in this particular season (Sastry, Rao, Rao, Ram, & Kumar, 2021). The harvested biomass (fresh leaves, stems and branches) is utilized for oil extraction via steam distillation or

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by hydro-distillation (Rao, 2002). Seasonal variation causes changes in oil composition and yield. For instance, cultivation in Gangetic plains revealed high oil yield in the months of Oct-Nov (Sastry, Rao, Rao, Ram, & Kumar, 2021) and in another study hot climate favored high citronellol/low geraniol content in contrast to low citronellol/high geraniol due to cold climatic conditions (Rao, 2002).

The cultivation of geranium for gaining economic benefits began in the early 1900's. Cultivation was initiated by two French nationals and acclimatized the plants for cultivation in southern India. At present, two varieties of geranium, viz. Algerian or Tunisian and Bourbon or Re-union, are widely cultivated in India. Another cultivar 'Kelkar' recently introduced by M/S SH Kelkar and Co, a multinational company in Mumbai, is the leading variety famously cultivated for its application in flavors and fragrance industry (Gauvin, Lecomte, & Smadja, 2004; Ram, Singh, Naqvi, & Kumar, 1997). The regions in India where geranium cultivation is piqued are Nilgiri, Pulney Hills of Tamil Nadu, Andhra Pradesh, Karnataka, Maharashtra and Uttar Pradesh. Significant amount of data is available on the scented geranium cultivated in different parts of India and its processing for essential oil (Jain, Aggarwal, Syamasundar, Srivastava, & Kumar, 2001).

The essential oil obtained by distillation of phototropic parts of the plant, viz. fresh flowers, leaf and stalks, is extensively utilized in perfumery, fragrance and cosmetic industry. Literature also suggests application of geranium in treating staunch bleeding, wound healing, treatment of ulcers, skin disorders, and in managing diarrhea, dysentery, and colitis (Ram, Ram, & Roy, 2003). The oil is known to have antibacterial and insecticidal properties (Ali *et al*., 2013; Hammami, Triki, & Rebai, 2011). Geranium oil has also been used in dispensable aromatherapy and shown to have mood-enhancing benefits (Upadhyay *et al*., 2016). Evidence suggests geranium is sedative and mood-uplifting, and has shown potential in treating extreme depression, panic, and anxiety (Braden, Reichow, & Halm, 2009). In addition to this, geranium has also been used for relief in menopausal stress, diabetes (Braden, Reichow, & Halm, 2009), blood disorders and throat infections, and endometriosis (Bussmann et al., 2013; Karato, Yamaguchi, Takei, Kino, & Yazawa, 2006). The cosmetic potential of geranium oil has also been explored for decades now, and it is found as the best remedy for congested, oily, and dry skin (Benazir, Suganthi, & Mathithumilan, 2013; Verma, Chandra Padalia, & Chauhan, 2016). Considering its applications in food, perfumery, flavor, cosmetics, and pharmaceutical industry, it is important that the high demand in the market for geranium oil has been met properly (Carvalho, Estevinho, & Santos, 2015; Neagu, Păun, Moroeanu, & Radu, 2010; Tabanca *et al*., 2013; Tuery, Bhat, Rather, & Kumar, 2008). Currently, the demand for the oil is more than 600 tonnes and is most met by countries like China, Morocco, Egypt, Reunion Island and South Africa. India produced approximately 20 tons of geranium oil on an area of 1,400 hectares (Narayana, Rao, Rao, & Sastry, 1986). Some data suggests that the consumption of geranium oil in India is 149 tons and production in India is approximately 5 tons per year (Ram, Ram, & Roy, 2003): the rest is imported from other countries (Saxena, Laiq-ur-Rahman, Verma, Banerjee, & Kumar, 2008).

Since decades, the Kelkar Education Trust's Scientific Research Centre (KET's SRC) has been involved in the cultivation of the best varieties of geranium, a cash crop of Kashmir (Shawl, Kumar, Chishti, & Shabir, 2006), using plant tissue culture technology. The SRC encourages farmers to cultivate the crops in areas with suitable soil and agro-climate conditions conducive for the growth of geranium. Nevertheless, the post distilled biomass is either discarded, or used for the preparation of fertilizer if carefully handled. The authors are now focusing on developing a technology that is based on the utilization of post-distillation biomass. The present study aims at the extraction of bioactive products from the post distillation geranium biomass (PDGB). The extractions were done with two solvents, namely methanol and ethyl acetate. Both of the extracts were tested for antimicrobial, antioxidant, and tyrosinase inhibition activities, and for toxicity. The extracts showed significant antioxidant ability as well skin whitening ability. The PDGB extract can be used in pharmaceutical and cosmetic industry for its bioactivities. Further purification of the active ingredients can increase the overall value-added benefits to the farmer.

2. Materials and Methods

2.1 Materials

For the present study, geranium plant material was obtained from a farmers' field from one of the villages in Karhad, Maharashtra, India, in mid-December. Chromatec-Crystal series of GC-MS (Russia) equipped with Autosampler and an MS detector was utilized for analysis of geranium oil. Microbial cultures were procured from Microbiologics USA kwik-stik: *Staphylococcus aureus ATCC 6538P* and *Escherichia coli ATCC 8739*. 2,2-diphenyl-1-picrylhydrazyl (DPPH) was procured from Himedia, Mumbai, India. Mushroom tyrosinase and tyrosine were procured from Thermo Fisher, USA. The 3T3/NIH 1658 fibroblast cells (Mouse) were procured from ATCC and maintained on Dulbecco's Modified Eagle Media DMEM (Himedia, Mumbai, India) with 10% Fetal Bovine Serum (FBS, Gibco, Thermo Fisher, USA) and IU/mL penicillin and 100 ug/mL streptomycin (HiMedia, Mumbai, India). The samples for toxicity were prepared in DMSO (Sigma Aldrich), and the cell viability assay was quantified using MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) (Himedia, Mumbai, India).

2.2 Collection of post distilled biomass of geranium and extraction of bio-actives

The geranium oil (GO) was extracted using steam-distillation and the post distilled biomass was oven-dried at 60º C for 2 days. The dried biomass was powdered using a mechanical grinder and stored in cold condition at 2-8^{0}C. Of the dried post distilled geranium biomass (PDGB), 10 g was used for extraction of phytochemicals by cold extraction: by soaking it overnight in 100 ml of the organic solvent methanol, or alternatively in ethyl acetate. The extracts were dried in a hot air oven at 50^{0}C and stored at 2-8^{0}C until further use. The extracts and GO were screened for their safety and potential efficacy as materials to be used in cosmetic formulations.

2.3 Phytochemical analysis

The alternative extracts of PDGB prepared for these studies were subjected to preliminary photochemical screening by using different reagents for identifying the presence of various phytocontstituents, viz. flavonoid, tannins, terpenoid, saponins, phenols, glycosides, carbohydrates, and proteins. These phytochemicals were tested per the standard methods (Edeoga, Okwu, & Mbaebie, 2005; Jamal & Yousef, 2018; Sawant & Godghate, 2013).

2.4 Gas chromatography-mass spectrometry (GC-MS) analysis of geranium oil

Chromatec-Crystal series of GC-MS (Russia) equipped with Autosampler and with an MS detector was utilized for the analysis of geranium oil. The ionization energy was 70 eV, used with a Time-of-Flight analyzer and Flame Ionization Detector. The column was TR-5MS GC. The capillary flow was 1 ml/min with a split ratio of 50:1 and the maximum oven temperature was 265.0ºC and run time 28 mins. The components were compared for their retention time with NIST (National Institute of Standards and Technologies, Mass Spectra Libraries) libraries.

2.5 Antioxidant activity (DPPH free radical scavenging activity)

The extract was added to methanol solvent at a concentration of 10 mg/ml. Dilutions of both the extracts were done in methanol to obtain concentrations. 0.5, 0.25, 0.125, 0.0625 and 0.03125 mg/ml. For GO, the final dilutions were 12.5, 25, 50, 100, and 200mg/ml in isopropanol (IPA). The sample was incubated with an equal volume of methanolic DPPH (2, 2-Diphenyl picryl hydrazyl) and DPPH diluted in IPA for GO. Appropriate controls and blanks were maintained throughout the experiment. Ascorbic acid was used as a standard. Negative control (Ac) was prepared similarly as a test sample, but the diluent was added instead of samples or standards. The tubes were incubated in dark for 30 mins. The samples were then read using a spectrophotometer (Cary 50) at 517nm (Dhawal, Satardekar, Hariharan, & Barve, 2017).

The DPPH radical scavenging activity of the extract was calculated using the equation (1):

$$\text{Percent DPPH radical scavenging activity} = \frac{(Ac - As)}{Ac} \times 100 \quad (1)$$

where, As is the absorbance of test sample or standard and Ac that of the negative control. The experiment was conducted in triplicates.

2.6 Tyrosinase inhibition

The samples were tested for melanin inhibition potential using an anti-tyrosinase assay (Sahasrabud & Deodhar, 2010). The reagents tyrosine and tyrosinase were solubilized in phosphate buffer at pH 6.8. The sample was solubilized in methanol followed by dilutions made in phosphate buffer and used at various concentrations (0.0625, 0.125, 0.25, 0.5, and 1.0 mg/ml). For GO, the sample concentrations used were 0.625, 1.25, 2.5, 5, and 10 mg/ml. For test solution 20 µL of the enzyme Tyrosinase was added to 100 µL of sample or standard for 15 minutes. The reaction was initiated by adding 80 µL of substrate tyrosine and incubating in the water bath at 37ºC for 15 minutes. After 15 min, the samples were placed in an ice bath to terminate the reaction. The absorbance of the plate was read at 475nm to determine the amount of L-dihydroxyphenylalanine-(L-DOPA)-quinone released. Appropriate control (enzyme-substrate) and blanks (without enzyme) were used in the experiments. Three independent experiments were conducted to study the tyrosinase inhibition activity and results are expressed as mean ± standard deviation. The tyrosinase inhibition activity of the extract was calculated using equation (2):

$$\text{Percent tyrosinase inhibition activity} = \frac{(Ac - As)}{Ac} \times 100 \quad (2)$$

where, As is absorbance of test sample or standard and Ac that of the negative control.

2.7 Antimicrobial activity

An overnight grown culture of *Staphylococcus aureus ATCC 6538P* and of *Escherichia coli ATCC 8739* were used in the assay. Disc diffusion assay was performed. The Petri dishes were inoculated with the culture via the spread plate technique. Soybean Casein Digest Agar was used for the experimental procedures. The extracts and GO were prepared in methanol to a concentration of 1 mg/ml. Sterile discs were added to the sample dilution and were dried at room temperature in a laminar airflow hood for 5 mins. The discs were then placed on the media plates containing respective bacteria. The plates were incubated at 37°C for 24 hours. The zone of inhibition was measured in mm (Syeda Sana, Govekar, Satardekar, Barve, & Dhawal, 2018).

2.8 MTT cell viability assay

Toxicity of both the extracts and IC_{50} value was estimated by using an MTT assay (Kakodkar *et al*., 2019). The 3T3 fibroblast cell lines ATCC 1440 were seeded at density of 0.5 x 10^4 cells /well into a 96-well plate (except blank) and incubated for 24 hrs at 37 °C under 5% CO_2. After 24 h the extracts and GO (0.125-1 mg/mL prepared in 1% DMSO) were added into wells with or without cells for test and blank, respectively, and the plates were incubated for 24 hrs at 37°C under 5% CO_2. After incubation, the media were removed from each well and 10µl of MTT reagent (5 mg/ml) was added to each well including the blank. The plates were incubated at 37^0C for 4 h in the dark. The MTT medium was discarded carefully and the Formazan crystals were extracted in 100µl of DMSO Reagent. The plate was kept at room temperature for 30 mins and shaken for 30 mins. The plate was read using Thermo MRX revelation 96 well plate reader at 570 nm. The experiment was conducted in triplicates.

2.9 Statistical analysis

All the experiments were conducted in triplicate for triplicate readings. Mean, standard deviation, regression coefficients and correlation between antioxidant activity,

tyrosinase inhibition and toxicity of the extracts were calculated using PASW (Predictive Analytics Software), earlier SPSS (Statistical Package) version 16.0.

3. Results and Discussion

3.1 Extraction of phytochemicals

Geranium oil has been studied in the past for antioxidant ability, photo-toxic effect on 3T3 cell line, antimicrobial properties, and anti-tyrosinase abilities (Lohani, Mishra, & Verma, 2018; Sienkiewicz *et al*., 2014). However, the present study highlights, for the first time, phytochemicals, antioxidant potential, antimicrobial analysis, and tyrosinase inhibition of the extracts isolated from post distilled biomass of geranium, for their use in cosmetological preparations. The phytochemicals present in the post-distilled biomass were extracted in two different solvents, viz. methanol and ethyl acetate.

The yields for both the extracts of PDGB were calculated and were found to be 0.5% for ethyl acetate extract and 0.9% for methanol extract. The methanolic extract of PDGB was sticky and brown in appearance, whereas the ethyl acetate extract was green in color and sticky in nature. The crude PDGB extracts were subjected to preliminary photochemical analysis followed by anti-oxidant, tyrosinase inhibition, antimicrobial, and toxicity analyses.

3.2 Phytochemical analysis

The phytochemical analysis of PDGB extracts, both in methanol and in ethyl acetate, is summarized in Table 1. In the current study, despite initial treatment of the sample for extraction of oil, the post distilled biomass still retained many essential phytochemicals such as flavonoids, tannins, polyphenols, carbohydrates, and proteins, which can be exploited for their medicinal and cosmetic abilities. In addition to these, ethyl acetate also extracted terpenoids from PDGB. Interestingly, saponins and glycosides were completely absent in both samples. Results of phytochemical analysis coincided with the results obtained for extracts in the solvents methanol, ethanol as well as acetone (Pradeepa, Kalidas, & Geetha, 2016). Literature mentions that ethanol, methanol, water, and acetone extracts of geranium leaves have shown flavonoids, phenols, and terpenoids amongst other phytochemicals.

3.3 Chemical composition of GO

The major chemical constituents and their relative percentages were determined using GC-MS analysis. The GC-spectrum is shown in Figure 1 and its relative constituents along with retention time are shown in Table 2. The GC-MS profile shows the presence of citronellol, neral, and geraniol, amongst others, in agreement with prior literature (Senthil Kumar *et al*., 2020).

3.4 Antioxidant capacity (DPPH free radical scavenging activity)

Both methanol and ethyl acetate extracts of PDGB showed great DPPH free radical scavenging activity

Table 1. Qualitative phytochemicals analysis of post distillation geranium biomass extracts

Phytochemical constituent	Ethyl acetate extract	Methanol extract
Flavonoids	+	+
Tannins	+	+
Terpenoids	+	-
Saponins	-	-
Polyphenols	+	+
Glycosides	-	-
Carbohydrates	+	+
Proteins	+	+

Key: + Positive test; - Negative test

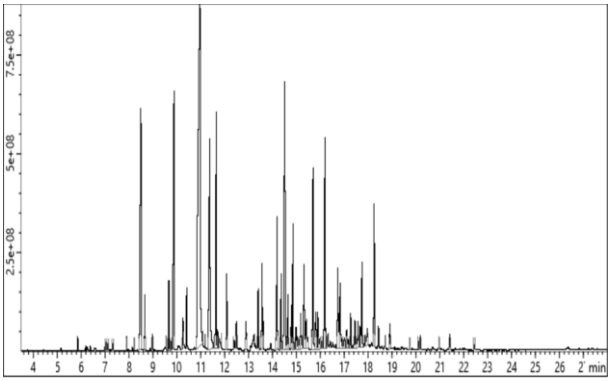

Figure 1. GC- MS spectrum for GO

Table 2. Major constituents in GO identified by using GC-MS

Retention time	Name
7.05	D-Limonene
7.32	β-Ocimene
8.5	Linalool
9.55	cis-Ocimenol
9.67	L-Menthone
9.77	trans-Ocimenol
9.88	Cyclohexanone, 5-methyl-2-(1-methylethyl)-, cis-
10.96	Citronellol
11.18	Neral
11.38	Geraniol
11.58	3-Cyclohexen-1-one, 2-isopropyl-5-methyl-
11.66	6-Octen-1-ol, 3,7-dimethyl-, formate
11.71	Benzoic acid, 3-methyl-, 2-methylpentyl ester
11.78	(-)-cis-Myrtanol
12.09	Geranyl formate
12.4	Bicyclo[3.1.0]hexan-3-ol, 4-methyl-1-(1-methylethyl)-, (1α,3β,4β,5α)-
12.49	trans-Geranic acid methyl ester
12.9	6-Octen-1-ol, 3,7-dimethyl-, acetate
13.41	Ylangene
13.57	(-)-β-Bourbonene
14.2	Caryophyllene
14.37	6-Octen-1-ol, 3,7-dimethyl-, propanoate
14.52	(1R,3aS,8aS)-7-Isopropyl-1,4-dimethyl-1,2,3,3a,6,8a-hexahydroazulene
14.59	6-Ethoxyquinaldine

indicating strong antioxidant potential. Optimal concentration of 0.5 mg/ml of methanol and ethyl acetate extracts of PDGB showed 83.78 ± 0.009% and 96.38 ± 0.006% DPPH free radical scavenging activity, respectively. The statistical analysis and coefficient of determination (R^2) at 0.9025 and 0.9337 for methanol and ethyl acetate extracts of PDGB showed good accuracy of antioxidant potential estimates. However, the GO sample showed relatively a very low DPPH activity with an IC50 of 77.49 mg/ml. Overall, the ethyl acetate extract of PDGB showed the highest DPPH free radical scavenging activity. The analysis of data in Figure 2 gave calculated IC_{50} for ethyl acetate and methanol extracts of PDGB as 0.188 mg/ml and 0.201 mg/ml, respectively.

The scavenging ability of the extract of geranium has been associated with the phenolic content in the extracts. However, the phytochemicals extracted from post distilled biomass have not been explored yet for their radical scavenging potential. In the past, antioxidant activity, analyzed via DPPH assay, for geranium leaf aqueous extract has been reported as 0.118 mg/ml (Singh, Kumar, Gupta, & Chaturvedi, 2012). In the present study, the IC_{50} of post distilled biomass extracts, both in methanol and in ethyl acetate, was found below 0.2 mg/ml, indicating an immense potential of the biomass even after oil distillation. Literature suggests that the polyphenols are extracted better in non-polar solvents due to their high affinity towards such solvents. This could possibly be the mechanism behind higher antioxidant activity seen with ethyl acetate extract. The R^2 was high for both the extracts demonstrating a strong association between increasing concentration with increasing antioxidant potential of the extracts (Figure 2).

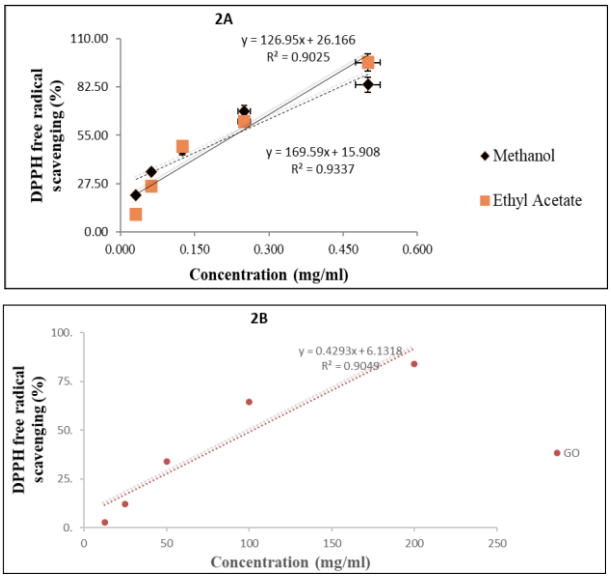

Figure 2. Antioxidant activities of post distilled geranium biomass extract (2A) and GO (2B) in DPPH radical scavenging. Error bars indicate standard deviations for triplicate readings.

3.5 Tyrosinase inhibition

The statistical analysis and coefficients of determination (R^2) at 0.9723 and 0.9860 for methanol and ethyl acetate extracts of PDGB showed consistency in determining tyrosinase inhibition potential. The data in Figure 3 gave calculated IC_{50} for methanol and ethyl acetate extracts of PDGB as 1 mg/ml and 0.97 mg/ml, respectively. However, as seen in the DPPH assay, the GO showed relatively very low tyrosinase inhibition activity with an IC_{50} of 7.44mg/ml.

Strong DPPH free radical scavenging and tyrosinase inhibition activities revealed correlation and exhibited very good antioxidant potential and the calculated Pearson's correlation index between DPPH free radical scavenging and the anti-tyrosinase assay was 0.922 for methanol extract and 0.952 for ethyl acetate extract. Once again ethyl acetate extract of PDGB exhibited slightly better activity compared to the methanolic extract. Tyrosinase inhibition has also been reported in the literature for several species of geranium (Singh, Kumar, Gupta, & Chaturvedi, 2012). For instance, the fresh leaves extract has shown IC_{50} of 30-32% when tested *in vitro* on several varieties of geranium species. The study also revealed that the phytochemicals ellagic acid and quercetin were the major tyrosinase inhibitors in the extracts (Ćavar & Maksimović, 2012). In the current study, despite the post distilled biomass being used for extraction of phytochemicals, the extracts retained tyrosinase inhibition potential. The extracts showed significant antioxidant and anti-tyrosinase abilities with higher IC_{50} for ethyl acetate extract than for methanol extract. Literature suggests the total tannin, phenol and flavonoid contents were higher in acetone extract than in the methanolic extract (Lohani, Mishra, & Verma, 2018) which would explain the higher tyrosinase inhibition activity of ethyl acetate extract, due to a low polarity solvent.

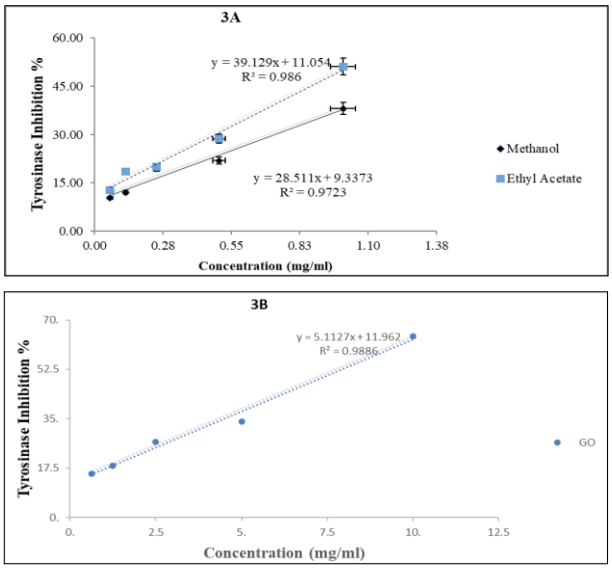

Figure 3. Tyrosinase inhibition activities of post distilled geranium biomass extract (3A) and GO (3B). Error bars indicate standard deviations for triplicate readings.

3.6 Antimicrobial activity

The samples showed no zone of inhibition against the tested microorganism at 1mg/ml. Geranium oil is found to have antimicrobial activity. However, the PDGB extracts exhibited no antimicrobial potential when tested in a disc diffusion assay as no zone of inhibition representing the

antibacterial activity of the extracts or of GO was found. Methanol, ethanol, acetone, and aqueous extracts of geranium fresh leaves have been analyzed in the past for anti-microbial ability against *E. coli*, *S. aureus*, *P. aeruginosa* and *K. pneumonia* and have shown inhibitory activities against the tested microbes (Pradeepa, Kalidas, & Geetha, 2016). However, in the current study the extracts did not show any antimicrobial ability, while geranium oil is a strong antioxidant and geraniol is the major antimicrobial agent present in the plant (Bigos, Wasiela, Kalemba, & Sienkiewicz, 2012; Singh, Kumar, Gupta, & Chaturvedi, 2012). Different extraction procedures such as subcritical extraction or Soxhlet extraction with various solvents need to be tested for the extraction of antimicrobial molecules from the after distilled biomass.

3.7 Cell survival and toxicity assay

The extracts were checked *in vitro* for toxic effects on 3T3 mouse fibroblast cell line. According to the graph of percent viability versus concentration of the extract (Figure 4), there was a negative correlation between viability and concentration of extract with a high R^2. Additionally, the toxicity of methanol showed a strong negative correlation with antioxidant (0.935) and anti-tyrosinase activities (0.889). Similar strong negative correlation was observed between toxicity for ethyl acetate extract with antioxidant (0.9567) and anti-tyrosinase activities (0.9412). The cell viability was nearly unaffected until 0.125 mg/ml concentration for both the extracts. Viability of 75% was observed at 0.25 mg/ml for both the extracts. The concentration where the cell viability is 50% is said to be non-toxic and gives the IC_{50} of the extract, after which the extract becomes toxic. For the current study, the IC_{50} of PDGB methanol extract was 0.433 mg/ml and for ethyl acetate extract it was 0.49 mg/ml. The GO also showed an IC_{50} roughly similar to the extracts at 0.37 mg/ml. Both of the extracts showed toxicity only at higher concentrations suggesting that they are safe for utilization by humans and can be used in cosmetic preparations.

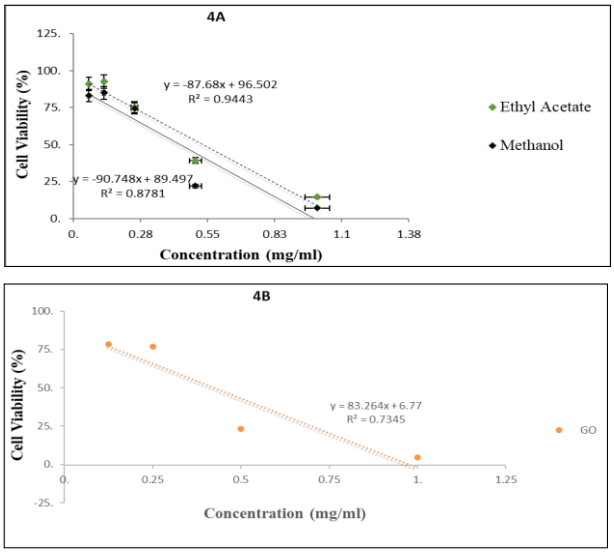

Figure 4. Toxicity testing of post distilled geranium biomass extract (4A) and GO (4B) on 3T3 mouse fibroblast cell line. Error bars indicate standard deviations for triplicate readings.

4. Conclusions

The post distilled biomass of geranium is currently used only for preparation of fertilizer. However, before that, many secondary bioactives are still present in the biomass and can be good candidates for cosmetics, drug, and pharmaceutical industry. The PDGB after distillation of geranium oil was studied *in vitro* for factors related to cosmetic efficacy and safety. The extracts possessed strong antioxidant and antityrosinase ability and were safe to use in cosmetic products. Further the post distilled biomass can also be studied for the presence of pigments, or use as organic fertilizer or for biogas production. Thus, the present results support increasing the income for farmers cultivating geranium crops by adding value to the post distilled biomass in Indian market.

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