Assessment of Stabilization of Canola Oil, Free Radical Scavenging and Cytotoxic Potential of *Peucedanum graveolens* (roots)

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Abstract- *Peucedanum graveolens* has been perceived as a wellspring of common cell reinforcement's aginst oxidative mechanism. The purposes of that study were to assess the antioxidative potential of different extracts of *P. graveolens* roots in different solvent systems by computing yield, TPC, TFC, DPPH and linoleic acid per-oxidation. Extracts yield observed in this study are found in range 6.90-10.80%.Total phenolic contents (TPC) and total flavonoid contents (TFC) were observed in the scale of 0.90-2.49 mg and 3.20-5.70 mg respectively. The DPPH-IC_{50} and %age-inhibition per-oxidation examined by different extracts of *P. graveolens* roots were come in the extent of 23.9-80.9 µg/mL and 40-71.9% respectively. By balancing out the canola oil as oxidative substrate, oxidative parameters like peroxide value (PV), free fatty acid (FFA) along with *para*-anisidine (PAV) were additionally analyzed. Cytotoxic potential investigated against the personage red blood corpuscles (RBCs) *in vitro* by measuring the haemolysis effect in different extracts of *P. graveolens* roots and range of % lysis 1.3-4.5%was explored. The outcomes from the present research work exhibited that petroleum ether and methanol extracts of *P. graveolens* roots exhibited enhanced anti-oxidative characteristics and lesser cytotoxic effect.

Index Terms- *Peucedanum*, Extract, Antioxidants, Stabilization, Free Fatty Acid, Canola oil

I. INTRODUCTION

Nature has gifted aerobic organisms with an inner defense system that hinder oxidative damage because of reactive oxygen species (ROS). However, boosting the natural defense mechanism with dietary antioxidants might offer better defense against the risk of certain cancers, inflammation and other degenerative diseases [1]. The introduction of synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxy-toluene (BHT) in lipid containing foods is often dispiriting because of their safety and discern carcinogenicity. On the other hand, employment of plant based antioxidant compounds in foods as well as in defensive medicine is acquiring a great deal of concentration due to their great health improvement effects [2-5]. It is well acknowledged that plants are the richest resorts of antioxidants. Antioxidants comprising compounds are Tocopherols, carotenoids, phenolics compounds and acids. Trace elements such as zinc and selenium, as well as compounds of medicinal plants such as polyphenols, flavonoids, terpeenes, anthraquinones and phytic acid [6]. *Peucedanum graveolens*, an annual or biennial aromas herb also suspected to have antioxidants in large concentration in their different parts like leaves, fruits, and seeds. Native to Europe is naturalized to North America and the West Indies. Principal dill manufacture areas are India and Pakistan, Egypt, Fiji, Mexico, the Netherlands, the United States, England, Hungary, Germany, and Holland also have commercially productive areas [7]. Its leaves are generally having been taken in salads and tea while its seeds are employed in tea, breads, soups, salads and preserves. The plant is a supply of protein, carbohydrate, phosphorus, iron, magnesium, sodium and potassium. It also contains a small amount of riboflavin, niacin and zinc. Dill oil has dominant characteristics of pesticide, aromatic, stimulant, and carminative properties' also reported [8]. The fruits are acrid, better, thermogenic, deodorant, digestive, stomachic, anthelmintic, anodyne, anti-inflammatory, diuretic, emmenagogue, galactagogue and expectorant. They are valuable in halitosis, flatulence, colic, dyspepsia, fever ulcers, skin disease, cough, asthma, bronchitis and cardiac debility [9]. Dill deliberated its performance as therapy for the gastrointestinal, releasing wind and soothing the assimilation. Dill's vital oil provides the relief against gastric contractions and moaning; consequently have been employed in complaint water mix. Dill has ability to enhance the productivity of milk in feeding mothers as they were taking frequent amount of it; provide assistances to avert indigestion in offspring [10]. The essential oil that was procured by distillation is employed in pharmaceutical industry, in the fabrication of liqueurs and in cosmetic preparation [7]. Dill is also applied as tranquilizer. The total volatile oil portion of dill has also been premeditated for its aptitude to thwart overgrowth of bacteria. In this admiration, dill as well as garlic is sharing same juncture, which has also been publicized to have "bacteriostatic" or bacteria-regulating effects [11]. It has been revealed that monoterpene constituents of dill prompt *glutathione-S-transferase*to fasten the anti-oxidant molecule-glutathione to oxidized molecules that causes harmness in the body. The low boiling point oils of dill's meet the criteria as "chemoprotective" nourishment (much like parsley); act as facilitator to deactivate scrupulous kinds of cancer causing agents, such as benzo-pyrenes [12]. The goal of the present research work was to assess the *in vitro* antioxidant,

phytochemical, and cytotoxicity learning of the *Peucedanum graveolens* roots by using different solvent systems and also involve to find out the antioxidant activity using canola oil (CO) as oxidation substrate.

II. RESEARCH METHDOLOGY

The roots of *Peucedanum graveolens* were acquired from the native zone of Faisalabad, Pakistan in July, 2015 and representative samples were recognized from Taxonomist, Department of Botany, and G.C. University Faisalabad. The current investigational study was conducted in the organic research laboratory, Department of Chemistry, University of Management and Technology, Lahore and in R&D Lab Shafi Reso Chemicals, Lahore Pakistan. All chemical-substances employed in that research effort were procured from BASF (Germany) and Fluka Chemische (Switzerland). All other solvents were also of analytical grade which were employed in this study.

Assembly of extract Construction: The cured taster was pulverizing by fleeting through 85 netting filters by means of commercial grinder (Bartec-728, Germany). 1.5 liters petroleum ether employed to drench 500g roots of *P. graveolens* in ash form for single week. Whatman No.1 filter paper was employed for the segregation of Petroleum ether concentrate from remnants by sifting over it. The same procedure was repeated for three times to get maximum amount of extract and all three extracts were mixed. Further extracts were prepared by sinking left behind scum in different solvent systems, based on their polarity differences such as petroleum ether (M-1), petroleum ether: chloroform (M-2), ethyl acetate (M-3), ethyl acetate: chloroform (M-4), chloroform (M-5), ethanol (M-6) and methanol (M-7).Scum was isolated as of all the extracts by means of Whatman filter paper No.1 and all extracts were to make concentrated and freed of solvent by means of a rotary evaporator under reduced pressure at 50 ºC. Weight of individual extracts was measured to estimate the quantity of each extract and heaped in fridge-freezer at 4ºC till for future research exertion [13, 14].

Phytochemical examination: Phytochemical analysis employed on different extracts of *P. graveolens* roots executing in different solvent systems such as petroleum ether (M-1), petroleum ether: chloroform (M-2), ethyl acetate (M-3), ethyl acetate: chloroform (M-4), chloroform (M-5), ethanol (M-6) and methanol (M-7). Standard methods were applied to recognize the chemical components in these extracts as reported [15, 16] with some moderation.

Estimation of Antioxidant Activity of Plant Extracts

Subsequent anti-oxidant evaluates were castoff for the estimation of antioxidative potential of different extract of roots of *Peucedanum graveolens*.

Total Phenolic Contents (TPC) Determination: By employing Folin-Ciocalteu reagent, quantity of TPC was calculated by subsequenting the course of action as narrated by [17].

Total flavonoid contents (TFC) Determination: Spectrophotometrically, TFC were evaluated by subsequenting the methodology as illustrated by [18].

DPPH Assay Determination: By succeeding the methodology as evoked by [19], DPPH assay was executed. Plot of percentage scavenging against concentration of different extracts in different solvent systems was used for enumerating the IC_{50} values. Three replicates of each sample were done.

%age-inhibition per-oxidation Determination: %age-inhibition per-oxidationin linoleic acid system was cast off by computing antioxidative potential of plant extracts as succeeding the process as elucidated by [20].

Employing of canola oil (CO) as oxidative substrate for the estimation of antioxidative potentialof roots extracts of *Peucedanum graveolens*

Oil (CO) acts as oxidative substrate. By utilizing escalated getting older of refined, bleached and deodorized (RBD) canola oil, antioxidative potential of different roots extracts of plan tin different solvent systems was estimated. Amount of 500 ppm of different root extracts of the plant substantial were aside introduced to preheat (50ºC) refined, bleached and deodorized canola oil. Continuously mixing was given to the oil tasters for 0.5 h at 50 ºC for unvarying distribution. In this research learning, synthetic antioxidative substance was employed to its permissible perimeter of 200 ppm to equate the potency of plant extracts. 100mL of control and stabilized oil samples have been located in obscure tan hermetic glass flagons along with tapered roll neck and put them through to enhance storing in an electrical forge at 60±2 ºC for forty days. Underneath the identical set of investigative considerations as in published methodology [13, 14], a control taster was similarly formulated. Afterward every ten days for the duration of storing time, investigation was carried out on oil tasters. By vindicating peroxide value (PV), free fatty acid (FFA %) contents and *para*-anisidine values (PAV), magnitude of oils towards the oxidative deterioration were evaluated [14].

Peroxide value and free fatty acid estimation: Subsequenting AOCS official method (Cd 8-53)[21], peroxide value as well as free fatty acid were estimated for control and stabilized canola oil tester.

***p*-Anisidine value:** By the estimation of *p*-anisidine value, mensuration of α- and β unsaturated aldehyde in the tester was done as they gives color composites by *p*-anisidine that resulted spectrophotometerically. These are familiar as secondary oxidative compounds [22].

Cytotoxicity studies: The cytotoxic effect of plant extract was calculated by the use of method described by [23, 24].

Statistical Investigation: All experimentations were done with minimum frequent of three times. The information was offered with mean ±SD tenets at 95% confidence interval. Data were analyzed using Statistical Analysis System program. Significant differences ($p < 0.05$) were resulted among means.

III. RESULTS AND DISCUSSION

Phytochemical examination: Alkaloids, flavonoids, saponins, tannins and anthraquinones that act as phytochemical constituents were scrutinized in different extracts of *P. graveolens* roots in different solvent systems. These chemical investigates exhibited that significant compounds identical to saponins, alkaloids, tannins and flavonoids were founded

although anthraquinone was not present in the plant tester underneath this research work. Phytochemical analysis of *P. graveolens* roots extract manifested that has affluent quantity of saponins tannins flavonoids as well as alkaloids.

ExtractYield: %yield exhibited by different extracts of roots of *P. graveolens* in different solvent systems was within the range of 6.90% to 10.80%. Figure 1 represented the Graphical representation of these results. Methanol extract (M-7) was observed with maximum yield (10.80%) while minimum (6.90%) yield was found with chloroform extract (M-5). The %age yield of different extracts in different solvent systems obtained by the roots of *P. graveolens* lessened in the following pattern, M-7 (10.80%) > M-1(10.41%) > M-2 (9.50%) > M-3 (8.64%) > M-6 (8.53%) > M-4 (7.99%) > M-5 (6.90%). [25] And [26] deliberated the antioxidant activity of fennel seed and *C. inerme* stem respectively and delineated the higher yield of methanol extract (12.11%) and (11.33%) respectively. In this present study, methanol extract (M-7) exhibited lower yield (10.80%) as compared to reported data. This fact pronounced by [27] as the naturally occurring antioxidative components are polar in nature, so as result when there is increasing polarity of the solvent, enlargement in concentration of constituents were also founded. Henceforth, for supreme abstraction of antioxidative constituents from plants, methanol is generally engaged due to its polar nature and better dissolution of several antioxidative constituents in it in comparison to other solvents. This research work exhibited the yield of petroleum ether extract (M-1) is 10.10% which is within the range of reported yield of petroleum ether extract (10.41%) of *C. inerme* by [26] which indicated that non-polar antioxidants like tocopherol, phospholipids and lipophilic are also sourcing by *P. graveolens*.

Total Phenolic Contents (TPC): The total phenolic contents gotten from extracts of different solvent systems of *Peucedanum graveolens* roots range from 0.90mg /100 g to 2.49mg /100g Gallic Acid Equivalent (GAE). Figure 2 represented the Graphical representation of these results. Lowest TPC value 0.90g/100g GAE was detected by chloroform extract (M-5) while the highest TPC value 2.49g /100g GAE was perceived by methanol. The gradation of TPC among different extract of *P. graveolens* roots lessened in the following pattern, M-7 (2.49) > M-1 (2.44) > M-2 (2.27) > M-3 (1.42) > M-4 (1.31) > M-6 (1.40) > M-5 (0.90). Total phenolic contents in dill roots extract was 2.63mg/100g reported by [28] which is high from presently investigated total phenolic content (2.49mg/100g) in the methanol extract (M-7) of *P. graveolens* roots which in turn was higher than in the dill seed extract which was 2.46mg/100g reported by [29]. In addition to methanol extract (M-7) of *P. graveolens* roots, remarkable total phenolic contents (2.44mg/100g) were founded in petroleum ether extract which is higher form the reported value of TPC (1.59mg/100g) in *C. inerme* extract of petroleum ether by [26] which indicated that present investigated plant is also a rich source of non-polar antioxidants. Difference between reported values and present work is due to method of cultivation, geographical position of land and soil effect.

Total Flavonoid Contents (TFC): TFC that acquired from different extracts of roots of *P. graveolens* in different solvent systems range from 3.20mg to 5.70mg catechin equivalent. Figure 3 represented the Graphical representation of these results. Highest TFC value (5.70mg) was perceived by methanol extract (M-7) while lowest TFC value (3.20mg) was detected by chloroform extract (M-5). The gradation of TFC among different extracts in different solvent systems of *P. graveolens* roots lessened in the following pattern, M-7 (5.70) > M-1 (5.09) > M-2 (4.41) > M-3 (3.80) >M-6 (3.50) > M-4 (3.31) > M-5 (3.20). Results of the present analysis of methanol extract (M-7) are comparable to some extent with the results of literature reported TFC values of seeds of methanol extract of dill seed. Difference between present work and reported value may be due to several reasons such as method of cultivation, geographical position of land, soil effect and fertilizers used. Presence of yield differences among different extracts of plants may be because of the accessibility of diverse extractable constituents, chemical configuration, and soil nature and agro meteorological circumstances. Efficacy of the solvent to excavation out the dissolve endogenous constituents is also a factor among other factors [30].

DPPH Assay: In 2, 2'-diphenyl-1-picrylhydrazyl scavenge examine, root extracts of *P. graveolens* in different solvent systems demonstrated the persuasive antioxidative potential. DPPH is very steady organic free radical with profound bluish-purple color. Its precise λ_{max} observed at 510-528nm. Misses its own chromospheres by accepting the H^+ from any hydrogen giver, henceforth shifting its color bluish-purple to yellow. It was measured that extract aptitude to scavenge free radical like DPPH is because of the phenolic composites existence in them. Consequently, as phenolic compounds extent enlarges, DPPH-radical scavenges action as well rises with its antioxidative potential as well [31]. By DPPH evaluation, free radical scavenging talents of different extract of roots of *P. graveolens* were accomplished and figure 4 exposed the graphical representation of outcomes. The IC_{50} value was ranging from 23.9µg/mL to 80.9µg/mL. Methanol extract (M-7) presented 23.9µg/mL which was lowermost and chloroform extract (M-5) exhibited highest which was 80.9µg/mL. IC_{50} progression among different extracts obtained from *P. graveolens* roots dwindled in the following pattern, M-5 (80.9) > M-6 (67.7) > M-4 (54.9) > M-2 (46.4) > M-3 (46) > M-1 (34.1) > M-7 (23.9) > BHT (20.5) µg/ml. These results dictated that methanol extract (M-7) of plant sample roots have IC_{50} (23.9µg/ml) value closer to the synthetic antioxidant standard BHT (20.5µg/ml). From this current research work, it had been determined that methanol extract (M-7) showed stronger free radical scavenging activity as well as petroleum ether extract (M-1) also revealed better consequences of free radical scavenging. In the inspection of DPPH; extract capacity was explored to work like benefactor of H-atoms, for the conversion of DPPH into DPPH-H. As a result, outcomes disclosed that extracts have ability to turn down bluish-purple colored (DPPH) to yellow colored (DPPH-H). Consequences of the current investigation are comparable to some extent with the outcomes of literature-testified.

%age-inhibition per-oxidation: %age-inhibition per-oxidations were castoff to examine the antioxidative potential of different roots extracts of *P. graveolens* in different solvent systems. The %age-inhibition per-oxidation executed in linoleic acid system was ranging 40% to 71.9%. Maximum inhibition demonstrated in methanol extract (M-7) which was 71.9% and lowest inhibition revealed in chloroform extract (M-5) which was

40%. Figure 5 represented the Graphical representation of these results. The progression of %age inhibition of linoleic acid oxidation among different extract of *P. graveolens* roots lessened in the following pattern, BHT (81%) > M-7 (71.9%) > M-1 (65%) > M-2 (58%) > M-4 (56%) > M-3 (53%) > M-6 (49%) > M-5 (40%). All extracts demonstrated significant inhibition of per-oxidation and were matched with BHT. Synthetic antioxidant (BHT) that act as standard has 81% inhibition per-oxidation. Lower peroxidation was observed with all extracts as compared to BHT. The %age inhibition of the methanol extracts of seeds of *P. graveolens* resolute by [29] was 70.2%. This value is within the range of the results of present analysis of both methanol (M-7) and petroleum ether extract (M-1) which are 71.9% and 65% respectively. Negligible difference founded between reported value and present work of methanol extract (M-7) and petroleum ether extracts (M-1).

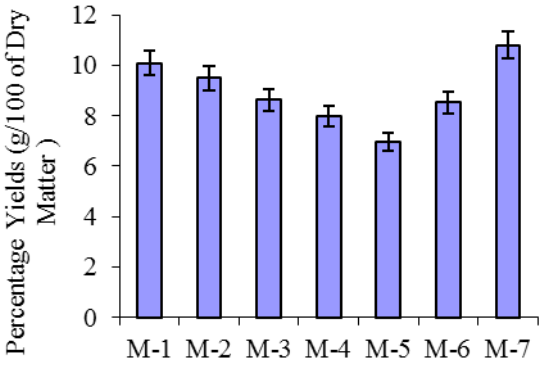

Figure 1. Different extracts of *P. graveolens* roots

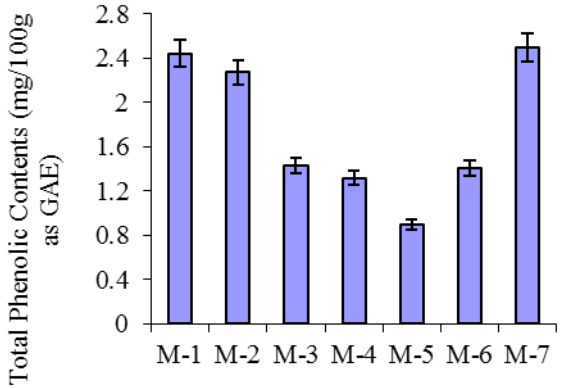

Figure 2. Different extracts of *P. graveolens* roots

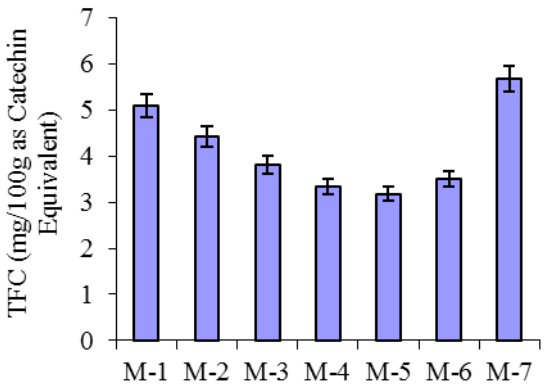

Figure 3. Different extracts of *P. graveolens* roots

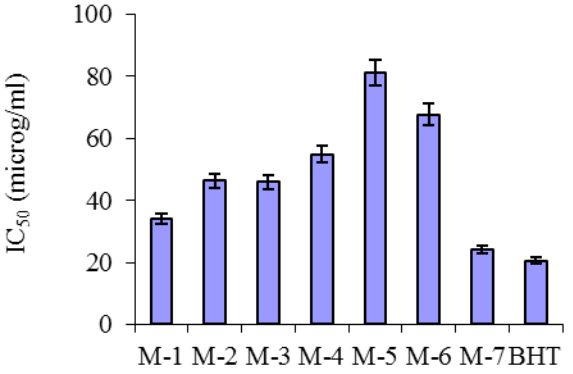

Figure 4. Different extracts of *P. graveolens* roots

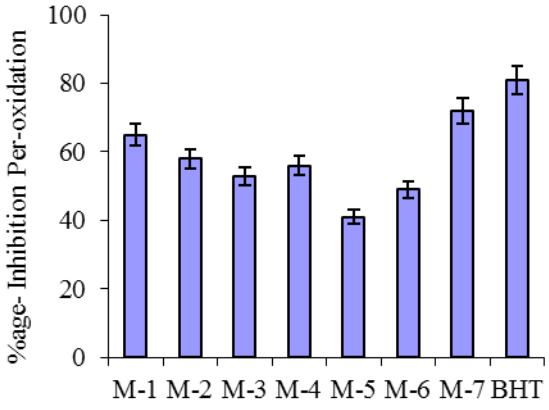

Figure 5. Different extracts of *P. graveolens* roots

Antioxidative potential of *P. graveolens* roots extracts for stabilization of canola oil

Peroxide value (PV): For the assessment of extent of primary-oxidation crop (primarily the per-oxides) in oils per-oxide value is frequently applied [32]. Figure 6 represented the comparative enlargement in PV of stabilized and control canola oil (CO) with diverse roots extracts and without extracts respectively. Rise in peroxide values were appreciably founded fewer than in oil samples that treated through various roots extracts of plant as compared to the control sample as well as presenting a lesser amount of deterioration. While the control

sample demonstrated higher peroxide values in consequence exhibiting larger degree of oxidation. The methanol extract of *P. graveolens* roots revealed low peroxide values because of the existences of extortionate phenolics quantity. According to literature exploration, extracts of ginger at 1600 ppm and 2400 ppm strength, hindered the raise in peroxide values significantly and current outcomes were to some extent analogous to conclusion [33] and current investigational findings were also founded in accordance with the methanol extract of soybean oil that investigated the 17.20 peroxide value.

Free fatty acid (FFA): A computation of FFA content was carried out by stabilizing oil samples with different extracts of *P. graveolens* roots in different solvent systems. Figure 7 revealed the correlation in progression in the free fatty acid concentration of treated canola oil and control samples. It is observed that initially, extracts were showed a sluggish enlarge in FFA contents. In contrast to other extracts, methanol extracts (M-7) manifested superior antioxidant activity in provisos of free fatty acid. The efficacy of *P. graveolens* roots extracts stipulated by measuring the decrease in the free fatty acid contents of stabilized oil taster than control oil sample as free fatty acid generation decelerated by natural antioxidant. During the storage phase, enlargement in oxidized and hydrolyzed components was assessd by the increscent of FFA value in canola oil taster of control (without extracts). According to the literature appraisal, for the deterioration of oils as well as free fatty acid development, reaction of oxygen in the existence of light is the first step; as a result numerous compounds based on organic nature as well as free fatty acid development takes place that are accountable for the spoilage creation in full of fat food stuff [25].

***p*-anisidine values (PAV):** Secondary lipid-oxidation products yield determined by through this chemical test. Relative enhancement in PAV was observed in the canola oil that treated with roots extracts of *P. graveolens* under esclated circumstance. Maximum PAV was explored by control taster. Lower PAV was estimated with the methanol extract (M-7) of *P. graveolens* roots then other extracts. Figure 8 revealed the graphical representation of outcomes of treated oil taster and control taster by different extracts of *P. graveolens* roots in different solvent systems. Aldehyde present in oil and *p*-anisidine reagent both react in acidic environment [34]. Yellow coloured products were obtained by the projected reaction of aldehyde and *p*-anisidine.

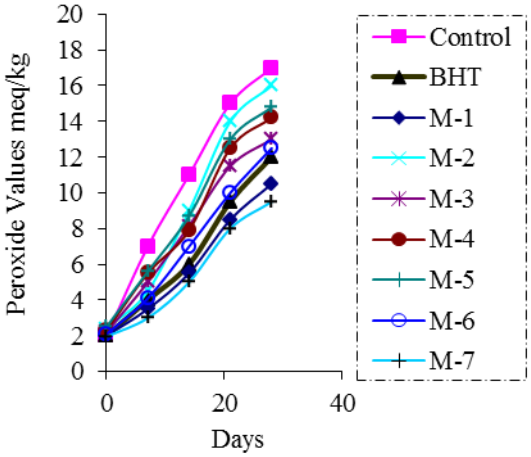

Figure 6

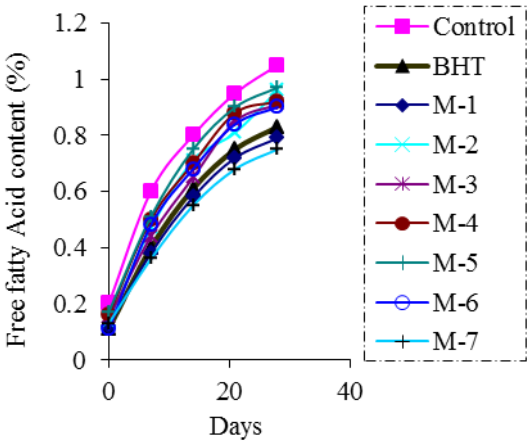

Figure 7

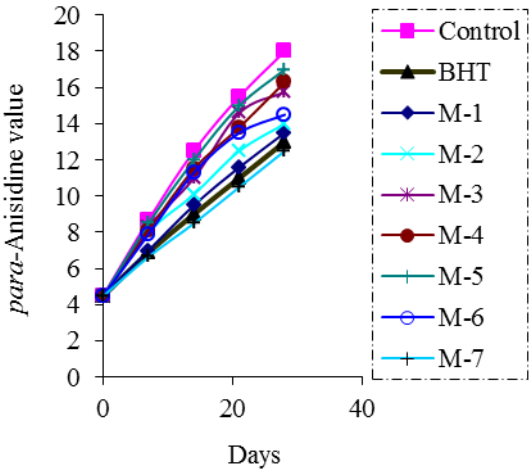

Figure 8

By means of dimension of PAV of oil, estimation of antioxidative potential for plant extracts was normally acknowledged [13].

Cytotoxic Potential: Cytotoxic potential of roots extract of *P. graveolens* was deliberated by the estimation of hemolytic

effect outcomes. This fallout was evaluated with roots extracts of *P. graveolens* in different solvent systems. Figure 9 represented the graphical representation of these results. The progression of %age of red blood cell (RBC) lysis among different extract of *P. graveolens* roots narrowed in the following pattern, M-9 (99.06) > M-6 (4.5%) > M-3 (3.8%) > M-1 (3.6%) > M-7 (2.6%) > M-2 (2.5%) >M-5 (1.6%) > M-4 (1.3%) > M-8 (0). Manifestation of cytotoxicity of plant extracts exhibited that it is less than 6%. So as a result it can be say that the plant extracts have no cytotoxic effect. Plants extracts which have a reduced amount of cytotoxic effect may be employed as herbal medicines [29].

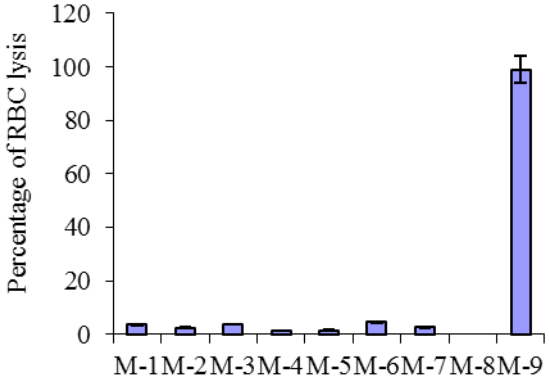

Figure 9 Different extracts of *P. graveolens* roots

IV. CONCLUSION

From the consequences of different antioxidant assessments as well as from different oxidative circumstances of treated canola oil with plant extracts, it was explicable that extracts of methanol (M-7) and petroleum ether (M-1) exhibited good antioxidant activity. However, methanol extract (M-7) exhibited the antioxidant activity considerably higher than other solvent extracts. The antioxidant activity of different extracts of *P. graveolens* roots in different solvent systems as evinced in this current investigation may be accredited to the occurrence of considerably elevated quantity of polyphenole and other antioxidative components. In the present study, different extract of roots of *P. graveolens* in different solvent systems disclosed exceptional antioxidative potential as well as also revealed the significant quantity of flavonoid contents and phenolic contents. Consequently, current learning would facilitate to find out the effectiveness of crude extracts roots of *P. graveolens* as prospective resource of natural antioxidants. Although, supplementary investigations are desirable to recognize constituents that are involve in the formation of antioxidative system and develop their approaches for pharma industry as wells food industry.

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