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In-vitro comparative studies of *Apium graveolens* L. extracts for antioxidant and anti-inflammatory activity

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Abstract

The purpose of the present study was to find out the secondary metabolites and to evaluate and compare the antioxidant and anti-inflammatory activity of various extracts (aqueous, methanol, chloroform and acetic acid) of Apium graveolens L. Preliminary phytochemical screening revealed the presence of the major class of phytochemicals. The TPC and the TFC were found to be highest in the aqueous extract (61.3 ± 0.8 mg GAE /g) and TFC (47.7 ± 0.4 mg QE /g) with acetic acid extract showing the lowest TPC (2.6 ± 0.3 mg GAE /g) and TFC (13.0 ± 1.1 mg QE/g), respectively. The same trend was found in the case of TAC, where aqueous extract exhibited the highest TAC (9.5 ± 0.06 AAE mg/g) and antioxidant activity with IC_{50}= 0.87 and 0.13 mg/ml for DPPH and NO scavenging activity, respectively. Extracts also showed in vitro anti-inflammatory activity by inhibiting the heat-induced protein denaturation with the IC_{50} values of 0.58, 0.69, 1.06, and 2.82 mg/ml for methanol, aqueous, chloroform and acetic acid extracts, respectively. The results of our study revealed that aqueous extract is the most effective solvent to proceed for the in vivo studies against oxidative stress and inflammation-associated diseases.

Keywords: *Apium graveolens* L., Flavonoid, Antioxidant activity, Anti-inflammatory activity.

Introduction

Since time immemorial, people over the world have been using the medicinal plants for relief of symptoms of diseases (Maqsood *et al.,* 2010). Even today, despite the great advances observed in modern medicine, medicinal plants are being used due to their easy access and low cost. Furthermore, the World Health Organization (WHO) encourages the use of traditional medicines for the treatment of diseases provided they are proven to be efficacious and harmless (WHO, 1985). Epidemiological studies have shown that regular consumption of a plant-based diet containing antioxidants reduces the risk of many oxidative stress-related diseases (Arabshahi-Delouee and Urooj, 2007). Thus, in recent years, interest in natural antioxidants, especially of plant origin, has increased manifolds (Jayaprakash and Rao, 2000). Natural antioxidants either in the form of raw extracts or their chemical constituents are very effective to protect organisms from

damage caused by free radical-induced oxidative stress (Zengin *et al.,* 2011). Presently, the probable toxicity of synthetic antioxidants such as butylated hydroxyl anisole or butylated hydroxyl aniline has been condemned (Zhang *et al.,* 2009). Therefore, a considerable interest in the development of natural antioxidants from plants has gained much interest in recent years, especially in the field of biomedical and nutritional areas.

The therapeutic properties of medicinal plants are mainly due to the presence of phytochemical compounds (Kumar *et al.,* 2009). These are naturally occurring compounds which include primary compounds such as chlorophyll, proteins, and common sugars and secondary compounds such as alkaloids, glycosides, quinones, saponins, terpenoids, flavonoids, tannins and phenolic compound (Zheng and Wang, 2001; Mallikaharajuna *et al.,* 2007; Gracelin *et al.,* 2012). They are also antioxidant compounds that possess anti-inflammatory, anti-tumor, anti-mutagenic, anti-carcinogenic, anti-bacterial, and anti-viral activities (Zheng and Wang, 2001; Sala *et al.,* 2002; Gracelin *et al.,* 2012). One of the most important compounds are polyphenols (E.g. flavonoids, phenolic acids, and transipropanoids). They have been directly associated with antioxidant activity due to their free radical scavenging activities (Nickavar *et al.,* 2007). All these anti-properties of medicinal plants, such as anti-oxidant, anti-cancer, anti-inflammatory, etc. are mainly due to the presence of the biologically active compounds (secondary metabolites) (Zheng and Wang, 2001; Sala *et al.,* 2002; Gracelin *et al.,* 2012). Since these biologically active compounds are present in a very low concentration, the method of extracting them solely dependent on the nature of extracting solvent, due to their chemical characteristics and polarities that may or may not be soluble in a particular solvent. Therefore, the choice of extraction methods and solvents is critical for obtaining these biologically active compounds for phytochemical screening as well as for both qualitative and quantitative studies.

Celery (*Apium graveolens* L.) belonging to the family of Apiaceae, is a medicinal herb used as a food and also in traditional medicine due to its many health benefits (Kooti and Daraei, 2017) and to our best knowledge, this is the first comparative study of this species using different solvent extracts. Therefore, the present study aimed to determine the most effective solvent for extracting phytochemical compounds present in this plant and also to evaluate and compare the antioxidant and anti-inflammatory activity of different solvent extracts.

Materials and methods

Plant material: Fresh leaves of celery (*Apium graveolens* L.) were procured from the local market at Iewduh, Shillong, Meghalaya, India. The leaves were washed thoroughly with tap water and air-dried at room temperature, which was then cut to small pieces and dried in an oven at 40 ºC for 3 days. The dried leaves were then crushed to a fine powder using an electric blender. The powdered sample was stored at 4 ºC in an airtight bottle.

Preparation of extracts: 10 g of powdered plant material was kept in a conical flask

and added 100 ml of a solvent such as water, 80% methanol, 80% acetic acid, and 80% chloroform individually at the ratio of 1:10 (powder/solvent). The mouth of the conical flask was covered with aluminum foil and was subjected to periodically shaking in an electric shaker for 24 h. The extract was filtered by using muslin cloth followed by Whatman # 1 filter paper to obtain the filtrate. The filtrate was then concentrated using a Rotary Evaporator (Model) and was lyophilized to obtain the powdered form of the extracts. All extracts were dissolved in cold saline water containing 0.1% DMSO at the appropriate concentrations and were stored in a refrigerator at 4 ºC for further use.

Chemicals: Standards, such as DPPH, ascorbic acid, gallic acid, tannic acid, and standard anti-inflammatory drug, i.e., Aspirin were purchased from Sigma-Aldrich. Folin-Ciocalteu's reagent was procured from Qualigens. Sodium carbonate, sodium nitrite, aluminum chloride, sodium hydroxide, potassium dihydrogen orthophosphate, sodium hydroxide, potassium ferricyanide, trichloroacetic acid, ferric chloride, and others chemicals used in the study were of analytical grade and purchased from Sigma-Aldrich and HiMedia laboratory, India.

Phytochemical screening

The plant extracts were assessed for the existence of the phytochemicals such as alkaloid (Evans, 2002), phenols (Maze, 1963), flavonoids, terpenoids, cardiac glycosides (Prabhavathi *et al.,* 2016), steroids (Vimalkumar *et al.,* 2014), saponins, tannins and quinones (Deyab *et al.,* 2016).

Quantitative analysis

Estimation of phenols: The amount of Total Phenolic Content (TPC) present in the plant extracts was determined with Folin-Ciocateu (FC) reagent with slight modification (Ainsworth and Gillespie, 2007). Gallic acid was used as a standard for plotting the calibration curves (50-300 µg/ml). The TPC was expressed as mg Gallic Acid Equivalent (GAE)/g dried weight and calculated by the formula, TPC = (C × V)/M where, TPC is total phenolic content (mg/g plant extract in GAE), C is the concentration of gallic acid established from the calibration curve (mg/ml), V is the volume of the extract (ml), M is mass of the extract of the plant (g). All samples were analyzed in triplicates and the results were mean values ± standard deviations.

Estimation of flavonoids: The amount of Total Flavonoid Content (TFC) present in the plant extracts was determined by using a standard method with slight modification (Zhishen et al., 1999). Quercetin was used as a standard for plotting the calibration curves (50-300 µg/ml). The TFC was expressed as mg Quercetin Equivalent (QE)/g dried weight and calculated by the formula, TFC = (C × V)/M where, TFC is total flavonoid content (mg/g plant extract in QE), C is the concentration of Quercetin established from the calibration curve (mg/ml), V is the volume of the extract (ml), M is mass of the extract of the plant (g). All samples were analyzed in triplicates and the results were mean values ± standard deviations.

Estimation of tannins: Total tannins content present in the plant extracts were determined by slightly modified Folin and Ciocalteu method (Ainsworth and Gillespie, 2007). Tannic acid was used as a standard for plotting the calibration curves (10-50 µg/ml). The TTC was expressed as mg Tannic acid Equivalent (TAE)/g dried weight and calculated by the formula, TAE = (C × V)/M where TTC is total Tannin content (mg/g plant extract in TAE), C is the concentration of Tannic acid established from the calibration curve (mg/ml), V is the volume of the extract (ml), M is the mass of the extract of the plant (g). All samples were analyzed in triplicates and the results were mean values ± standard deviations.

Total antioxidant capacity

The total antioxidant capacity (TAC) of the plant extracts was determined by the Phosphomolybdenum method with slight modification (Preito *et al.,* 1999). Ascorbic acid was used as a standard for plotting the calibration curves (10-60 µg/ml). The TAC was expressed as mg Ascorbic Acid Equivalent (AAE)/g dried weight and calculated by the formula, TAC = (C × V)/M where TAC is total Antioxidant capacity (mg/g plant extract in AAE), C is the concentration of Ascorbic acid established from the calibration curve (mg/ml), V is the volume of the extract (ml), M is the mass of the extract of the plant (g). All samples were analyzed in triplicates and the results were mean values ± standard deviations.

***In-vitro* antioxidant activity**

DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay: The ability of the plant extracts to scavenge the DPPH radical was determined by the method with slight modification (Karadag et al., 2009). An aliquot (1 ml) of the plant extracts or standard solution of ascorbic acid (50-300 µg/ml) was added to 2 ml of 1 mM DPPH radical solution prepared in methanol. The reaction mixture was incubated in dark for 30 min and the absorbance was read at 517 nm. A control was prepared by adding 0.5 ml methanol and 0.5 ml saline water containing 0.1 % DMSO to 2 ml of 1 mM DPPH. All samples were analyzed in triplicates and the results were mean values ± standard deviations. The % of DPPH scavenging activity of both samples was calculated using the formula:

$$\% \text{ inhibition} = A_c - A_s / A_c * 100$$

Where, A_c = absorbance of control, A_s = absorbance of sample/standard. The decreasing absorbance indicates a high DPPH scavenging activity.

Total reducing power assay

The total reducing ability of the samples was evaluated (Deore *et al*., 2009) with slight modification. An aliquot (1 ml) of extracts or standard solutions of ascorbic (50-300 µg/ml) were added to 1 ml of phosphate buffer (PB) (0.2 M, pH 6.6) and 1 ml of 1% potassium ferric cyanide [$K_3Fe(CN)_6$]. The mixture was vortex and incubated at 50 °C in a water bath for 30 min and the reaction was terminated by the addition of 1 ml of 10% TCA and the mixture was centrifuged at 3000 rpm for 10 min. 1 ml of the supernatant was transferred into the tube containing 1 ml distilled water and 0.2 ml of 0.1% ferric

chloride ($FeCl_3.6H_2O$). After 5 min, the absorbance was measured at 700 nm against a blank containing 0.5 ml saline containing 0.1 % DMSO and 0.5 ml PB, whereas the control containing all reagent except standard or extracts. All samples were analyzed in triplicates and the results were mean values ± standard deviations. An increase in absorbance indicates their reducing power.

Nitric Oxide scavenging assay

The ability of the plant extracts to scavenge the nitric oxide (NO) was determined (Kumari *et al.,* 2016) with slight modification. An aliquot (1 ml) of extracts or standard solutions of ascorbic (50-300 µg/ml) was added to 2 ml of 10 mM sodium nitroprusside. The mixture was incubated at 25 °C for 2.5 h, 1 ml of the above reaction mixture was taken, followed by mixing with 1 ml of 1% sulfanilamide prepared in 20% glacial acetic acid and after 5 min, 1 ml of 0.1% naphthyl ethylenediamine dichloride prepared in 2% phosphoric acid were added. The absorbance was measured at 546 nm against the blank containing 0.5 ml saline containing 0.1% DMSO and 0.5 ml PBS, whereas the control containing all reagent except standard or extracts. All samples were analyzed in triplicates and the results were mean values ± standard deviations. The % of NO scavenging activity of both samples was calculated using the formula:

$$\% \text{ inhibition} = A_c - A_s / A_c * 100$$

Where, A_c = absorbance of control, A_s = absorbance of sample/standard. The decreasing absorbance indicates a high NO scavenging activity.

In-vitro **anti-inflammatory activity**

Inhibition of protein denaturation: The anti-inflammatory activity of the plant extracts was determined by the inhibition of protein albumin denaturation method with slight modification (Padmanabhan and Jangle, 2012). An aliquot (1 ml) of extracts (100-300 µg/ml) and standard Aspirin (100-200 µg/ml) were added to 0.5 ml of 5% albumin obtained from fresh hen egg and the final volume was raised to 3 ml with PBS (pH 6.4). The reaction mixture was incubated at 37 °C for 20 min and then heated at 80 °C for 5 min. After cooling, the turbidity was measured at 660 nm against the blank containing 0.5 ml saline containing 0.1 % DMSO and 0.5 ml PBS, whereas the control containing all reagent except standard or extracts. All samples were analyzed in triplicates and the results were mean values ± standard deviations. The ability of the plant extracts to inhibit heat-induced protein denaturation were compared with the control and a standard drug aspirin through the formula:

$$\% \text{ inhibition} = A_c - A_s / A_c * 100$$

Where, A_c = absorbance of control, A_s = absorbance of sample/standard. The decreasing absorbance indicates a high inhibition of heat-induced protein denaturation.

Statistical analysis

All experiments were done in triplicates and the results were mean values ± standard deviations. Linear regression analysis was used to calculate IC_{50} for each plant extract. Data were processed with graph pad prism version 8.02 software.

Results

Preliminary phytochemicals screening revealed the presence of all tested parameters in different plant extracts as shown in table 1. The phytochemical screening was performed with aqueous, methanol, chloroform and acetic acid extracts of *Apium graveolens* L. Quinones did not show a positive result for their presence in methanol extract as shown in table 1. The presence or absence of the phytochemicals depends upon the solvent medium used for extraction; therefore, the choice of extraction solvent is critical for phytochemicals screening as well as for both qualitative and quantitative studies.

Table 1: Qualitative analyses of phytochemical substances in different extracts of *Apium graveolens* L.

Sl. No.	Tests	Methanol	Chloroform	Acetic acid	Aqueous
1	Alkaloid	+	+	++	++
2	Flavonoid	++	++	+	++
3	Phenolic	++	+	+	++
4	Saponin	+	+	+	+
5	Tannin	++	+	+	+
6	Glycosides	+	+	+	+
7	Terpenoid	+	+	+	+
8	Quinones	-	++	+	++

++: intensely present, +: Present, - : Absent

The Total Phenolic Content, Total Flavonoid Content, Total Tannin Content and Total Antioxidant Capacity in all extracts was calculated using the linear equation obtained from the standard calibration curve as shown in figure 1, where y is the absorbance and x is the amount of Gallic acid equivalent (GAE), Quercetin equivalent (QE), Tannic acid equivalent (TAE) and Ascorbic acid equivalent (AAE) for TPC, TFC, TTC, and TAC respectively. The TPC (61.3 ± 0.8 mg of TAE/g of extract), TFC (47.7 ± 0.4 mg of QE/g of extract), TTC (12.9 ± 0.7 mg of TAE/g of extract), and TAC (9.5 ± 0.06 mg of AAE/g of extract) was found to be higher in an aqueous extract with acetic acid extract showing the lowest TPC (2.6 ± 0.3 mg GAE /g of extract) and TFC (13.0 ± 1.1mg QE / g of extract), TTC (5.2 ± 0.3 mg TAE/g) and TAC (4.3 ± 0.5 mg of AAE/g of extract), respectively as shown in table 2. The TPC, TTC, and TAC of the four extracts were found to decrease in the order aqueous > methanol > chloroform > acetic acid whereas the TFC was found

to decrease in the order aqueous > chloroform > methanol > acetic acid at a particular concentration, respectively.

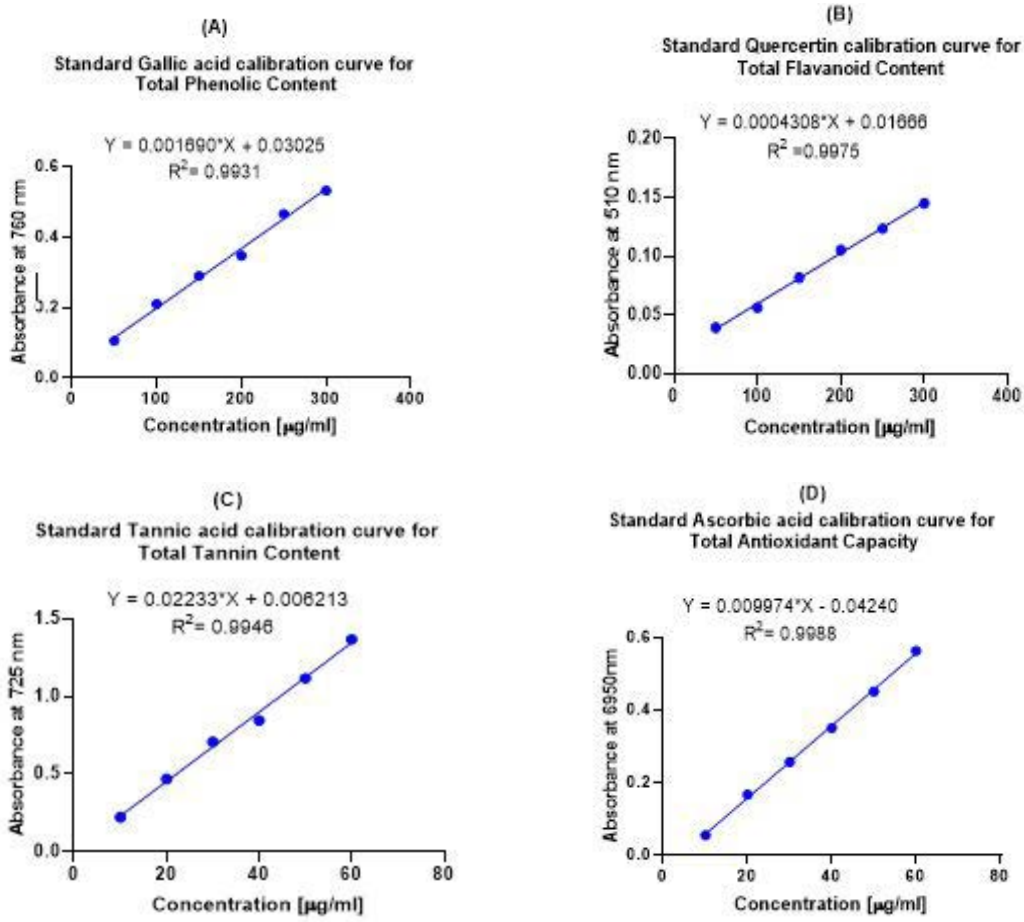

Figure 1: Calibration curve for Gallic acid (A), Quercetin (B), Tannic acid (C) and Ascorbic acid (D) for TPC, TFC, TTC and TAC.

Extracts	**Concentration [mg/ml]**	**Total Phenolic Content (mg GAE/g)**	**Total Flavonoid Content (mg QE/g)**	**Total Tannin Content (mg TAE/g)**	**Total Antioxidant Capacity (mg AAE/G)**
Aqueous	0.5	61.3±0.8	47.7±0.4	12.9±0.7	9.5±0.06
Methanol	0.5	30.9±0.9	20.1±0.7	10.5±0.1	6.1±0.03
Chloroform	0.5	14.1±0.3	24.1±1.5	7.1±0.3	5.4±0.1
Acetic Acid	0.5	2.6±0.3	13.0±1.1	5.2±0.3	4.3±0.5

Table 2: TPC, TFC, TTC, and TAC of different extracts of *Apium graveolens* L. Data represented as Mean ± SD (n = 3).

DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay is based on the principle that DPPH radical in the presence of an antioxidant, accept one or more electron donated by the antioxidant compound and in turn reduces from violet to yellow color which can be quantitatively measured from the change or decrease in absorbance. Here ascorbic acid, a well-known antioxidant was used as a positive control and the calculated % inhibition values of both standard and extracts was plotted against their respective concentration as shown in figure 2(A) and the results were expressed as IC_{50} i.e., the concentration of both samples and standard required to scavenge 50% of the DPPH radical as shown in table 3, which was calculated from the curve of % inhibition plotted against their respective concentration. Low IC_{50} value indicates the strongest scavenging activity. The aqueous extract exhibited the smallest IC_{50} (IC_{50} = 0.87 mg/ml), which indicated that aqueous extract has the highest scavenging activity when compared with methanol (IC_{50} = 1.1 mg/ml), chloroform (IC_{50} = 2.4 mg/ml) and acetic acid (IC_{50} = 5.3 mg/ml) extracts, respectively, although standard ascorbic acid (IC_{50} = 0.023 mg/ml) has the highest scavenging activity in all tested concentration, the extracts still showed good scavenging activities in a dose-dependent manner.

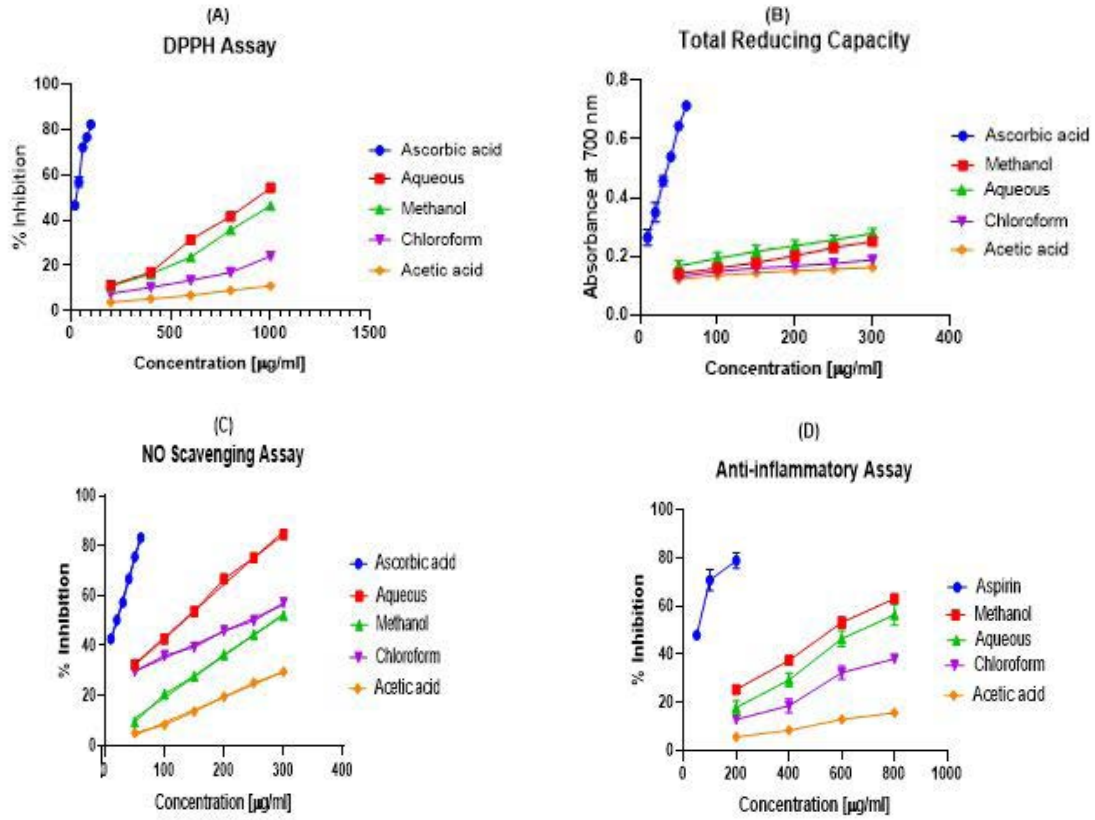

Figure 2: Percentage inhibition of various extracts and standard (Ascorbic acid) on DPPH (A), NO (C), Anti-inflammatory (D), and Absorbance of the total reducing capacity (B).

Table 3: Results of DPPH scavenging activity

Standard/ Extracts	**Concentration [µg/ml] % Inhibition (Mean ± SD)**					**IC_{50} [mg/ml]**
Ascorbic Acid	20 µg	30 µg	40 µg	50 µg	60 µg	0.023
	46.5 ± 0.1	56.9 ± 2.1	72. 1 ± 1.5	76.5 ± 1.3	82.2 ± 0.1	
Aqueous	200 µg	400 µg	600 µg	800 µg	1000 µg	
	11.3 ± 0.05	17.06 ± 0.32	31.6 ± 1.4	42.6 ± 0.79	54.6 ± 0.58	0.87
Methanol	10.9 ± 0.11	16.3 ± 0.4	23.6 ± 0.47	35.7 ± 0.6	46.3 ± 0.76	1.1
Chloroform	7.73 ± 0.4	10.3 ± 1.02	13.4 ± 1.51	17.0 ± 0.75	24.1 ± 1.18	2.4
Acetic Acid	3.9 ± 0.26	5.3 ± 0.05	6.8 ± 0.51	9.0 ± 0.55	11.1 ± 0.32	5.3

The total reducing capacity or the electron-donating ability of the extracts was determined by using Fe^{3+} reduction as an indicator that is based on the chemical reduction of ferricyanide Fe^{3+} complex to Fe^{2+} form in the presence of the extracts or standard and the concentration of ferrous form was determined spectrophotometrically at 700 nm. In this particular assay, the reducing power of both extracts and standard increases with an increase in absorbance as shown in figure 2 (B), and as indicated by their absorbance values as shown in table 4. An increase in absorbance indicates their reducing power. It is obvious that ascorbic acid, a well-known antioxidant compound has the highest reducing power but when compared among extracts, the aqueous extract exhibited the strongest reducing power followed by methanolic, chloroform and acetic acid extracts, respectively.

Table 4: Results of Total Reducing Capacity Assay

Standard/ Extracts	**Concentration [µg/ml]/Absorbance (Mean ± SD)**					
Ascorbic Acid	10 µg	20 µg	30 µg	40 µg	50 µg	60 µg
	0.264 ±0.02	0.349 ±0.03	0.456 ±0.01	0.539 ±0.01	0.644 ±0.01	0.7139 ±0.1
Aqueous	50 µg	100 µg	150 µg	200 µg	250 µg	300 µg
	0.166 ±0.01	0.193 ±0.02	0.2162 ±0.1	0.236 ±0.1	0.255 ±0.01	0.278 ±0.1
Methanol	0.140 ±0.1	0.159 ±0.02	0.178 ±0.2	0.201 ±0.7	0.229 ±0.9	0.250 ±0.1
Chloroform	0.130 ±0.7	0.149 ±0.01	0.159 ±0.09	0.166 ±0.03	0.175 ±0.5	0.187 ±0.1
Acetic Acid	0.122 ±0.3	0.134 ±0.01	0.143 ±0.1	0.150 ±0.4	0.155 ±0.06	0.162 ±0.8

NO scavenging assay is based on the principle that SNP in aqueous solution produces NO, and under aerobic condition, NO react with O_2 to produce a stable product nitrite or nitrate, which can be determined by Griess reagent and the ability of the extracts to scavenge NO is by competing with oxygen for NO, this lead to a reduction in nitrite or nitrate formation, which can be determined spectrophotometrically at 546 nm from the changed or decrease in absorbance. Here ascorbic acid, a well-known antioxidant was used as a positive control and the calculated % inhibition values of both standard and extracts were plotted against their respective concentration as shown in figure 2 (C) and the results were expressed as IC_{50} i.e., the concentration of both samples and standard required to scavenge 50% of NO as shown in table 5, which was calculated from the curve of % inhibition plotted against their respective concentration. Low IC_{50} value indicates the strongest scavenging activity. Aqueous extract exhibited the smallest IC_{50} (IC_{50} = 0.13 mg/ml), which indicated that aqueous extract has the highest scavenging activity followed by chloroform (IC_{50} = 0.24 mg/ml), methanol (IC_{50} = 0.28 mg/ml) and acetic acid (IC_{50} = 0.50 mg/ml) extracts, respectively.

The anti-inflammatory activity of different plant extracts was studied by using inhibition of egg albumin denaturation technique. This assay is based on the principle that when egg albumin induces to heat it denature, and the ability of the plant extracts to

decrease the degree of denaturation was compared with a standard drug aspirin, which can be quantitatively measured from the change or decrease in absorbance. Here aspirin, a well-known anti-inflammatory drug was used as a positive control and the calculated % inhibition values of both standard and extracts was plotted against their respective concentration as shown in figure 2 (D) and the results were expressed as IC_{50} i.e., the concentration of both samples and standard required to inhibit 50% of the denaturation process, as shown in table 6, which was calculated from the curve of % inhibition plotted against their respective concentration. Low IC_{50} value indicates the strongest inhibition activity.

Table 5: The result of Nitric Oxide Scavenging Assay

Standard/ Extracts	Concentration [µg/ml]/ % inhibition (Mean ±SD)						IC_{50} [mg/ml]
Ascorbic Acid	10 µg	20 µg	30 µg	40 µg	50 µg	60 µg	
	42.7 ±2.8	50.3 ±2.8	57.2 ±1 .7	66.7 ±3.1	75.5 ±1.1	83.2 ±4.7	
Aqueous	50 µg	100 µg	150 µg	200 µg	250 µg	300 µg	0.01
	32.3 ±2.2	42.7 ±7.2	53.6 ±6.9	66.7 ±4.6	75.2 ±3.4	84.5 ±3.8	0.13
Chloroform	29.7 ±1 .9	36.0 ±1.6	39.4 ±1 .0	45.9 ±3.9	49.9 ±3.9	57.2 ±2.7	0.24
Methanol	9.4 ±4.2	20.6 ±2.1	27.7 ±1.7	36.3 ±2.6	44.3 ±0.9	52.0 ±0.8	0.28
Acetic Acid	5.0 ±1.9	8.2 ±0.8	13.6 ±1.4	19.5 ±0.5	25.1 ±2.0	29.5 ±1.9	0.50

The methanolic extract exhibited the smallest IC_{50} (IC_{50} = 0.58 mg/ml), which indicated that methanolic extract has the highest inhibition activity when compared with aqueous (IC_{50} = 0.69 mg/ml), chloroform (IC_{50} = 1.08 mg/ml) and acetic acid (IC_{50} = 2.82 mg/ml) extract, respectively, but when compared with aspirin (IC_{50} = 0.04 mg/ml) it is obvious that aspirin a well-known anti-inflammatory drug has the highest inhibition activity in all tested concentration, but the extracts still show good inhibition activities in a dose-dependent manner.

Discussion

The use of medicinal plants to tackle human diseases is as old as mankind. This is because medicinal plants possess great varieties of biologically active compounds or secondary metabolites such as alkaloids, glycosides, quinones, saponins, terpenoids, flavonoids, tannins and phenolic compounds. They are directly responsible for different activities such as antioxidant, anti-inflammatory, anticancer, etc. each through different mechanisms. Phenolics compounds account for most of the antioxidant activity in plants or plant products (Sulaiman *et al.,* 2013). Therefore, the extraction of these biologically active compounds from plant materials is greatly influenced by their solubility in the extraction solvent. In this study, aqueous extracts gave the highest extraction yield of the total extractable compounds, while the acetic acid, being the least polar, gave the

lowest yield. Phytochemicals screening revealed the presence of various phytochemical constituents in the plant extracts (Table.1). Thus, extraction with polar solvents such as aqueous, methanol, acetic acid, and chloroform being the least polar among them all would give a good extraction yield of the biologically active compounds.

Table 6: The result of Protein denaturation Assay.

Standard/ Extracts	Concentration [µg/ml]	%Inhibition (Mean ±SD)	IC_{50} [mg/ml]
Methanol	200	25.2 ±**0.4**	0.58
	400	37.4 ±**1.8**	
	600	53.1 ±**2 .3**	
	800	63.0 ±**1.1**	
Aqueous	200	17.7 ±**2.7**	0.69
	400	29.4 ±**2 .9**	
	600	46.3 ±**3.3**	
	800	56.3 ±**4 .3**	
Chloroform	200	12.7 ±**0.7**	1.06
	400	18.4 ±**2.9**	
	600	32.3 ±**2 .7**	
	800	38.0 ±**1.6**	
Acetic Acid	200	5.5 ±**0 .8**	2.82
	400	8.2 ±**1.2**	
	600	12.8 ±**1.8**	
	800	15.5 ±**0.4**	
Aspirin	50	47.8 ±**1.5**	0.04
	100	67.4 ±**2 .3**	
	200	78.8 ±**3 .2**	

The TPC, TTC, TAC in different extracts were showing that aqueous extract has highest with a descending order of aqueous > methanol > chloroform > acetic acid, and the TFC of different extracts shows that aqueous extract has the highest flavonoid content with a descending order of aqueous > chloroform > methanol > acetic acid. Thus, in all cases aqueous was a better solvent for extraction of polyphenol compounds.

In our present study, several *in vitro* model systems have been used for assessing the free radical scavenging activity. One of the most common and rapid methods is the DPPH free radical scavenging activity. The DPPH radical scavenging activity of different plant extracts of *Apium graveolens* L. is denoted in figure 4. All the extracts showed different levels of DPPH radical scavenging activity and were found to decrease in the order aqueous > methanol > chloroform > acetic acid in a dose-dependent manner. In this assay, the aqueous extract exhibited strongest DPPH free radical scavenging activity

compared to other extracts. Similarly, we also assayed the reducing power of the plant extracts, and in this particular assay, it was observed that higher the absorbance, the stronger is the antioxidant activity; thus, the reducing power of the extracts also increases with the increase in concentration. When compared to other extracts, the aqueous extract exhibited the highest reducing power ability. The reducing power ability of the four extracts was found to decrease in the order aqueous > methanol > chloroform > acetic acid. Both cases follow the same trend as in the case of TPC, TTC, and TAC, indicating that the free radical scavenging activity and the reducing power of *Apium graveolens* L. extracts is highly related to the presence of phenolic compounds which caused the reduction of DPPH radical and Fe^{3+}/ ferricyanide complex.

NO is an important signaling molecule involved in various normal physiological processes (Parul *et al.,* 2013). At low concentration, NO do not harm macromolecules such as DNA, proteins or lipids but excess production of NO can lead to several diseases (Ialenti *et al.,* 1993). Excess NO reacts with O_2 to produce an unstable intermediate (NO_2, N_2O_4, and N_3O_4) that are highly toxic to the cell (Parul *et al.,* 2013). Therefore, antioxidant compounds from plants have gained much interests due to their ability to tackle free radicals in the biological systems. The nitric oxide radical scavenging activity of different plant extracts of *Apium graveolens* L. was presented in figure 4. All the extracts effectively reduced the generation of nitric oxide from sodium nitroprusside. The aqueous extract showed highest nitric oxide radical inhibition compared to other extracts and was found to decrease in the order aqueous > chloroform > methanol > acetic acid, which follows the same trend as in the case of the TFC, indicating that flavonoids might be directly contributing toward the NO scavenging activity which further support the earlier report (Parul *et al.,* 2013). Thus, in all the antioxidant assays, the results correlated between phenolics, flavonoids, and antioxidant activity.

Besides oxidative stress, inflammation is also a major cause of many chronic diseases (Godhandaraman and Ramalingam, 2016). It is considered as a normal protective response and as a part of a host defense system associated with pain and involves occurrences such as the increase in vascular permeability, increase of protein denaturation and membrane alterations (Umapathy *et al.,* 2010). If inflammation is left untreated, it leads to the onset of many chronic diseases (Godhandaraman and Ramalingam, 2016). The most common synthetic drugs used in the present are known as NSAIDs such as aspirin, diclofenac but these compounds are associated with many unwanted side effect (Amir *et al.,* 2010). Since protein denaturation leads to inflammatory and arthritis diseases (Williams *et al.,* 2008), thus, the prevention of protein denaturation may help in inflammatory conditions. Hence, a simple and viable protein denaturation assay was selected to determine the anti-inflammatory activity of *Apium graveolens* L. extracts. All extracts and standard drug aspirin exhibited dose-dependent percentage inhibition of heat-induced protein denaturation in fresh egg albumin and was found to decrease in the order aspirin > methanol > aqueous > chloroform > acetic acid, When compared among extracts, the methanolic extract showed higher anti-inflammatory activity at increasing

concentration. It may due to the presence of active principles of phytocompounds such as tannins, phenols, flavonoids, and related polyphenols that might be responsible for this anti-inflammatory activity. Hence, *Apium graveolens* L. might be used as a putative anti-inflammatory agent.

Conclusion

The results of our study revealed that *Apium graveolens* L. extracts possess various bioactive phytochemical compounds such as alkaloids, flavonoids, phenols, tannins, etc. Extraction solvents have an effect on extraction yield and the total extractable compounds from *Apium graveolens* L. Most of the antioxidant compounds were present in the aqueous extract and it showed the highest activity in most of the assays. Therefore, water appears to be the best extraction solvent for the extraction of various phytochemical compounds from *Apium graveolens* L. The plant extracts also showed remarkable inhibiting activity against heat-induced protein denaturation. Therefore, we can conclude that *Apium graveolens* L. is a potential candidate for a natural source of antioxidants and might be used as an anti-inflammatory agent. However, further detailed investigations are needed to ascertain the specific mechanism and phytochemicals compounds responsible for its anti-inflammatory activities. Nevertheless, consumption of *Apium graveolens* L. can be beneficial in preventing oxidative stress and inflammation-associated diseases.

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