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Research Article

PRELIMINARY PHYTOCHEMICAL SCREENING AND *IN- VITRO* ANTIOXIDANT POTENTIAL OF DIFFERENT EXTRACTS OF *APIUM LEPTOPHYLLUM* PERS

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ABSTRACT

The aim of the present study was to evaluate the preliminary phytochemical screening and *In-Vitro* antioxidant activity of aqueous and ethanolic extract of *Apium leptophyllum* Pers. Ethanolic and aqueous seed extracts of *Apium leptophyllum* Pers were prepared as per standard procedure and screened for the presence of phytoconstituents. The extracts were also evaluated for the *In –Vitro* antioxidant activity against various free radicals. Phytochemical screening showed the presence of alkaloids, glycosides, flavonoids, tannin, phenol, terpenoids, saponins, and steroids. The findings of the present study suggest both extracts of *Apium leptophyllum* Pers possess good antioxidant potential against free radicals such as DPPH, NO, Reducing power assay, H_2O_2, and ABTS assay.

Keywords: *Apium leptophyllum* Pers., Phytochemicals, *In vitro* antioxidant.

INTRODUCTION

Plant derived drug compounds have been used in versatile therapeutic options for various diseases. Secondary metabolites found in plants are the bioactive principles which are either administered as an isolated single drug molecule or as a holistic drug. These phyto chemicals along with other nutrients form an integrated part of defence mechanism and stress conditions. The flavonoids, terpenoids, tannins, steroids, alkaloids etc. found in very small quantities in the plant are the secondary metabolites[1]. Extraction of the medicinally active principle involves the diffusion of the compounds from the plant and solubilize in the solvent system with similar polarity and used for the extraction[2].

Free radicals are formed during oxidation reduction reaction involving transfer of an electron or when a covalent bond is broken and one electron from each pair remains with each group. The unpaired electrons make the free radicals effective and involve in a number of pathophysiological conditions such as atherosclerosis, cancer, cardiovascular disease, inflammatory joint disease, asthma and diabetes[3]. The mechanism of biological ageing, peroxidation of lipid in cell membranes and inactivation of the membrane bound enzymes occurs due to the damage caused by free radicals[4]. Antioxidants are present in abundance in medicinal plants and are a boon to the mankind. The antioxidants react with free radicals, inactivating it and thereby prevent the oxidation of cellular molecules. Hence it implies that these molecules have health promoting effects leading to the prevention of degenerative diseases[5]. It has been suggested that antioxidant activity of plants might be due to the presence of phenolic compounds[6].

Studies on oxidative stress and its adverse effects on human health are gaining momentum and researchers show great interest in exploring this area[7]. The process of oxidation is a natural metabolic reaction in cell, resulting in the generation of free radicals such as hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCL)[8]. Cigarette smoke, automobile exhaust, radiation, pesticides and air pollution are mainly involved for the production of free radicals[9]. The free radicals play a crucial role in neurodegenerative disorders, ageing, Damage of cell membrane, genetic materials, proteins and oxidation of fats resulting in oxidative stress[10]. Studies have proved that plant based active compound-phenols can prevent the oxidative stress caused by oxygen and sunbeam[11].

Medicinal plants which are abundant sources of natural antioxidants, such as flavonoids, vitamin C, tocopherol and other phenolic compounds[12]. Secondary metabolites are used to protect living organism from damage caused by uncontrolled production of ROS, DNA strand breaking, protein damage and concomitant lipid peroxidation[13]. Medicinal plants are now reported for free radical scavenging activity because of the presence of some important active compounds such as phenol, tannins, flavonoid, coumarins, terpenoids etc[14]. The antioxidant activity of *Apium leptophyllum* was evaluated by using *In-Vitro* methods which involve the prevention of oxidation and its damage by inhibiting free radicals[15]. The plant has been used as an anti-inflammatory carminative, nervine, sedative, antiemetic, tranquilizer, anticonvulsant, antifungal and antispasmodic[16].

MATERIALS AND METHODS

Collection and Identification of Plant Material

The dried seed of *Apium leptophyllum* was procured from Crude drug sellers from Madhya Pradesh and the seed part of the plant was authenticated from Botanical Survey of India (BSI) TNAU

campus, Coimbatore. The authentication reference number of plant *Apium* is BSI/SRC/ 5/23/ 2019 /Tech/140.

Preparation of Plant Extract

The washed and air dried seeds of *Apium leptophyllum* was powdered in a pulveriser and about 250 g. of this powder were subjected to Soxhlet hot extraction using 750 ml of the series of solvents in the order of polarity. Each of the extract was reduced to dry residue and was stored in aseptic conditions in the refrigerator for further studies.

Phytochemical screening

Preliminary Phytochemical Screening

S. No	Name of the Test	Experimental Procedure	Observation
1.	**Test for Carbohydrates**: Molisch's test	2-3 ml of extract, add two drops of alpha naphthol solution in alcohol shake and add conc. H_2SO_4 from sides of test tube	Violet colouration occurs
2	**Test for Proteins:** a)Biuret test	To 3 ml test solutions add 4% NaOH and few drops of 1% $CuSO_4$ solution	Pink colour is obtained.
	b)Million' s test	Mix 3 ml test solution with 5 ml Million's reagent	White precipitate turns brick red or the precipitate dissolves given red coloured solution
3	**Test for Phytosterol.** i.Salkowski test	To 2 ml of extract add 2 ml chloroform and 2 ml Conc H_2SO_4. Shake well.	Chloroform layer appears red and acid layer shows greenish yellow florescence.
	ii.Libermann Burchard's Test	Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride boiled and cooled. Concentration of Sulphuric acid was added.	Formation of brown ring at the junction indicates the presence of phytosterols.
4	**Test for Saponin:** Foam test	To test solution add drop of sodium bicarbonate. Shake well	Honey comb like froth is obtained.
5.	**Test for Alkaloids:** i.Dragendorff test	To 2-3 ml test solution add few drops Dragendroff's reagent	Orange brown precipitate
	ii.Wagner's test	2-3 ml test solution with few drops Wagner's reagent	Reddish brown precipitate
	iii.Mayer's test	2-3 ml test solution with few drops of Mayer's reagent	White or pale precipitate
6.	**Test for Tannins:** i.Lead acetate test	3-5 ml test solution with few drops of 1%Lead acetate.	Red precipitate is obtained.
	ii.Ferric chloride test	To 2-3 ml test solution add 2 ml of $FeCl_3$	Blue precipitate is obtained.
7.	**Testfor Phenolic compounds:** Ferric Chloride test:	To 1-2 ml test solution adds 2 ml of water and 10% aqueous ferric chloride solution	Green colour solution is obtained.
8	**Test for Flavonoids:** Shinoda test:	The test solution, add 5 ml of alcohol. Few drop of Conc HCl and Magnesium turnings and boiled for few minutes.	Pink coloration occurs.
9.	**Test for terpenoids:**	To test solutions add 2 ml of chloroform and 1 ml of of Conc H_2SO_4	Reddish brown coloration occurs.
10.	**Test for coumarin:**	A small quantity of substance was mixed with few drops of 10% NaOH. Put two spots on filter paper and examined under UV light.	Yellow fluorescence indicates the presence of coumarin
11.	**Test for Quinones:**	The substance was mixed with few drops of concentrated sulphuric acid.	Red colouration was observed.
12	**Test for Lignin:**	The substance was mixed with alcoholic solution of Phloroglucinol and added few drops of concentrated hydrochloric acid	Colour reaction was observed it indicates the presence of lignin

The Phytochemical Screening of the extracts was conducted using procedures described by Trease and Evans[17]

***In vitro* Antioxidant Activity**

Determination of DPPH radical scavenging activity

The Free radical scavenging activity of aqueous and ethanolic solvent extract of *Apium leptophyllum* Pers was assessed by DPPH scavenging assay[18]. For assessing the scavenging activity of free radicals DPPH about 0.2 mM solution of DPPH in ethanol was made and 2 ml of this solution was mixed to 4 ml of each extract at various concentration level (25, 50,75,100,------ 1000 µg/ml). After half an hour, the absorbance was read at 517 nm. Scavenging activity of DPPH was determined and calculated.

Determination of Nitric oxide radical scavenging activity

The aqueous and ethanolic extract of *Apium leptophyllum* Pers was screened by NO radical scavenging assay[19]. Nitric oxide was formed from sodium nitroprusside and analysed by Greiss method. Sodium nitroprusside (5 mM) in Phosphate Buffer Saline (PBS) was made and mixed with 5 ml of various concentration (25, 50, 75, 100,------ 1000 µg/ml) of each extract and incubated at 25 C for 2 h. After incubation, 1 ml of Greiss reagent (1% Sulphanilamide, 2% H_3PO_4 and 0.1%N-(1-Naphthyl) ethylenediamine dihydrochloride was mixed to each sample and the absorbance was read at 546 nm using UV Spectrophotometer. The percentage of inhibition was calculated.

Determination of Hydroxyl radical scavenging activity

The antioxidant activity of aqueous and ethanolic extract of *Apium leptophyllum* Pers to scavenge hydrogen peroxide was determined by the method of Ruchetal[20,21]. The solutions of $FeCl_3$ (10 mM), EDTA (1 mM), ascorbic acid (1 mM), H_2O_2 (10 mM) and deoxyribose solutions (10 mM) were made in deionised water. The assay was carried out by adding 0.1 ml EDTA, 0.01 ml of $FeCl_3$, 0.1 ml of H_2O_2, O.36 ml of deoxyribose, 1 ml of each extract of various concentration (25, 50, 75, 100,------ 1000 µg/ml) were mixed with 0.33 ml of phosphate buffer solutions (50 mM, pH 7.4) and 0.1 ml of ascorbic acid in uniform manner.

The mixture solution was incubated at 37° C for 60 minutes about 1 ml portion of each incubated mixture was taken and added with 1 ml of 10% TCA and 1.0 ml of 0.5% TBA to form pink colour. The hydrogen peroxide radical scavenging activity of each extract was evaluated as percentage of inhibition of deoxyribose degradation and was measured.

Reducing Power Assay

The reducing power of seed extracts was determined by Ferric reducing assay[22]. In brief, 1 ml of different concentration of extracts (25, 50, 75, 100,------ 1000 µg/ml) in 1 ml of methanol were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide [$K_3Fe(CN)_6$, 1%]. The tubes were incubated at 50 C for 20 minutes in water bath, cooled and 2.5 ml of trichloroacetic acid (10%) and 0.5 ml of 0.1% ferric Chloride ($FeCl_3$) were added to each type. The absorbance of reaction mixtures was measured at 770 nm. Increased absorbance of the reaction mixture on increasing the concentration of extracts indicated increased reducing power.

ABTS radical scavenging activity

The Efficacy of seed extracts to scavenge free radicals was determined using ABTS radical scavenging assay with minor modification[23]. The ABTS radical was generated by mixing (7 mM). ABTS stock solution with 2.45 Mm. Potassium persulfate and the mixture was left in the dark for 12-16 hours at room temperature. The resulting solution was diluted with distilled water to an absorbance of 0.70 at 730 nm. 1 ml of different concentrations of seed extracts (25, 50, 75, 100,------ 1000 µg/ml) were added to 4 ml of ABTS solution in labelled tubes and the tubes were incubated for 30 minutes followed by measuring the absorbance at 730 nm.

RESULTS AND DISCUSSION

Table 1: Phytochemical Screening of different solvent extracts of *Apium leptophyllum* Pers

S. No.	Active compounds	Hexane	Chloroform	Ethyl Acetate	Ethanol	Water
1	Alkaloids	-	-	-	+	+
2	Flavanoids	-	+	+	++	+
3	Terpenoids	+	+	-	++	-
4	Coumarin	+	+	-	++	+
5	Quinone	-	+	+	+	+
6	Phenol	-	-	++	++	+
7	Sterol	+	+	+	+	-
8	Sugar	-	-	-	+	+
9	Lignin	-	-	-	+	+
10	Saponin	-	-	-	+	+
11	Protein	-	-	-	+	+
12	Tannin	+	-	+	+	+

Aqueous and Ethanolic extract of *Apium leptophyllum* Pers showed that the presence of phenol, flavonoid, coumarin, terpenoids, alkaloids

Assessment of Antioxidant activity

DPPH Radical Scavenging activity

The percentage of scavenging effect of DPPH on different extracts of *Apium leptophyllum* was found to be dose dependent. The result of DPPH radical assay showed that the ability of plant drug to act as a free radical scavenger.[24] DPPH is a protonated radical with absorbance maximum at 517 nm and decreases with the scavenging of free radical. This property has been widely used to evaluate the free radical scavenging effect of natural antioxidants[25].

The IC 50 values of aqueous and ethanolic extract were found to be 180 µg/ml and 98 µg/ml respectively.

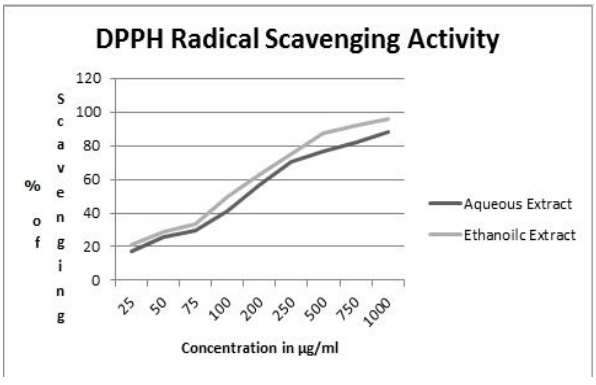

Figure 1: DPPH free radicals scavenging activity of aqueous and ethanolic extracts of *Apium leptophyllum pers*
All values were expressed in triplicate as mean ± SD

Nitric Oxide Radical Scavenging

In this study the two different extracts of *Apium leptophyllum* were screened or inhibitory effect nitric oxide production and revealed the best result in the arresting nitric oxide radical generation in a dose dependent manner. It is a diffusible free radical, which plays many roles as an effectors molecule in diverse biological system including neuronal messenger, vasodilation, antimicrobial and antitumor activities[26].

The IC 50 values of Aqueous and Ethanolic extract were found to 180 µg and 110 µg. The ethanolic extract has high antioxidant activity.

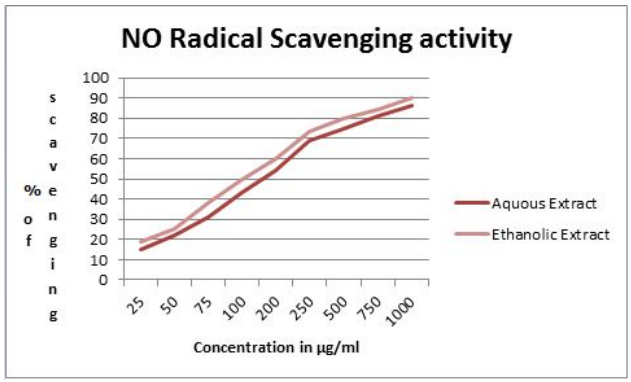

Figure 2: Nitric Oxide free radicals scavenging activity of aqueous and ethanolic extracts of *Apium leptophyllum* Pers
All values were expressed in triplicate as mean ± SD

Hydroxyl Radical Scavenging

The percentage of scavenging effect of Hydroxyl radical on different extracts of *Apium leptophyllum* revealed that inhibition on hydroxyl radical activity in dose dependant. The ability of the extracts to quench hydrogen peroxide radicals seems to be directly relevant to the prevention of propagation of the process of lipid peroxidation and seems to be a good scavenger of Reactive Oxygen Species. Hydrogen peroxide is a weak oxidizing agent that inactivates a few enzymes directly, usually by oxidation of essential thiol (-SH) groups[27,28].

The IC 50 values of aqueous and ethanolic extract were found to 250 µg and 218 µg.

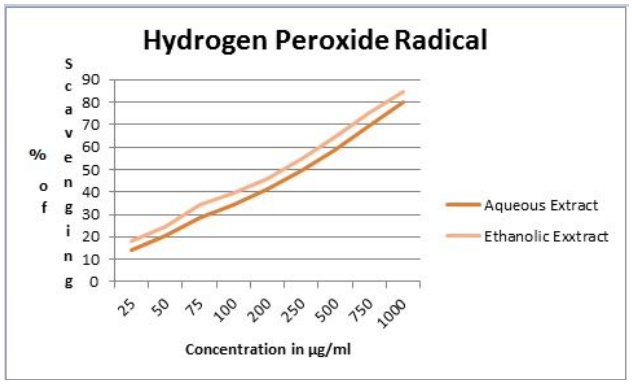

Figure 3: H_2O_2 free radicals scavenging activity of aqueous and ethanolic extracts of *Apium leptophyllum* Pers
All values were expressed in triplicate as mean ± SD

Reducing Power Assay

The IC 50 values of aqueous and ethanolic extract were found to 170 µg and 100 µg and the percentage of reducing power was dose dependent. Electron donating capacity of the plant extracts convert free radicals in to more stable products and terminates the radical chain reaction[29]. Presence of the polyphenolic compounds in the plant extracts is responsible for the reducing power capacity[30].

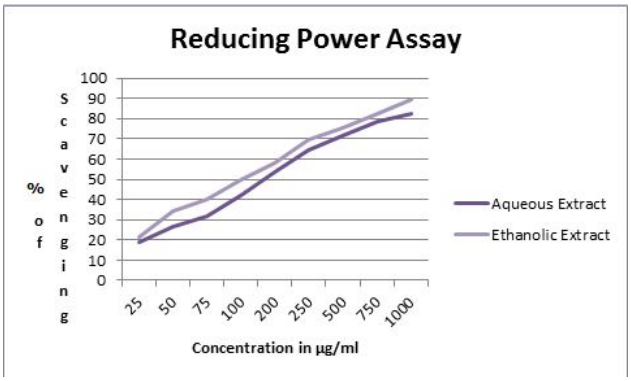

Figure 4: Reducing power assay of aqueous and ethanolic extracts of *Apium leptophyllum pers*

All values were expressed in triplicate as mean ±SD

ABTS radical scavenging activity

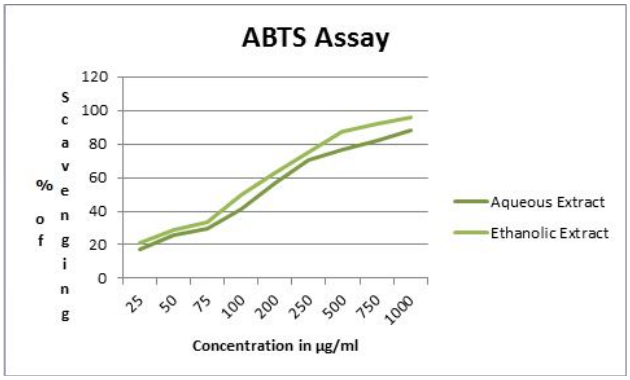

Figure 5: ABTS radical scavenging assay of aqueous and ethanolic extracts of *Apium leptophyllum* Pers
All values were expressed in triplicate as mean ± SD. The ethanolic extract has high antioxidant activity against ABTS radical

The IC 50 values of aqueous and ethanolic extract were found to be 148 µg and 105 µg and the ABTS radical scavenging was done in a dose dependent manner.

CONCLUSION

The study revealed the presence of several secondary metabolites and the antioxidant potentials of the plant. Hence it is suggested that the plant may use as a therapeutic for various diseases.

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