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ACETYLCHOLINESTERASE FROM HUMAN ERYTHROCYTES MEMBRANE: A SCREEN FOR EVALUATING THE ACTIVITY OF SOME TRADITIONAL PLANT EXTRACTS

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Abstract

The extraction of plant constituents is essential to isolate biologically active compounds and understanding their role in disease prevention, treatment and in knowing their toxic effects as well. However, meager information is available about the properties and biological activities of phytochemicals derived from certain plants found in Allahabad and adjoining areas. Keeping this information in view, we prepared aqueous extracts and determined their biochemical properties including their impact on the activity of human RBC's acetylcholinesterase (AChE). The UV-Visible spectrophotometric profiles of the aqueous extracts of different parts of the four plant species viz. *Calotropis procera*, *Datura metal*, *Cannabis sativa, Argemone mexicana* and *Thevitia peruviana* displayed two major peaks at 302 and 336 nm corresponding to the presence of different flavonoids in these preparations. These extracts indicated presence of protein in the range of 1.12 to 19.25mg/g wet weight of the plant tissues. The impact of different phytochemicals present in these extracts was studied on the activity of AChE isolated from human erythrocytes (RBCs). The extracts derived from *Argemone mexicana* and *Datura metal* exhibited strong AChE inhibitory potential, whereas others did not show significant inhibition even at higher concentrations. The results indicate that human RBC's can be used as a potential biomarker towards evaluation of the efficacy and toxic potential of varied plant extracts.

Key words: Acetylcholinesterase inhibition, Alzheimer's disease, Aqueous plant extract, *Calotropis procera, Datura metal, Cannabis sativa, Argemone mexicana, Thevitia peruviana*.

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INTRODUCTION

Phytochemicals, as plant components with discrete bioactivities towards animal biochemistry and metabolism are being widely explored for their ability to provide health benefits (35). Research supporting beneficial roles for phytochemicals against cancers, coronary heart disease, diabetes, high blood pressure, inflammation, microbial, viral and parasitic infections, psychotic diseases, spasmodic conditions, ulcers, etc. is based on chemical mechanisms using in vitro and cell culture systems. Several toxins have been derived from different plants and their efficacy and toxicity or medicinal evaluations have been reported (27). Toxins are the substances that can cause disturbance to organisms usually by chemical reactions or other activity at the molecular and biochemical levels, when a sufficient quantity is absorbed by an organism.

Acetylcholinesterase (AChE) is an essential component of cholinergic synapses since it hydrolyzes acetylcholine released from presynaptic nerve terminals. However, it is well documented that AChE is also expressed in a variety of noncholinergic tissues including hematopoietic cells (17). Digestion with proteolytic enzymes has shown that AChE is present on the outside of the erythrocyte membrane (19). The normal functions of AChE in erythrocytes are unknown although a better understanding of the functional significance of AChE of hematopoietic cells may be relevant for the future design of novel therapeutic strategies. Inhibition of AChE activity is considered as a promising strategy for the treatment of neurological disorders such as Alzheimer's disease (33), senile dementia, ataxia, myasthenia gravis and Parkinson's disease (3, 31). Plants in nature have been reported to serve as potential sources of AChE inhibitors (27). In traditional practices, numerous plants have been used to treat cognitive disorders, including neurodegenerative diseases and different neuropharmacological disorders. Ethnopharmacological approach and bioassay-guided isolation have provided a lead in identifying potential AChE inhibitors from plant sources, including those for memory disorders (27). Several methods for screening of AChE inhibitory activity from natural resources has been reported based on Ellman's reactions (13).

Calotropis procera, *Datura metal*, *Cannabis sativa*, *Argemone mexicana* and *Thevitia peruviana* are known common traditional Indian plants exploited for various purposes; for example *C. procera* has both the medicinal and toxic constituents, *D. metal* for its narcotics and toxic substances, *A. sativa* for containing popular narcotics, *A. mexicana* as an agent causing dropsy and *T. peruviana* known for its medicinal and ornamental applications. *Calotropis procera* is also known as apple of Sodom or mudar. It belongs to Apocynaceae family and found in many countries such as Africa, Western and South Asia, as well as Indochina. It is known for its medicinal and pharmacological properties (23). The milky sap of this plant is known to contain three toxic glycosides (i) calotropin, (ii) uscharin and (iii) calotoxin as well as steroidal heart poisons, known as cardiac aglycones (7). The crude extract of this plant and its protein fraction possess high fibrinolytic and anticoagulant activity in rabbit and human plasma (40). Aqueous extracts of different parts of this plants are shown to exert mild diuretic and cardiac as well as respiratory stimulating effects in experimental animals (11).

Datura metal, a well known traditional Indian plant, is found throughout the warmer parts of the world and contains both the ornamental and medicinal properties. All parts of *Datura* plants contain high levels of tropane alka-

loids, which are highly toxic to humans and other animals. This plant is known to possess analgesic (32, 46), antioxidant and antimicrobial properties (1).

Argemone mexicana, known as Mexican poppy or Mexican prickly poppy, is a species of poppy found in Mexico and now in the United States, India and Ethiopia. It is poisonous, but has been used medicinally by natives of the western US and parts of Mexico (15). It possesses the alkaloid sanguinarine reported to be responsible for epidemic dropsy (9, 39). *A. mexicana* is reported to have antimicrobial activity (21), wound healing capacity in rat (10), larvicidal and chemosterilant activity (34), nematicidal and allelopathic potential (36).

Cannabis sativa being multi-purpose in nature can be used to make food, fiber, fuel, and medicine. The root is remembered by some as an old folk remedy for arthritis or joint pain and cathartic properties (5). In *Cannabis sativa*, various phytochemicals have been identified, yet according to broadly diversified scientific community opinion much of the *Cannabis* preparations can be evaluated solely on tetrahydrocannabinol (THC) content (44). The rapid and broad increase in the use and abuse of drugs obtained from *Cannabis* being controlled by international treaties. Cannabidiol, which is also a *Cannabis sativa* constituent acts as an antipsychotic drug **(**47). Terpenes have been detected and isolated from essential oil from flowers, leaves and roots (38). The terpenes are responsible for the flavor of different varieties of cannabis (16). Alkaloids are another chemical constituents found in cannabis. From different parts like roots, leaves, stems, pollen, and seeds, piperidine and pyrrolidine were identified and isolated (14).

Thevitia peruviana is a large glabrous evergreen shrub which produces milky juice. It is native to Iran, Mediterranean region and India as well. Various parts of *T. peruviana* plant are used for the treatment of human ailments. The leaves are used as a cardiotonic, anti-bacterial and diuretic agent. It is used in cutaneous eruptions and it is also effective as an antidote against snake venom (41). Its root is used for curing different types of cancers, ulcers and leprosy while the root-bark is used specifically against ring worm and the aqueous extracts of the leaves, branches, roots and flowers are toxic to certain insects (45). Several phytochemicals are found in various parts of the plant, which contain mainly glycosides, terpenoids, cardiotonic substances and steroids (41).

The information available on these important medicinal plants indicates that not much attention has been paid towards studying their physico-chemical properties as well as biological activities in humans. The present paper illustrates extraction of phytochemicals from different parts of certain traditional Indian plants such as *Calotropis procera*, *Datura metal*, *Cannabis sativa*, *Argemone mexicana* and *Thevitia peruviana* in aqueous medium, characterization in terms of the responses of phytochemicals towards absorbance of monochromatic beam of radiations in the ultraviolet (UV) and visible ranges as well as their impact on to the activity of AChE isolated from healthy human erythrocytes. Here AChE has been used as a biomarker of neurotransmission system in an organism and as a screen to evaluate the activity of molecules derived from plants. The data suggested that aqueous extracts of these plants shared common properties of displaying two peaks in UV region. However, they significantly differed in their activities against the activity of AChE from healthy human erythrocytes. The results indicate that AChE from healthy humans RBCs may be used as a screen for evaluation of the medicinal potential of different plant products.

MATERIALS AND METHODS

Chemicals and materials

In this study, Tris buffer [Tris(hydroxymetyhyl)aminomethane] was purchased from Merk . Acetylthiocholine iodide and 5, 5'-dithiobis (2-nitro benzoic acid) were purchased from Polysciences and Himedia, respectively. All other reagents used were of analytical grade.

Collection and identification of plant materials

Different parts of some plants belonging to different families have been used. *Argemone Mexicana, Datura metal*, *Calotropis procera*, *Thevitia peruviana* and *Cannabis sativa*, were belonging to the Papaveraceae , Solanaceae, Asclepiadaceae, Apocynaceae and Cannabaceae families, respectively. These plant samples were collected from Allahabad and adjoining areas during March and April in the year 2011. The genus and species of the plants were authenticated by Dr. Satyanarayana of Department of Botany, University of Allahabad, India.

Preparation of plant extracts

The collected fresh plant parts were taken, washed with tap water and cut into several small pieces, minced well in pestle mortar and extracted with 50 ml of 50mM Tris-HCl buffer at pH 7. Freezing and thawing is done twice at the intervals of 2h each followed by mechanical jerk in pestle mortar in order to rupture the plant cell wall. The 10% homogenate of each of the plant materials was prepared at 4-6 ^{0}C. The homogenate was filtered using Whatman's filter paper. The volume of the filtrate was recorded. The filtrate was centrifuged at 1000xg for 10 min. The clear supernatant was used to evaluate the effects of the extracts on the activity of AChE. The difference of the weights of the starting material and the residues was considered as the amount of the plant present in the extract.

UV-Visible spectroscopy of the aqueous extracts of plants

Different concentrations of the plant extracts were taken in a quartz glass cuvette (1cm light-path, 3ml volume) and mixed with 50mM Tris-HCl buffer, pH 7.0. The UV-Visible Spectrum profiles of different plant extracts were monitored in the UV-Visible range between 240 to 700 nm using Thermo Scientific Spectroscan UV 2700 double beam UV-Vis spectrophotometer.

Collection of blood and separation of erythrocytes

The erythrocytes were isolated from human blood collected from volunteers at the Department of Biochemistry, University of Allahabad, India. For each experiment, 5ml blood was collected into buffered dextrose (ACD solution) into 6ml sterilized Haemochek polymed vial by venipuncture from non-smoking healthy donor not exposed to radiation or drugs. Subsequently erythrocytes were separated by centrifugation at 800xg for 10 min and subsequently washed three times with 0.9% NaCl buffer with 10mM Tris–HCl, pH 7.4. Washed erythrocytes were resuspended in the same buffer, and used for preparation of ghosts.

Preparation of human erythrocytes membrane

Erythrocytes ghosts were prepared from healthy human erythrocytes according to the procedure described previously (12). In brief, to remove the hemoglobin content from human erythrocyte ghosts, the suspended erythrocytes were mixed with hypotonic solution (5 mM Tris-HCl and 1mM EDTA, pH-7.4) and kept at -20°C for overnight. After thawing the erythrocytes, it is centrifuged at 12000xg for 30 min. The supernatant was discarded and erythrocytes were washed with hypertonic solution (50 mM Tris-HCl, 1mM EDTA, 500 mM NaCl, pH 7.4). This procedure of washing with buffer was done alternatively with hypotonic buffer and hypertonic buffer till the clear, colourless or haemoglobin free supernatant is obtained. A hemoglobin free ghost is obtained at the bottom of centrifuged tubes after centrifugation.

Extraction and assay of AChE activity

In order to extract the membrane bound AChE from the human erythrocytes membrane, the ghost suspension was solubilized in non-ioninc detergent i.e. 0.1% Triton X-100 (v/v) by gentle shaking and incubating at 4-6^{0}C for 30 min. The extract was further diluted with 50m M Na_2HPO_4/ NaH_2PO_4 (pH 7.4) to obtain the required protein concentration so as to measure the steady state kinetics of the enzyme.

In vitro effect of plant extracts on the activity of AChE

In this study 19 aqueous extracts of five different plants (*Argemone maxicana, Datura metal, Calotropis procera, Canabis sativa*, *Thevitia peruviana*) from different families were examined for their effects on the activity of AChE. The amounts of each extract used were 0.75, 1.50, 2.25, 3.75 and 7.50 µg. The spectrophotometric method developed by Ellman et al. (13) with some modifications was applied to measure the increase in optical density using Thermo Scientific Spectroscan UV 2700 double beam UV-Vis spectrophotometer with quartz cuvette (1cm light-path, 3ml). The assay mixture of 3ml volume contained 50 mM phosphate buffer (pH 7.4), 0.5mM acetylthiocholine iodide (ATI), 0.5mM 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) and different concentrations of plant extract. The reaction was started by addition of the enzyme protein (100-200µg) to the mixture. The change in the absorbance at 412 nm was recorded spectrophotometrically at each interval of 30sec at room temperature 26±1°C. A reaction mixture containing all the components except the plant extract is used as control. The blank contained no enzyme protein or ATI in the reaction mixture.

Determination of IC_{50} value

A linear regression analysis using Microsoft excel program is done for calculating IC_{50} values (the concentration inhibiting the enzyme activity by 50%) for different plant extracts against the activity of human erythrocytes' AChE.

Determination of protein

The concentration of protein in the enzyme preparation was done by the method of Lowry et al. (25) using bovine serum albumin (BSA) as a standard.

Statistical analysis of data

Data obtained were analyzed using graph pad Prism version 5.01. Each experiment is carried out in triplicate and values are expressed as mean±SD. One-way ANOVA with Dennett post-test was used to compare the changes at each of the extract concentrations from that of control. *, ** and *** represent the values significant at $p < 0.05$, $p < 0.01$and $p < 0.001$, respectively.

RESULTS

UV-Visible spectrophotometric analysis of aqueous extracts of different parts of the plants

The UV-Visible absorption spectrophotometric profiles were determined for the aqueous extracts of different parts of the plants towards their absorptivity for UV-Visible radiations. The results presented in Figures 1, 2, 3, 4 and 5 showed two prominent absorption peaks at 302 and 338 nm from each of the aqueous extracts of different parts of plants, *Argemone mexicana Datura metal, Calotropis procera , Thevitia peruviana* and *Cannabis sativa* respectively. These peaks appearing at 302 and 338 nm are the characteristics of flavonoids; some of them possibly would have got solubilised in aqueous medium. A summary of UV-Visible spectrophotometric profiles of the different extracts from these plants is presented in Table 1. However, no peaks were detected for nucleic acids and proteins at 260 and 280 nm, respectively, which could be due to the excessive dilution of the aforementioned preparations. The probable reason for all the extracts giving only two peaks at 302 and 338 nm could be attributed to the fact that very few phytochemicals are soluble in aqueous medium.

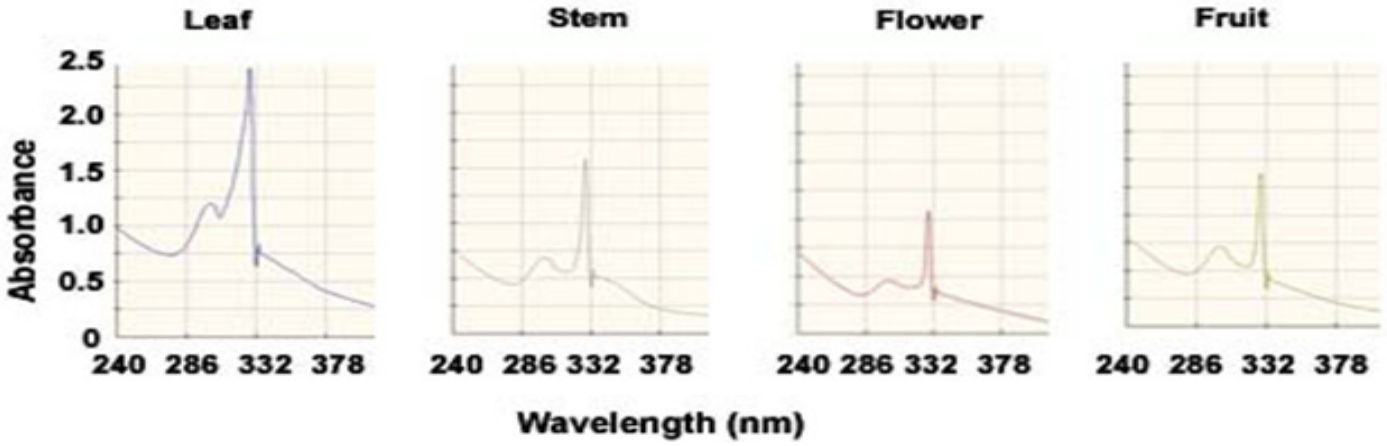

Figure 1. UV-Visible spectrophotometric profile of aqueous extracts of *Argemone Mexicana.*

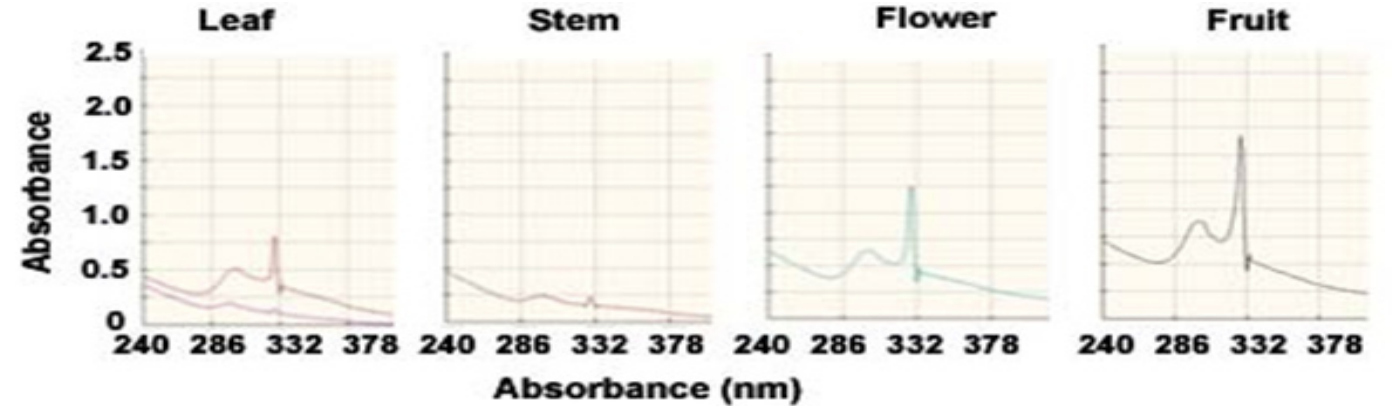

Figure 2. UV-Visible spectrophotometric profile of aqueous extracts of *Datura metal.*

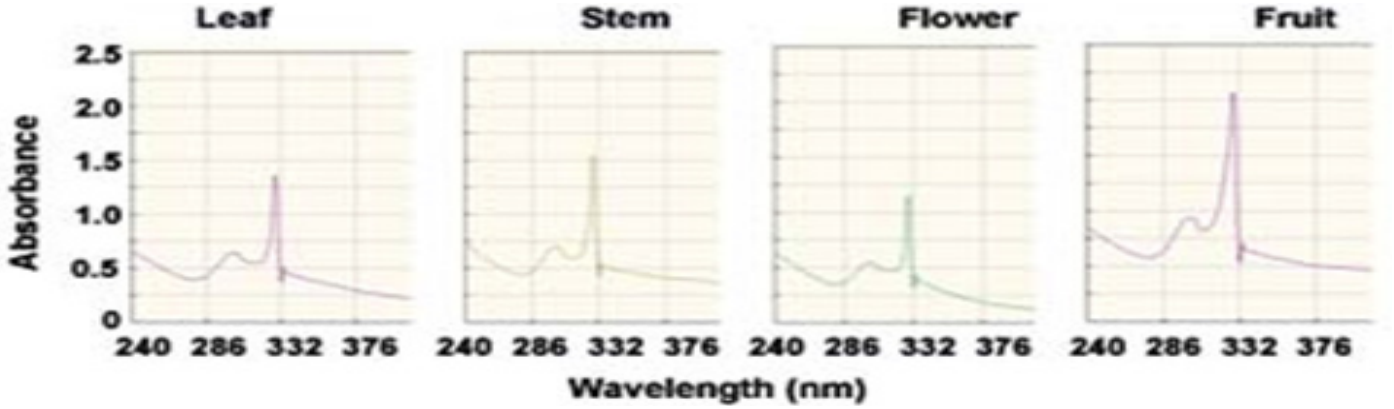

Figure 3. UV-Visible spectrophotometric profile of aqueous extracts of *Calotropis procera.*

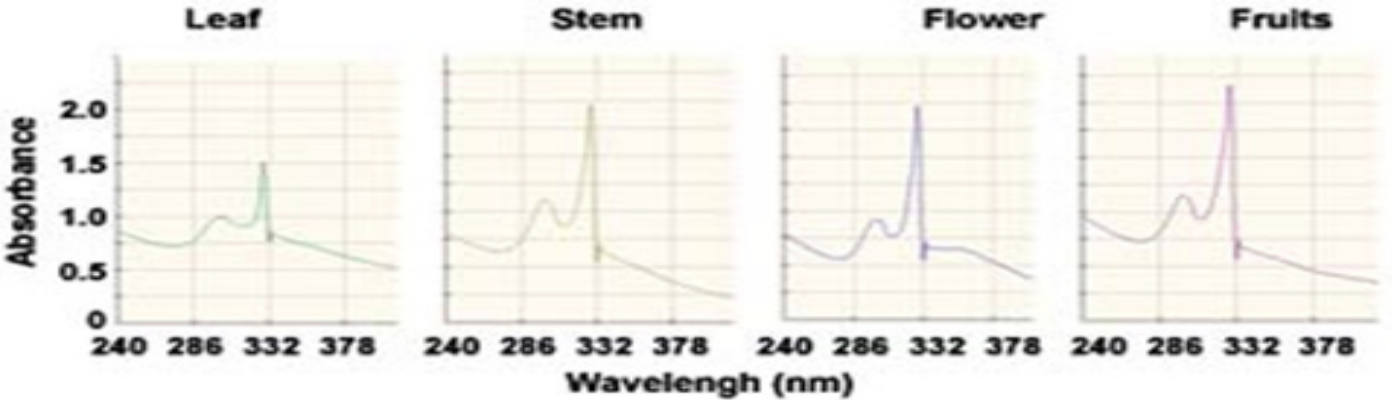

Figure 4. UV-Visible spectrophotometric profile of aqueous extracts of *Thevitia peruviana.*

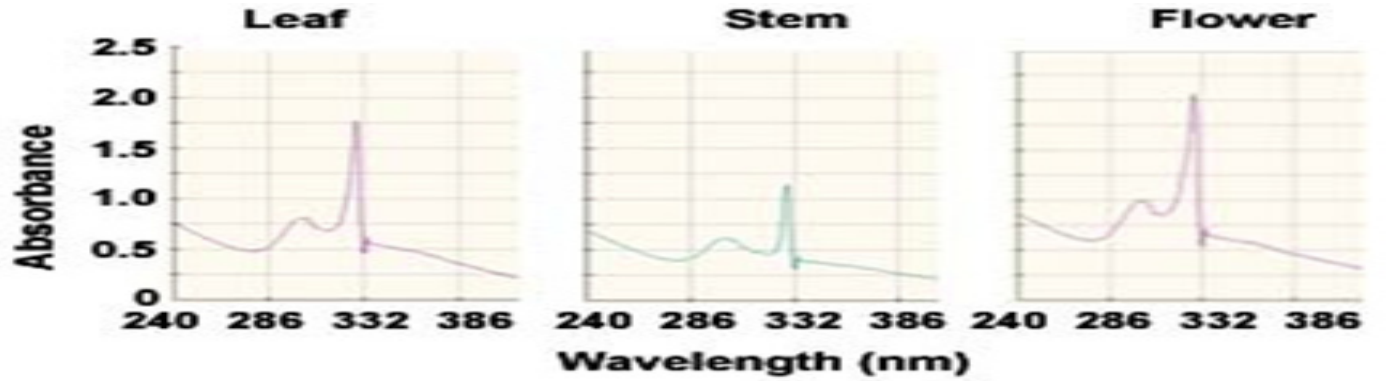

Figure 5. UV-Visible spectrophotometric profile of aqueous extracts of *Cannabis sativa.*

Levels of protein content in the aqueous extracts of different parts of the plants

A profile of soluble proteins isolated from different parts of the plants has been displayed in Table 2. The results depicted the presence of different protein concentrations in different parts of the above mentioned plants. The maximum protein content in all the five experimental plants showed tissue wise difference. In *Cannabis sativa*, the maximum protein content (19.25 mg/g wet weight) was present in the flower followed by *Datura metal* (12 mg/g wet weight) in fruit, *Argemone maxicana* (11.5 mg/g wet weight) in the leaves, *Thevitia peruviana* (10.4 mg/g wet weight) in the fruits and *Calotropis procera* (8mg/g wet

Table 1. UV-Visible spectroscopy analysis of aqueous extracts of different parts of the plants.

S.No.	Plant	Parts of plants	Concentration Of extract (µg/ml mix)	Number of peaks Obtained	λ-max	Probable plant Molecules	References
1	*Aregemone mexicana* (Papaveraceae)	Leaf	1.16	02	302, 330	Flavonoids	4, 43
		Stem	2.33	02	302, 330	Flavonoids	4, 43
		Flower	1.80	02	302, 330	Flavonoids	4, 43
		Fruit	1.83	02	302, 330	Flavonoids	4, 43
2	*Datura metal* (Solanaceae)	Leaf	1.30	02	302, 330	Flavonoids	4, 43
		Stem	2.90	02	302, 330	Flavonoids	4, 43
		Flower	2.30	02	302, 330	Flavonoids	4, 43
		Fruit	3.33	02	302, 330	Flavonoids	4, 43
3	*Calotropis procera* (Asclepiadeceae)	Leaf	3.70	02	302, 330	Flavonoids	4, 43
		Stem	1.30	02	302, 330	Flavonoids	4, 43
		Flower	0.70	02	302, 330	Flavonoids	4, 43
		Fruit	3.00	02	302, 330	Flavonoids	4, 43
4	*Thevitia peruviana* (Apocynaceae)	Leaf	1.38	02	302, 330	Flavonoids	4, 43
		Stem	3.33	02	302, 330	Flavonoids	4, 43
		Flower	1.66	02	302, 330	Flavonoids	4, 43
		Fruit	2.70	02	302, 330	Flavonoids	4, 43
5	*Cannabis sativa* (Cannabaceae)	Leaf	0.80	02	302, 330	Flavonoids	4, 43
		Stem	1.20	02	302, 330	Flavonoids	4, 43
		Flower	0.23	02	302, 330	Flavonoids	4, 43

Table 2. Protein content in the aqueous extracts of different parts of the plants The protein content in the different aqueous extracts of the parts of the plants were determined by precipitating the protein with equal volume of chilled 10% TCA followed by dissolving the protein into minimum volume of 0.1N NaOH. This solution was used for quantification of the protein by Lowery et al. (1951) as described in the Materials and Methods section. The data presented are the average values of three independent experiments.

S. No.	Plant	Part	Extract	Protein (mg/gm) of wet weight
1	*Argemone mexicana*	Leaf	Aqueous	11.5 ± 0.036
		Stem	Aqueous	1.80 ± 0.004
		Flower	Aqueous	5.50 ± 0.013
		Fruit	Aqueous	9.00 ± 0.000
2	*Datura metal*	Leaf	Aqueous	3.00 ± 0.001
		Stem	Aqueous	2.00 ± 0.006
		Flower	Aqueous	2.60 ± 0.003
		Fruit	Aqueous	12.0 ± 0.005
3	*Calotropis procera*	Leaf	Aqueous	8.00 ± 0.004
		Stem	Aqueous	3.00 ± 0.004
		Flower	Aqueous	6.40 ± 0.007
		Fruit	Aqueous	3.20 ± 0.001
4	*Thevitia peruviana*	Leaf	Aqueous	4.20 ± 0.004
		Stem	Aqueous	1.20 ± 0.000
		Flower	Aqueous	9.60 ± 0.044
		Fruit	Aqueous	10.4 ± 0.016
5	*Cannabis sativa*	Leaf	Aqueous	6.40 ± 0.000
		Stem	Aqueous	10.50 ± 0.007
		Flower	Aqueous	19.25 ± 0.002

weight) in the leaves. Excepting *Cannabis sativa*, all other plants exhibited minimum protein content in their stems; the values being 1.8, 2.0, 3.0 and 1.2 mg/g wet weight of *A. Mexicana*, *D. metal*, *C. procera* and *T. peruviana*, respectively. The minimum protein content (6.4 mg/g wet weight) in *C. sativa* was found in the leaves (Table 2).

Effect of aqueous extracts of different parts of the plants on the activity of human erythrocytes' acetyl cholinesterase (AChE)

The existing literature indicates the efficacy of certain

plant extracts on the activity of some marker enzymes isolated from human tissues (20,27). Keeping this fact in view, we attempted to evaluate the efficacy of the plant extracts at different quantities such as 0.75, 1.50, 2.25, 3.75 and 7.50 µg on to the activity of AChE isolated from the membrane of healthy human erythrocytes. The results are presented in Figures 6, 7, 8 and 9 for different plants. The data suggested that the aqueous extracts from all the parts of *A. mexicana* displayed maximum inhibitory activity (2-90%, Figure 6) followed by *D. metal* (7-55 %, Figure 7) and *Calotropis procera* (0- 30% Figure 8). The extent of enzyme inhibition by the aqueous extracts of each of these plants was concentration dependent. The strongest inhibitory activity was shown by the aqueous extract of different parts from *Argemone mexicana.* Among the four parts of *A. Mexicana*, leaves and stem showed maximum inhibitory potential against the activity of AChE followed by flowers and fruits. However, at highest concentration tested (7.80 µg), the fruit of *A. mexicana* displayed exceptionally high inhibitory potential (up to 90%) against human erythrocytes' AChE activity.

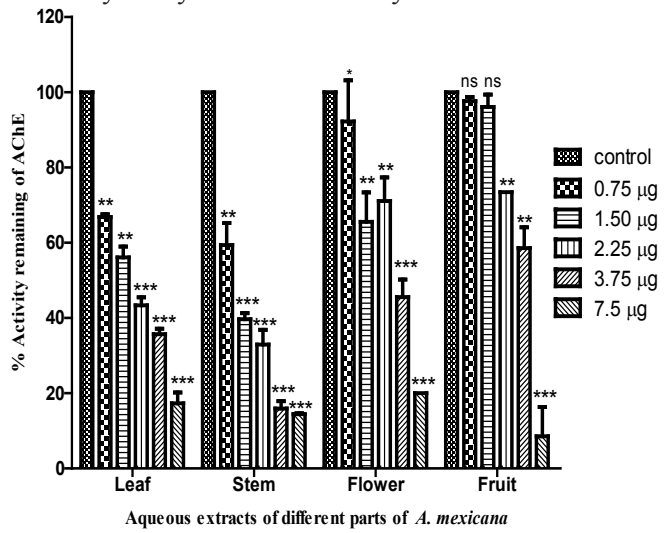

Figure 6. Effect of the aqueous extract of different parts of the plant, *Argemone mexicana*, on the activity of AChE isolated from human RBCs' membrane. Each experiment is carried out in triplicate and values are expressed as mean±SD. AChE inhibition caused by the aqueous extract of different parts of the *A. mexicana*, was compared against control, using One-way ANOVA with Dennett post-test was used to compare the changes at each of the extract concentrations from that of control. *, ** and *** represent the values significant at $p < 0.05$, $p < 0.01$and $p < 0.001$, respectively. ns=non significant.

In case of *Datura metal*, the aqueous extract prepared from its leaves was highly active against AChE activity at all concentrations tested as compared to the extracts prepared from stem, flower and fruit; the values being in the range of 12-55%. In fact the extracts from stem, flower and fruit of *D. metal* showed similar inhibitory activity (7-43%) against AChE at all the five concentrations tested. Up to 45% when treated with 7.50 µg of aqueous extract while in case of leaf up to 55% of inhibition was seen with same quantity (Figure 7). With *C. procera*, the lowest concentration of the extract (0.75µg) from leaves and fruits is not effective, whereas the stem and flowers showed 10-12% inhibition of enzyme activity (Figure 8). The extracts from flower and the fruit of *C. procera* displayed more significant inhibition in the enzyme activity (20-30%) at higher concentrations (2.25, 3.75 and 7.5µg) as compared to that from leaves and stem. The extracts prepared from the leaves, stem, flower and fruits of *T. peruviana* did not show any significant inhibition in enzyme activity even at the highest concentration (7.5 µg) tested (Figure 9). Similarly, the extracts from *C. sativa* also did not exhibit any inhibitory effect against AChE activity at all concentrations tested (data not shown).

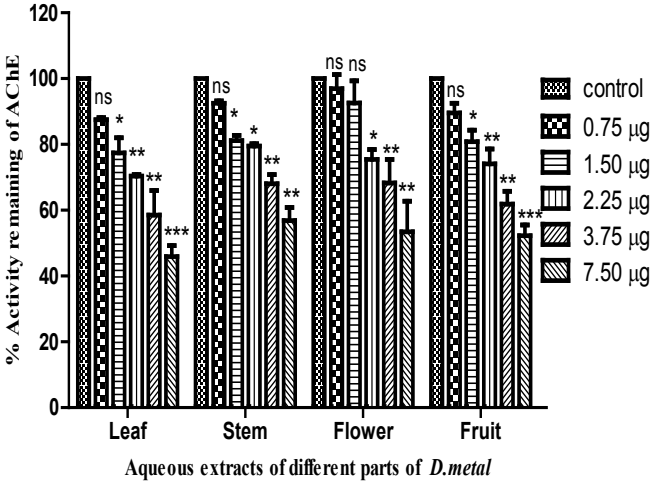

Figure 7. Effect of the aqueous extract of different parts of the plant, *D. metal*, on the activity of AChE isolated from human RBCs' membrane. Each experiment is carried out in triplicate and values are expressed as mean±SD. AChE inhibition caused by the aqueous extract of different parts of the *D. metal,* was compared against control, using One-way ANOVA with Dennett post-test was used to compare the changes at each of the extract concentrations from that of control. *, ** and *** represent the values significant at $p < 0.05$, $p < 0.01$and $p < 0.001$, respectively. ns=non significant.

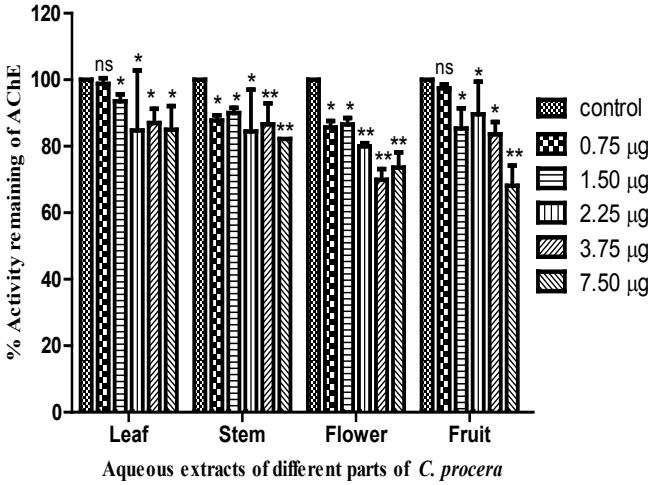

Figure 8. Effect of the extract of different parts of the plant, *C. procera*, on the activity of AChE isolated from human RBCs' membrane. Each experiment is carried out in triplicate and values are expressed as mean±SD. AChE Inhibition caused by the aqueous extract of different parts of the *C. procera,* was compared against control, using One-way ANOVA with Dennett post-test was used to compare the changes at each of the extract concentrations from that of control. *, ** and *** represent the values significant at $p < 0.05$, $p < 0.01$and $p < 0.001$, respectively. ns=non significant.

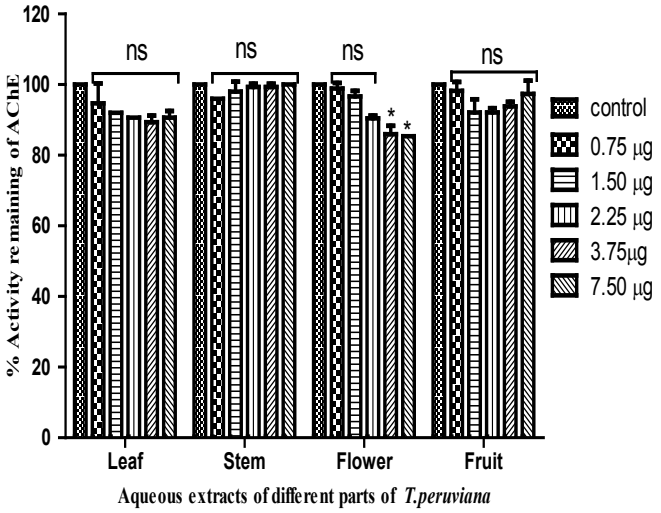

Figure 9. Effect of the extract of different parts of the plant, *T. peruviana*, on the activity of AChE isolated from human RBCs' membrane. Each experiment is carried out in triplicate and values are expressed as mean±SD . AChE Inhibition caused by the aqueous extract of different parts of the *T. peruviana* , was compared against control, using One-way ANOVA with Dennett post-test was used to compare the changes at each of the extract concentrations from that of control. *, ** and *** represent the values significant at $p < 0.05$, $p < 0.01$and $p < 0.001$, respectively. ns=non significant.

Evaluation of IC_{50} values for aqueous extracts of different parts of the plants

The IC_{50} values for the aqueous extracts from different parts of the five plants tested was carried out by assaying the enzyme activity in absence (control) or presence of increasing concentrations of the extract with the enzyme in the activity assay system as described in Materials and Methods. The enzyme activity in control was considered as 100%. A linear regression analysis of the data obtained using excel program is carried out for calculating IC_{50} values (the concentration at which 50% enzyme activity remains) for the plant extracts that showed significant inhibitory effect. The results shown in Table 3 indicated that stem of *A. mexicana* was most active (IC_{50}=0.30µg/ml reaction mix) followed by leaves, fruits and flower; the IC_{50} values being 0.56, 1.15 and 1.44µg/ml reaction mix, respectively. In case of *D. metal*, the leaves exhibited maximum inhibitory potential against AChE activity with IC_{50} value being 2.1 µg /ml reaction mix. The other parts of this plant such as flower stem and fruit showed relatively higher IC_{50} values viz. >2.3, >2.8 and >3.3 µg /ml reaction mix, respectively. The extent of inhibition in the enzyme activity by these aqueous extracts of the plants was concentration dependent. However, the IC_{50} values for the remaining three plants such as *C. procera*, *T. peruviana* and *C. sativa* could not be determined (Table 3) as there was no significant inhibition in enzyme activity even at higher concentrations of the extracts tested.

DISCUSSION

The aqueous extracts prepared from several plants have shown the presence of water soluble flavonoids (mostly anthocyanins) as well as phenolics with antioxidant potential (43.). In the present investigation, the spectrophotometric ananlysis of all the aqueous extracts prepared from different parts of *A. mexicana*, *D. metal, C. procera*, *T. peruviana* and *C. sativa* have demonstrated the occurrence of two different peaks in the ultra-violet region; the wavelengths being 302 and 338nm. These absorption wavelengths correspond to the characteristics of flavonoids pre-

Table 3. Impact of aqueous extracts from different plants against the activity of human Erythrocytes' AChE.

S. No.	Plant	Part	IC_{50} (µg/ml Reaction mix)	Concentration of plant extract (µg/ml)
1	*Argemone mexicana* (Papaveraceae)	Leaves	0.56	70.0
		Stem	0.30	70.0
		Flower	1.44	80.0
		Fruit	1.15	55.0
2	*Datura metal* (Solanaceae)	Leaves	2.1	78.0
		Stem	>2.8	88.0
		Flower	>2.3	71.0
		Fruit	>3.3	100.0
3	*Calotropis procera* (Asclepiadaceae)	Leaves	ND	113.0
		Stem	ND	78.0
		Flower	ND	77.0
		Fruit	ND	92.0
4	*Theviatia peruviana* (Apocynaceae)	Leaves	ND	83.0
		Stem	ND	102.0
		Flower	ND	103.0
		Fruit	ND	81.0
5	*Cannabis sativa* (Cannabaceae)	Leaves	ND	13.0
		Stem	ND	37.0
		Flower	ND	07.0

The IC_{50} values of the aqueous extracts from different parts of the plants have been determined against AChE isolated from the healthy human erythrocytes as described in Materials and Methods. The IC_{50} value represents the concentration of the plant extract at which 50% of the enzyme activity remains.

sent in the aqueous extracts of some medicinal plants have shown that the plant flavones display different peaks with UV absorption maxima at 278 and 330 nm, respectively (43). Generally, flavonoids show two characteristic bands in the UV-region from 240 to 280 nm and from 300 to 340 nm (4).

Proteins are known to be the integral component of a plant cell playing crucial roles in many molecular forms. Despite providing a structural support to the cell, it is also utilized as a source of energy after the carbohydrates and fats are metabolized. In the present investigation, the protein profile from different parts of the five species of plants such as *A. mexicana*, *D. metal*, *C. procera*, *T. peruviana* and *C. sativa* suggested its tissue specific synthesis and distribution. It was found maximum in the flower of *C. sativa* and the minimum in the stem of *T. peruviana*. In the leaves of *Cannabis sativa* L, Lone and Lone (24) reported the presence of cannabinoid protein into the aqueous as well as acetone extracts; the values being 0.6mg/ml and 1.6mg/ml, respectively.

Acetylcholinesterase (AChE, EC: 3.1.1.7) is reported to be present both at the terminal exonic ends of neurons as well as the non-neuronal tissues such as red blood corpuscles (RBCs) (26). The AChE present in the membrane of RBCs serves as a valuable model system to evaluate the impact of any factor on the neurotransmission system. The erythrocytes AChE resembles in its biochemical characteristics with that of the neuronal enzyme and it can be easily and quickly isolated (28). Although erythrocytes AChE remain an integral constituent of the membrane, the exact physiological function of this enzyme in these cells is still unclear. Callahan and Kruckenberg (6) have reported that among all the mammalian systems tested, the activity of AChE from human RBCs is highest. Thus, the evaluation of the level of the AChE activity from human erythrocytes not only indicates about the intactness of the RBCs but also act as a viable biomarker to assess the extent of exposure of these blood cells to the natural or anthropogenic chemicals.

Plants showing their therapeutic potential towards treatment of neurodegenerative diseases such as Alzheimer's, Parkinson and epilepsy are being explored for a long time. There has been a continuous search for new drugs. Galanthamine, an alkaloid from snowdrop, was approved for use in Alzheimer therapy (18, 20). There are some reports which indicate the biological effects of plants as AChE inhibitors *in vitro* and also as memory enhancers *in vivo* (20, 42, 18). The results of the present investigation revealed that out of five plants tested, only two of them namely *A. mexicana* and *D. metal* showed inhibitory potential when tested *in vitro* against the AChE activity; the crude aqueous extract of stem of *A. mexicana* being most effective (lowest IC_{50} value) and the fruits of *D. metal* least effective (highest IC_{50} value). The chemical constituents isolated from *A. mexicana* have been shown to cause similar inhibition of AChE activity *in vitro* in the nervous tissue of *L. acuminate* (37). The significant levels of anti-acetyl cholinesterase activities of the extracts from *A. mexicana* (2, 22, 29) and *D. metal* (8) have been reported.

The crude aqueous extracts prepared from five different plants displayed similar absorption profile in the ultra-voilet region indicating common phytochemical constituents present in the preparation. The levels of proteins in different parts of the plants were found to be expressed in tissue dependent manner; the flower of *C. sativa* consisting highest and the stem of *T. peruviana* containing the minimum. Only two plant species *A. mexicana* and *D. metal* could exert anti-acetylcholinesterase effect; the former being stronger inhibitor than the later. These two plants proved to act as strong neurotoxic substances and hence may be exploited as potential sources of isolation and development of effective chemotherapeutics against different neurodegenerative disorders. The data also suggest that AChE from the healthy human erythrocytes can be used as a viable biomarker to evaluate the neurotoxic potential of different plant products.

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Other articles in this theme issue include references (48-75).

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