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In-Vitro Evaluation of Ethanolic Extract of *Convolvulus pluricaulis* Choisy for Anti-Inflammation & Memory Enhancing Activity

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Abstract

The aim of the present investigation is to evaluate *In-Vitro* Evaluation of Ethanolic Extract of *Convolvulus pluricaulis* Choisy for Anti-Inflammation & Memory Enhancing Activity. Whole plant materials were dried under shade and subjected to coarse powder for extraction process. Accurately weighed quantity of whole plant material was extracted using 95 % ethanol by soxhlet apparatus for 72 h. Qualitative chemical tests of ethanolic extracts were subjected to various chemical tests to detect various phytoconstituents. The human red blood cells (HRBC) membrane stabilization had been used as a method to study anti-inflammatory activity. In this method, blood was collected from healthy human volunteers who had not been taken anti-inflammatory drug for 2 weeks prior to the experiments. AChE activity was measured using a modified 96-well microplate assay (14) based on Ellman's method (15). The enzyme hydrolyses the substrate acetylthiocholine resulting in the product thiocholine which reacts with Ellman's reagent (DTNB) to produce 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate which can be detected at 412 nm.

The preliminary phytochemical analysis revealed that different active constituent present in different extracts such as carbohydrates, proteins, amino acids, fat, oils, steroids, terpenoids, glycosides, alkaloids, tannins and other phenolics compounds. At a concentration of 500 µg/ml, the extract produced 72.60% protection of RBC haemolysis as compared with 73.84% produced by prednisolone. The ethanolic extract of selected plant showed 40.22% inhibition. The Diclofenac sodium showed 48.50% inhibition against denaturation of protein. The ethanolic extract of selected plant materials showed 42.65% inhibition respectively. The Diclofenac sodium showed 63.89% inhibition against proteinase inhibitory activity. In conclusion, it can be stated that the ethanolic extract has beneficial effects in long lasting in membrane stabilizing method, inhibition of protein denaturation method and proteinase model.

Key words: *In-Vitro* Evaluation, Ethanolic Extract, *Convolvulus pluricaulis* Choisy, Anti-Inflammation Activity, Memory Enhancing Activity

Introduction

The use of herbal medicines continues to expand rapidly across the world. Many countries now turn towards herbal medicines or herbal products for their health care in national health-care settings. According to WHO, 80% if the rural population in developing countries depend on traditional medicines to meet their primary health care needs (1).

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Authentication and standardization are prerequisite steps while considering source materials for herbal formulation in any system of medicine (2). In traditional systems of medicine, the drugs are primarily dispensed as water decoction or ethanol extract. Fresh plant parts, juice, or crude powders are a rarity rather than a rule. Thus medicinal plant parts should be authentic and free from harmful materials like pesticides, heavy metals, microbial or radioactive contamination, etc. (3). It is very important that a system of standardization should establish for every plant medicine in the market because the scope for variation in different batches of medicine is enormous. World Health Organization (WHO) encourages, recommends, and promotes traditional / herbal remedies in national health care programmes because these drugs are safe, people have faith in them and easily available at low cost. The WHO is continuously emphasizing to ensure quality control of medicinal plant products by using modern techniques and applying suitable standards (4). India has a rich heritage of traditional medicine constituting with its different components like Ayurveda, Siddha, Unani, Homoeopathy and naturopathy. Traditional health care has been flourishing in this country for many centuries (5).

Inflammation is the protective mechanism of our body and during this process there is a migration of WBCs from the blood vessels. Similarly, memory is also an integral part of our life and subsides with the age. There is an important mediator or neurotransmitter in our brain and controls the memory.

The aim of our study is to evaluate the effect of ethanolic extract *In-vitro* models of inflammation and memory.

Material and Method

Plant materials

The whole plant parts of *Convolvulus pluricaulis* Choisy were purchased from the local market and also collected from college campus of Oriental College of Pharmacy, Indore.

Authentification of plant materials

All the plant materials were taxonomically identified by Dr. S. K. Sharma, Senior Scientist, KNK College of Horticulture, Mandsaur. The herbarium sheets were submitted in Department of Pharmacognosy, Oriental of Pharmacy, Indore.

Preparation of total crude extract

Whole plant materials were dried under shade and subjected to coarse powder for extraction process. Accurately weighed quantity of whole plant material was extracted using 95 % ethanol by soxhlet apparatus for 72 h. The ethanolic extracts were dried under the reduced pressure to get crude methanolic extracts. The ethanolic extracts were dried completely under reduced pressure. After drying, the respective extracts were weighed and percentage yield was determined (6).

Preliminary phytochemical tests

Qualitative chemical tests of ethanolic extracts were subjected to various chemical tests to detect various phytoconstituents (7, 8).

In-Vitro Anti-inflammatory activity membrane stabilizing activity of ethanolic extract

The human red blood cells (HRBC) membrane stabilization had been used as a method to study anti-inflammatory activity. In this method, blood was collected from healthy human volunteers who had not been taken anti-inflammatory drug for 2 weeks prior to the experiments. This blood was mixed with equal volume of sterilized alsever solution (2 % dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42%sodium chloride). The blood was centrifuged at 3000 rpm and packed cells were washed with isosaline and a 10% suspension was made with iso saline. Various concentrations of test samples in concentration of 100-500 µg/ml were prepared using ethanol and to each concentration 1 ml phosphate buffer, 2 ml hypo saline and 0.5 ml HRBC suspension were added. These were incubated at 37^0 C for 30 min. and centrifuged 3000 rpm for 20 min. The hemoglobin content in supernatant solution was estimated spectrophotometrically at 560 nm. Prednisolone 100 µg/ml was used as a standard. The % heamolysis was calculated by assuming the heamolysis produced by the control group as 100 %. The percentage of HRBC membrane stabilization or protection was calculated using the following formula (9, 10).

$$\% \text{ Protection} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Inhibition of protein denaturation method

The ethanolic extract of selected plant materials were suspended in distilled water. The following procedure was followed for evaluating the percent of inhibition of protein denaturation:-

Control solution (50 ml) consists of 2ml of Egg albumin (from fresh hen's egg) and 28 ml of phosphate buffer (PBS, pH 6.4) and 20ml distilled water.

Standard drug (50 ml) consists of 2ml of Egg albumin and 28 ml of phosphate buffer and 20 ml of various concentrations of standard drug Diclofenac sodium (20, 40, 60, 80 & 100 µg/ml).

Test solution (50 ml) consists of 2ml of Egg albumin and 28 ml of phosphate buffer and 20 ml of various concentrations of ethanolic extract in a concentration of 100, 200, 300, 400, 500 µg/ml.

All of the above reaction mixtures were adjusted to pH 6.4, using a small amount of 1N HCl. The samples were incubated at 37°C for 15 minutes and heated at 70°C for 5 minutes. After cooling, the absorbance of the above solutions was measured using UV- spectrophotometer at 660 nm. The percent inhibition of protein denaturation was calculated using the following formula (11, 12).

Percent inhibition = (Vt/Vc-1) x 100)

Where, Vt= absorbance of test sample, Vc= absorbance of control

Proteinase inhibitory activity

The ethanolic extract of whole plant materials were suspended in distilled water.

The following procedure was followed for evaluating the Proteinase Inhibitory Activity.

Control solution (50 ml) 20 ml consists of 0.06 mg Trypsin, 10 ml 20mM Tris HCl buffer (pH 7.4) and 10 ml distilled water.

Standard drug (50 ml) 20 ml consists of 0.06 mg Trypsin, 10 ml 20mM Tris HCl buffer (pH 7.4) and 10 ml of various concentrations of standard drug Diclofenac sodium. (20, 40, 60, 80 & 100 µg/ml).

Test solution (50 ml) 20 ml consists of 0.06 mg Trypsin, 10 ml 20mM Tris HCl buffer (pH 7.4) and 10 ml of various concentrations of methanolic extract in a concentration of 100, 200, 300, 400, 500 µg/ml.

All the reactions were incubated at 37° C for 5 minutes and then 10 ml of 0.8% (w/v) casein was added. Again the samples were incubated for an additional 20 min and 10 ml of 70% perchloric acid was added to arrest the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed as triplicate. The percent inhibition of proteinase inhibitory activity was calculated (13).

Percent inhibition = (Abs Control – Abs Sample) x 100 / Abs control.

In-vitro memory enhancing acivity

Determination of Acetylcholinesterase Inhibitory Activity

AChE activity was measured using a modified 96-well microplate assay (14) based on Ellman's method (15). The enzyme hydrolyses the substrate acetylthiocholine resulting in the product thiocholine which reacts with Ellman's reagent (DTNB) to produce 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate which can be detected at 412 nm. 50 mM Tris–HCl pH 8.0 was used as a buffer throughout the experiment unless otherwise stated. AChE used in the assay was from electric eel (type VI-S lyophilized powder, 518 U/mg solid, 844 U/mg protein). The enzyme stock solution (518 U/ml) was kept at 280uC. The further enzyme-dilution was done in 0.1% BSA in buffer. DTNB was dissolved in the buffer containing 0.1 M NaCl and 0.02 M MgCl2. ATCI was dissolved in deionized water. In the 96-well plates, 100 ml of 3 mM DTNB, 20 ml of 0.26 U/ml of AChE, and 40 ml of buffer (50 mM tris pH 8.0), 20 ml of extract in various concentrations (25, 50, 100, 250 and 500 mg/ml) dissolved in buffer containing not more than 10% methanol were added to the wells. After mixing, the plate was incubated for 15 min (25uC) and then the absorbance was measured at 412 nm in Tecan infinite 200 microplate reader and the readings were used as blank. The enzymatic reaction was initiated by the addition of 20 ml of 15 mM ATCI and the hydrolysis of acetylthiocholine was monitored by reading the absorbance every 5 min for 20 min. Physostigmine was used as positive control. All the reactions were performed in triplicate. The percentage inhibition was calculated as follows:

%Inhibition= (E S)/E ×100

Where-

E is the activity of the enzyme without extract and S is the activity of enzyme with the extract.

Results and Discussion

Preliminary phytochemical screening

The preliminary phytochemical analysis revealed that different active constituent present in different extracts such as carbohydrates, proteins, amino acids, fat, oils, steroids, terpenoids, glycosides, alkaloids, tannins and other phenolics compounds.

Table No 1: Qualitative chemical analysis of extracts by chemical tests

S. No	Phytoconstituents	Chemical Tests	*Convolvulus pluricaulis* Choisy
1	Alkaloids	Wagner's test	+
		Dragendorff's test	+
		Mayer's test	+
		Hager's test	-
2	Amino Acid	Millon's test	+
		Ninhydrine test	-
3	Flavonoids	Shinoda test	+
		Alkaline reagent test	+
		Zinc hydrochloride test	_
4	Phenolics (Tannins)	Gelatin test	+
		Phenazone test	-
		Ferric chloride test	+
5	Protein	Biuret test	+
		Hydrolysis test	+
		Test with trichloroacetic acid	-
6	Triterpenoids & Steroids	Libermann-Burchard test	+
		Salkowski test	+
7	Carbohydrates	Benedict's test	+
		Fehling's test	+
		Molish's test	-
8	Anthraquinone glycosides	Borntrager's test	+
		Modified Borntrager's test	+
9	Coumarin glycosides	_____	-
10	Saponin glycosides	_____	+
11	Cardiac glycosides	Baljet's test	+
		Legal's test	+
		Keller-killiani test	+

Where, (-) Negative, (+) Positive

Membrane Stabilizing Activity of Extracts on Rat Erythrocytes

The ethanolic extract was analyzed by membrane stabilizing property at concentration range of 100-500 µg/ml. Ethanolic extract significantly protect the rat erythrocyte membrane against lysis induced by hypotonic solution. At a concentration of 500 µg/ml, the extract produced 72.60% protection of RBC haemolysis as compared with 73.84% produced by prednisolone.

Table No. 2: Membrane stabilizing activity of extract at different concentration

S. No.	Fractions	Concentration (µg/ml)	% Protection
1	Control	----	100
2	Prednisolone	100 µg/ml	73.84
3	Ethanolic Extract	100 µg/ml	54.54
		200 µg/ml	57.95
		300 µg/ml	65.90
		400 µg/ml	68.18
		500 µg/ml	72.60

Protein Denaturation Methods

The ethanolic extract of selected plant showed 40.22% inhibition. The Diclofenac sodium showed 48.50% inhibition against denaturation of protein. The results are summarized in Table No. 3.

Table No. 3: Effect of ethanolic extract on Protein Denaturation

S. No.	Treatment	Concentration µg/ml	% Inhibition
1	Control	----	---
2	Ethanolic Extract	100	18.23
		200	19.98
		300	28.42
		400	34.53
		500	40.22
3	Diclofenac Sodium	20	21.50
		40	30.55
		60	40.75
		80	48.50

Proteinase inhibitory action:

The ethanolic extract of selected plant materials showed 42.65% inhibition respectively. The Diclofenac sodium showed 63.89% inhibition against proteinase inhibitory activity. The results are summarized in Table No. 4.

Table No. 4: Effect of ethanolic extract on proteinase inhibitory activity

S. No.	Treatment	Concentration µg/ml	% Inhibition
1	Control	----	-------
2	Ethanolic Extract	100	19.20
		200	25.60
		300	31.10
		400	37.50
		500	42.65
3	Diclofenac Sodium	20	26.88
		40	32.50
		60	41.55
		80	50.50
		100	63.89

Acetylcholinesterase Inhibitory Activity of the Extracts

The ethanolic extracts of selected plant material used in Ayurveda for nervous system disorders were tested for AChE inhibitory activity using Ellman's colorimetric method in 96-welled microplate. The inhibition curves have been presented in Table 5 representing the % inhibition at 100 mg/ml and IC50 for extracts. Physostigmine was used as the standard AChE inhibitor in this study which showed IC50 of 0.07560.003 mg/ml. IC50 for AChE inhibitory activity was 7763.1 mg/ml respectively. The plant showed very high antioxidant activity also in the DPPH assay.

Table No. 5: % Inhibition and IC50 values of ethanolic plant extracts AChE inhibition assays

S No.	% Inhibition of DPPH at 0.1 mg/ml	IC50 for DPPH scavenging assay (mg/ml)	IC50 for AChE inhibition assay (mg/ml)	% AChE inhibition at 0.1 mg/ml
	86±9.6	54.56±7.2	77±6.2	62.46±5.3

There is an emergent interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine. Thus, in the present investigation, an attempt was made to evaluate the anti-inflammatory and memory enhancing activity of selected medicinal plants (*Convolvulus pluricaulis* Choisy) on the basis of ayurveda and their traditional uses in a suitable experimental in vitro model.

In the preliminary study, dried powders of all selected plant were extracted by using methanol. The extracts were dried and screened for the presence of various active constituents. The extracts showed the presence of alkaloids, terpenoids, flavonoids, glycosides, phenolic compounds, tannins, steroids and fatty acids. For the preliminary assessment, the plant extract was evaluated by in-vitro models for anti-inflammatory and memory enhancing activity.

Cellular filtration is the main key aspect of inflammatory response and leukocytes play very important role in this infiltration process. During the process of inflammation, these cells release their lysosomal contents such as bactericidal enzymes and proteases causing further tissue damage and inflammation (16, 17). Such injury to cell membrane will further render the cell more susceptible to secondary damage through free radical induced by lipid peroxidation. The physical property of the cell membrane is controlled by specific membrane proteins and they contribute to the regulation of the volume and water content of cells by controlling the movement of sodium and potassium ions (18). Since the RBC membrane is similar to that of lysosomal membrane, inhibition of RBC heamolysis will therefore, provide good insights into the inflammatory process especially as both events are also consequent of injury. Injury to lysosomal membrane usually triggers the release of phospholipases A2 that mediates the hydrolysis of phospholipids to produce inflammatory mediators. In our study, *In vitro* assessments of the effect of extract of *Convolvulus pluricaulis* Choisy on membrane stabilization showed that it inhibited heat and hypo tonicity induced lysis of red blood cells. Ethanolic extract at higher concentration showed 71.59 % at a concentration of 500 µg/ml protection. It is already reported that NSAIDs with membrane stabilizing properties are well known for their activity with the early phase of the inflammatory mediators release, namely, the prevention of phospholipases release that trigger the formation of inflammatory mediators.

Serine proteinases from inflammatory cells, including neutrophils, are implicated in various inflammatory disorders such as Rheumatoid arthritis and pulmonary emphysema. Neutrophils are known to be a rich source of serine proteinase and are localized at lysosomes. Deficiency of protease inhibitors in circulation is the major risk factor for development of inflammatory disorder.

In the present investigation, the ability of ethanolic extract to inhibit protein denaturation as well as protienase inhibitory were studied. The ethanolic extract of *Convolvulus pluricaulis* Choisy were found to be effective in inhibiting heat induced albumin denaturation & protienase inhibition at different concentrations. The

inhibition of denaturation and protienase may be probable mechanism of ethanolic extract as anti-inflammatory activity.

Acetylcholinesterase (AChE) is a key enzyme in the cholinergic nervous system. Therapies designed to reverse the cholinergic deficit in AD is mostly based on inhibitors of AChE, which enhance cholinergic transmission with modest and transient therapeutic effects. Several studies revealed that cholinesterase inhibitors could act on multiple therapeutic targets such as prevention of the formation of b-amyloid plaques, antioxidant activity and modulation of APP processing.

Ayurveda, the ancient Indian system of medicine, dates back to 2000 BC in which various plants effective for treating CNS disorders and aging are well documented. In the present study, ethanolic extracts of *Convolvulus pluricaulis* Choisy plant materials considered to be 'nootropic' or brain boosting were prepared and evaluated for their anti-cholinesterase and anti-oxidant effects.

Conclusion

In conclusion, it can be stated that the ethanolic extract has beneficial effects in long lasting in membrane stabilizing method, inhibition of protein denaturation method and proteinase model. It also showed a protective effect on inflammation and memory enhancing effect. The mechanism may be mediated via the inhibition of prostaglandin synthesis in acute inflammatory reaction as well as inhibition of various lysosomal enzymes in chronic inflammatory responses this, justifies the claim made by Siddha and Ayurveda. The mechanism for the memory enhancing activity may be inhibition of cholinesterase enzymes in brain.

References

1. Bannerman, R. H., Burton, J., and Ch'en, W. C. (1983). Traditional medicine and health care coverage: a reader for health administrators and practitioners. *Traditional medicine and health care coverage: a reader for health administrators and practitioners.*
2. Ahmad, M., Khan, M. A., Rashid, U., Zafar, M., Arshad, M., and Sultana, S. (2009). Quality assurance of herbal drug valerian by chemotaxonomic markers. *African Journal of Biotechnology, 8*(6), 1148-1154.
3. Kamboj, V. P. (2000). Herbal medicine. *Current Science-Bangalore-*, *78*(1), 35-38.
4. Raina, M. K. (2003). Quality control of herbal and herbo-mineral formulations. *Indian Journal of Natural Products* 19: 16-23.
5. Kumar, K. J. (2011). Bottle Necks in Standardization of Traditional System of Medicines. *Research Journal of Medicinal Plant, 5*(4), 443-447.
6. Majno GM (1975). Healing Hand: Man and Wound in the Ancient World. Harvard University Press, Cambridge, MA.
7. Nagy, G., Clark, J.M., Buzas, E.I., Gorman, C.L., Cope, A.P., 2007. Nitric oxide, chronic inflammation and autoimmunity. *Immunology Letters*, Vol. 111, Issue 1, pp. 1-5.
8. Mukherjee, P.K., 2002. *Quality Control of Herbal Drugs-an Approach to Evaluation of Botanicals*. New Delhi, Business Horizons Pharmaceutical Publishers.
9. Kokate, C.K., 1996, *Practical Pharmacognosy*. Delhi, Vallabh Prakashan.
10. Khandelwal, K.R., 2006. *Practical Pharmacognosy*. Pune, Nirali Prakashan.
11. Vijayalakshmi, A., Ravichandiran, V., Jayakumari, S., Malarkodi, V., Nirmala, S., 2011. Anti-anaphylactic and anti-inflammatory activities of AFPA from the root bark of *Plumeria acutifolia* Poir. *Indian Journal of Pharmaceutical Education and Research*, Vol. 45, Issue 4, pp. 339-345.
12. Vijayalakshmi, A., Ravichandiran, V., Velraj, M., Hemalatha, S., Sudharani, G., Jayakumari, S., 2011. Anti-anaphylactic and anti-inflammatory activities of a bioactive alkaloid from the root bark of *Plumeria acutifolia* Poir. *Asian Pacific Journal of Tropical Biomedicine*, Vol. 1, Issue 5, pp. 401-405.

13. Chandra S, Chatterjee P, Dey P, Bhattacharya S. Evalauation of anti-inflammatory effect of Ashwagandha: A preliminary study invitro. Pharmacog J 2012; 4(29):47-49.
14. Chomarat P and Banchereau J. An update on interleukin-4 and its receptor. Eur Cytokine Netw 1997; 8(4):333-344.
15. Sakat S, Juvekar AR, Gambhire MN. *In vitro* antioxidant and inflammatory activity of methanol extract of *Oxalis corniculata* Linn. Int J Pharma and Pharm Sci 2010; 2(1): 146-155.
16. Okoli, C.O., Akah, P.A., Onuoha, N.J., Okoye, T.C., Nwoye, A.C., Nworu, C.S., 2008. *Acanthus montanus*: An experimental evaluation of the antimicrobial, anti-inflammatory and immunological properties of a traditional remedy for furuncles. *BMC Complementary and Alternative Medicine*, Vol. 8, Issue 27, pp 1-11.
17. Chou, C.T., 1997. The anti-inflammatory effect of *Tripterygium wilfordii* Hook F on adjuvant-induced paw edema in rats and inflammatory mediators release. *Phytotherapy Research*, Vol. 11, Issue 2, pp. 152-154.
18. Rowman, R.M., 1996. The blood and bone marrow. In *Muir's Textbook of Pathology*. India, Thomas Press Ltd.

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