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In-Vitro Anti-inflammatory Activity of Methanolic Extract of *Convolvulus pluricaulis* Choisy

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ABSTRACT

Aim: *In-Vitro* Anti-inflammatory Activity of Methanolic Extract of *Convolvulus pluricaulis* Choisy.

Material & Methods- The whole plant parts of *Convolvulus pluricaulis* Choisy were purchased from the local market. 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 % methanol by soxhlet apparatus for 72 h. Qualitative chemical tests of methanolic extracts were subjected to various chemical tests to detect various phytoconstituents. Solvent systems ethyl acetate: methanol: water (77:13:10) were found to be most satisfactory solvent system. After development of plates, they were air-dried and number of spots, color and R_f values were recorded. The % heamolysis was calculated by assuming the heamolysis produced by the control group as 100 %.

Results: 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 71.59% protection of RBC haemolysis as compared with 72.73% produced by prednisolone. The methanolic extract of selected plant showed 39.70% inhibition. The Diclofenac sodium showed 55.88 % inhibition against denaturation of protein.

Conclusion: In conclusion, it can be stated that the methanolic extract has beneficial effects in long lasting in membrane stabilizing method, inhibition of protein denaturation method and proteinase model.

Keywords: *In-Vitro*, Anti-inflammatory Activity, Methanolic Extract, *Convolvulus pluricaulis* Choisy, Protein Denaturation Method

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 (Bannerman et al., 1983). Authentication and standardization are prerequisite steps while considering source materials for herbal formulation in any system of medicine (Ahmad et al., 2009). 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. (Kamboj, 2000). 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 (Raina, 2003). 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 (Kumar, 2011). The aim of our study is to evaluate the polar extract in various in-vitro models of inflammation.

Materials and Methods

Plant Materials

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

Authentification of plant materials

All the plant materials were taxonomically identified by Dr. S. K. Sharma, Senior Scientist, KNK College of Horticulture, ……. The herbarium sheets were submitted in Department of Pharmacognosy, Jaipur College of Pharmacy, Jaipur under voucher specimen no. JCP, Jaipur/2020/01-*Convolvulus pluricaulis* Choisy dated……..

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 % methanol by soxhlet apparatus for 72 h. The methanolic extracts were dried under the reduced pressure to get crude methanolic extracts. The Methanolic extracts were dried completely under reduced pressure. After drying, the respective extracts were weighed and percentage yield was determined (Mukherjee, 2002).

Preliminary phytochemical tests

Qualitative chemical tests of methanolic extracts were subjected to various chemical tests to detect various phytoconstituents (Kokate, 2003; Khandelwal, 2006).

Chromatographic study

Chromatographic separation techniques are multistate separation methods in which the components of an extracts are distributed between two phases, one of which is stationary, while the other is mobile. The stationary phase may be a solid or a liquid supported on a solid or a gel. The stationary phase may be packed in a column, spread as a layer, or distributed as a film, etc. The mobile phase may be gaseous or liquid or supercritical fluid. The separation process is based on adsorption or may be based on differences in the physico-chemical properties of the molecules such as size, mass, volume etc (Mukherjee, 2002).

Thin Layer Chromatography (TLC)

TLC for the separation of various bioactive compounds from methanolic extract was developed to find out the probable number of compounds present in them. On the pre coated TLC plate, test samples (after dissolving in respective solvents) were applied in the form of spots with the help of fine capillary. Spots were marked on the top of the plate for their identification. Rectangular glass chambers were used for chromatography. To avoid insufficient chamber saturation and undesirable edge effect, a smooth sheet of filter paper was placed in TLC chamber and was allowed to be in the developing solvent. A number of developing solvent systems were tried during the study. Each time plate was sprayed with Anisaldehyde sulphuric acid and vanillin sulphuric acid and heated at 115^0C for 5 minutes. The solvent system in which there is a satisfactory resolution was taken as a final solvent system. Solvent systems ethyl acetate: methanol: water (77:13:10) were found to be most satisfactory solvent system. After development of plates, they were air-dried and number of spots, color and R_f values were recorded (Mukherjee, 2002).

R_f value=Distance travelled by solute/Distance travelled by solvent

In-vitro anti-inflammatory activity

Membrane stabilizing activity of methanolic 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 (F1-F6) in concentration of 100-500 µg/ml were prepared using chloroform 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 (Vijayalakshmi *et al*., 2011; Vijayalakshmi *et al*., 2011).

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

Absorbance of control

Inhibition of protein denaturation method

The methanolic 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 methanolic 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 (Chandra et al., 2012; Sakat et al., 2010).

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

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

Proteinase inhibitory activity

The methanolic 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^{o} 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 (Sakat et al., 2010).

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

Results

Extractive value determination

Dried whole plant material of *Convolvulus pluricaulis* Choisy were extracted by methanol. The percentage yields of all dried extracts were determined by using the following formula.

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

Table 1: Different extracts with their appearance and % yield (in gm)

S. No.	Extracts	Color of dried extracts	Consistency of dried extracts	% Yield (W/W)
1	Methanolic extracts of *Convolvulus pluricaulis* Choisy	Dark Green	Sticky	21 %

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 2: 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

THIN LAYER CHROMATOGRAPHIC STUDIES OF BIOACTIVE EXTRACTS

TLC study has shown the presence of different components present in methanolic extract of *Convolvulus pluricaulis* Choisy when the extract were run in specific solvent system. Before reaching to most optimum solvent system a number of systems were employed.

TLC of methanolic extract of *Convolvulus pluricaulis* Choisy

Table 3: Summary of TLC

S No.	Extract	Solvent systems	Detecting reagents	Color	No. of spots	R_f value of Spots
1	Methanolic	Ethyl acetate: methanol: water (77:13:10)	Vanillin sulphric acid, heated at 110^0C for 5 Min.	Green & orange	6	0.23, 0.32, 0.50, 0.61, 0.72, 0.81

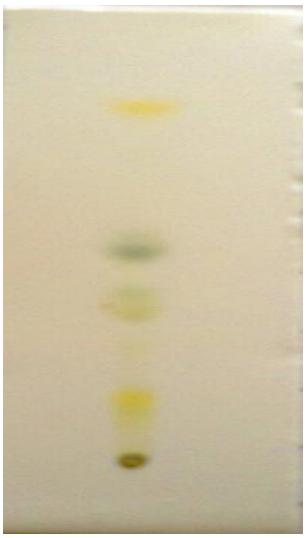

Figure 2: TLC of methanolic extract

Membrane Stabilizing Activity of Extracts on Rat Erythrocytes

The methanolic extract was analyzed by membrane stabilizing property at concentration range of 100-500 µg/ml. Methanolic extract significantly protect the rat erythrocyte membrane against lysis induced by hypotonic solution. At a concentration of 500 µg/ml, the extract produced 71.59% protection of RBC haemolysis as compared with 72.73% produced by prednisolone.

Table 3: Membrane stabilizing activity of extract at different concentration

S. No.	Fractions	Concentration (µg/ml)	% Protection
1	Control	----	100
2	Prednisolone	100 µg/ml	72.73
3	Methanolic Extract	100 µg/ml	54.54
		200 µg/ml	57.95
		300 µg/ml	65.90
		400 µg/ml	68.18
		500 µg/ml	71.59

Protein Denaturation Methods

The methanolic extract of selected plant showed 39.70% inhibition. The Diclofenac sodium showed 55.88 % inhibition against denaturation of protein. The results are summarized in Table No. 4.

Table 4: Effect of methanolic extract on Protein Denaturation.

S. No.	Treatment	Concentration µg/ml	% Inhibition
1	Control	----	---
2	Methanolic 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 methanolic extract of selected plant materials showed 41.50% inhibition respectively. The Diclofenac sodium showed 62.26% inhibition against proteinase inhibitory activity. The results are summarized in Table No. 5.

Table 5: Effect of methanolic extract on proteinase inhibitory activity.

S. No.	Treatment	Concentration µg/ml	% Inhibition
1	Control	----	-------
2	Methanolic 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	62.26

Discussion

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of diseases, although relatively modest acquaintance about their mode of action is existing. 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 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.

Bioactive extract was then tested chemically to know the presence of different chemical constituents. TLC studies were also performed to know the number of constituents present in both the fractions and to establish finger print profile. In the present investigation, phytochemical screening showed the presence of steroids, terpenoids, tannins, flavonoids, glycosides in methanolic extract. TLC findings were in agreement with the data of qualitative chemical tests and the spots characteristic for steroids, terpenoids and flavonoids were observed.

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 (Umukoro *et al*., 2006; Aitadafoun *et al*., 1996). Stabilization of the membranes of these cells inhibits lysis and subsequent release of the cytoplasmic contents which in turn limits the tissue damage and exacerbation of the inflammatory response (Okoli et al., 2008). It is therefore expected that compounds with membrane stabilization activity should offer significant protection of cell membrane against injurious substances. Exposure of red blood cell to ruinous substances such as hypotonic medium and phenyl hydrazine results in lysis of its membrane accompanied by haemolysis and oxidation of hemoglobin (Augusto *et al*., 1982; Ferrali *et al*., 1992). The hemolytic effect of hypotonic solution is associated to excessive accumulation of fluid inside the cell, consequential in the rupturing of its membrane and they are also sensitive to damage through free radicals induced by lipid peroxidation.

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. Methanolic 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 (Aitadafoun *et al*., 1996). Since our fractions also inhibit the lysis of rat erythrocyte at a primary step of inflammation. The probable mechanism of action might be prevention of phospholipases release that acts as an inflammatory mediator.

The arthritic disease progression was correlated with the fragility of the lysosomal membranes, denaturation of proteins & release of inflammatory mediators. Denaturation of proteins is a well known cause of inflammation. The majority of biological proteins lose their biological functions when denatured. In vivo denaturation of proteins causes production of auto-antigens in arthritic disorders (Brown & Mackey 1968). Agents that can prevent protein denaturation therefore would be worthwhile for anti-arthritic activity.

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. Previous researchers reported that, leukocytes proteinase play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors (Das et al., 1995; Hiemstra et al 2002). In the present investigation, the ability of methanolic extract to inhibit protein denaturation as well as protienase inhibitory were studied. The methanolic 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 methanolic extract as anti-inflammatory activity.

Conclusion

In conclusion, it can be stated that the methanolic 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.

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. Brown JH, Mackey HK. Inhibition of heat-induced denaturation of serum proteins by mixtures of nonsteroidal anti-inflammatory agents and amino acids. Proc Soc Exp Biol Med 1968; 128:225.
7. 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.
8. Das SN and Chatterjee S. Long term toxicity study of ART-400. Indian Indg Med 1995; 16(2):117-123.
9. Hiemstra PS. Novel roles of protease inhibitors in infection and inflammation. Biochemical Society Transactions 2002; 30(2):116-120
10. 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.
11. Aitadafoun, M., Mounieri, C., Heyman, S.F., Binitisc, C., Bon, C., 1996. 4-alkoxy benzamides as new potent phospholipase A2 inhibitors. *Biochemical Pharmacology*, Vol. 51, Issue 6, pp. 737-742.
12. Augusto, O., Kunze, K.L., Montellano, P.R., 1982. N-phenylprotoporphyrin formation in the haemogolobin phenylhydrazine reaction. *The*

Journal of Biological Chemistry, Vol. 257, pp. 6231-6241.
13. Ferrali, M., Signorni, C., Ciccoli, L., Comporti, M., 1992. Iron release and membrane damage in erythrocytes exposed to oxidizing agents, phenylhydrazine, divicine and isouramil. *Biochemical Journal*, Vol. 285, Issue 1, pp. 295-301.
14. Khandelwal, K.R., 2006. *Practical Pharmacognosy*. Pune, Nirali Prakashan.
15. Kokate, C.K., 1996, *Practical Pharmacognosy*. Delhi, Vallabh Prakashan.
16. ukherjee, P.K., 2002. *Quality Control of Herbal Drugs-an Approach to Evaluation of Botanicals*. New Delhi, Business Horizons Pharmaceutical Publishers.
17. Ooli, 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.
18. Umukoro, S., Ashorobi, R.B., 2006. Evaluation of the anti-inflammatory and membrane stabilizing effect of *Eupatorium odoratum*. *International Journal of Pharmacology*, Vol. 2, Issue 5, pp. 509-512.
19. 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.
20. 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.