



Original Research Article

***In vitro* anthelmintic and anti-inflammatory activity of ethanolic extract of *Randia uliginosa* DC Leaf**

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A B S T R A C T

Ethanolic extract of *Randia uliginosa* DC Leaf (EERUL) was assessed for its anthelmintic and anti-inflammatory activity by *in vitro* methods. Anthelmintic activity was performed on adult Indian earthworms, *Pheretima posthuma* due to its anatomical and physiological resemblance with that of intestinal round worm parasite of human beings, the bioassay involved determination of time of paralysis and time of death of the worms. Albendazole was used as standard reference. EERUL showed significant activity at the concentration of 100mg/ml with 44.50 and 58 minutes for paralysis and death respectively. *In vitro* anti-inflammatory activity was evaluated using albumin denaturation assay, human red blood cell membrane stabilization assay and proteinase inhibitory activity at different concentrations. Diclofenac Sodium was used as a standard reference drug. The extract showed *in vitro* anti-inflammatory activity by inhibiting the heat induced albumin denaturation, the percentage of inhibition was found to be 48- 85% EERUL and for the standard it was 47- 87% at the concentration range of 100 to 600 µg/ml. The EERUL and the standard showed maximum human red blood cells membrane stabilization of 67% and 78% at the concentration of 600 µg/ml respectively. Proteinase activity was also significantly inhibited by the EERUL it exhibited significant antiproteinase activity. It showed maximum inhibition of 66% at 600µg/ml and that of standard was found to be 73% at the same concentration. From the results, it can be concluded that the anthelmintic activity and anti-inflammatory activity of EERUL may be due to the presences of secondary metabolites in the extract

Keywords

Pheretima posthuma, NSAIDS, HRBC, albumin denaturation, Proteinase

Introduction

The medicinal value of plants has been documented in almost all ancient civilizations. As plants are the store house and natural source of drugs, most of the present drugs are derived directly or

indirectly from these botanicals. Each part of the plant like leaves, stem, flowers, fruits, bark, roots and seeds are known to have various medicinal properties. These plant based systems continue to play an essential

role in health care, and it has been estimated by the World Health Organization that approximately 80% of the world population still depends mainly on traditional medicines for their primary health care (Farnsworth et al. 1985). The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment and would overcome the resistance produced by the pathogens as they exist in a combined form or in a pooled form of more than one molecule in the protoplasm of the plant cell (Lai and Roy, 2004; Tapsell *et al.*, 2006)

Helminthiasis is a disease in which a part of the body is infested with worms such as pinworm, roundworm or tapeworm. These worms inhabit in the gastrointestinal tract and may also burrow into the liver and other organs. Infected people excrete helminth eggs in their feces, which then contaminate the soil in areas with inadequate sanitation (Idika, *et al.*, 2012). Other people can then be infected by ingesting eggs or larvae in contaminated food, or through penetration of the skin by infective larvae in the soil (hookworms). Helminthic infections are one of the most widespread infections in humans, affecting a huge population of the world, these infections cause enormous hazard to health and resulting in undernourishment, anaemia, eosinophilia and pneumonia. As per the reports of WHO most of the drugs used against these worms are synthetic but these synthetic drugs are out of reach of millions of people and have a lot of side effect. Inflammation is the response of living tissues to injury, infection or irritation during which lysosomal enzymes are released which produces many disorders which resulting in tissue injury by damaging the macromolecules and lipid peroxidation of membranes which are believed to be responsible for some of the pathological conditions such as heart

attacks, septic shocks and rheumatoid arthritis etc. The extra cellular activity of these enzymes is said to be related to acute or chronic inflammation. Stabilization of lysosomal membrane is important in limiting the inflammatory response by inhibiting the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release. The currently available drugs like opioids and non –steroidal anti-inflammatory drugs (NSAIDs) are not useful in all cases of inflammatory disorders, because of their side effects and potency (Ahmadiani *et al.*, 1998). As a result, a search for other alternatives seems necessary and beneficial. The study of botanicals which are used traditionally to cure inflammation is still fruitful and logical research strategy in the source of new anti-inflammatory drugs (Kumarappan *et al.*, 2006).

Phytochemicals from medicinal plants showing anti-inflammatory activities have the potential of filling this need because of structures that are different from those of the more studied and their mode action may also differ (Fabricant and Fansworth, 2001). In this growing interest, many of the phytochemical bioactive compounds from medicinal plants have shown many pharmacological activities (Prachayasittikul et al., 2008; Chen et al., 2008; Pesewu et al., 2008; Turker and Usta, 2008). Our earlier studies on ethanolic extract of *Randia uliginosa* DC Leaf has proved its potential as antimicrobial and antioxidant and the phytochemical analysis of the extract showed the presence of important phytoconstituents (Gulnaz.A.R *et al* 2014). Lay publications have documented the anthelmintic and anti-inflammatory activity of this plant some of the researchers have proved the anti-inflammatory activity of the whole plant of *Randia uliginosa*

(Sandhyarani *et al*, 2014) but there are no reports on anthelmintic and anti-inflammatory activities of *Randia uliginosa* DC Leaf. Hence in the present study an attempt was made to investigate anthelmintic and anti-inflammatory activities of ethanolic extract of *Randia uliginosa* DC Leaf (EERUL) to provide scientific validation to this plant.

Materials and Methods

Anthelmintic Activity:

In vitro anthelmintic activity of EERUL at various concentrations was carried against *Pheretima posthuma* by bioassay which involved the determination of time of paralysis and time of death of the worms. Albendazole was used as standard reference. The assay was performed on adult Indian earthworms, *Pheretima posthuma* due to its anatomical and physiological resemblance with that of intestinal round worm parasite of human beings (Suresh *et al*, 2001; Vidyarthi, 2011; Chatterjee, 1967) and also because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds *in vitro* (Das *et al*, 2002; Shivkar and Kumar, 2003). The earthworms were collected, from moist soil and washed with normal saline to remove all fecal matter and were used for the anthelmintic study. The earthworms of 5-7 cm in length were used for all experimental protocol. The anthelmintic assay was carried as per the method of (Gulnaz & Savitha, 2013) with slight modifications. Briefly test samples of the extract was prepared at the concentration of 5, 25, 50 and 100 mg/ml in Tween 20 (1%) solution. Four groups of *Pheretima posthuma* (consisting of three earth worms each in triplicate) were released in to 30 ml of experimental formulation, first group serves as normal control which is treated

only with normal saline, second group is treated with Tween-20 along with normal saline, which serves as negative control, group three receives standard drug Albendazole at a concentration of 5mg/ml and it serves as standard, group four was treated with different concentrations of ethanolic extract of EERUL. All the test solutions and standard solutions were prepared freshly before starting the experiment. The mean time for paralysis was noted when no movement of any sort could be observed, except when the worm was shaken vigorously. The time death of worm (min) was recorded after ascertaining that worms neither moved when shaken nor when given external stimuli by putting motionless worms in 50^oC warm water. No movement of worms confirms death (Dey *et al.*, 2012). Death was concluded when the worms lost their motility followed with white secretion and fading away of their body colors (Karale *et al.*, 2010).

Anti-inflammatory activity

The human red blood cell (HRBC)

Membrane stabilization method: It was carried out as per the method of Sadique, *et al* (1989), with slight modification in brief, fresh whole human blood (10 ml) was collected from the volunteers who had not taken any anti-inflammatory drugs for 2 weeks prior to the experimental plan and transferred to the heparinized centrifuged tubes. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline and reconstituted as 10% v/v suspension with normal saline. The test sample consisting of stock erythrocyte (RBC) suspension of 0.03ml and 0.02ml of extract/ standard drug, Diclofenac sodium, of different concentration 100-600 µg/ml is mixed with 5ml of hypotonic saline. The control sample

was 0.03 ml RBC suspension mixed with hypotonic buffered solution alone. The mixtures were incubated at 10 minutes at room temperature, centrifuged for 10 minutes at 3000rpm and absorbance of the supernatant was measured spectrophotometrically at 540 nm. The experiment was carried out in triplicate and the percentage inhibition of membrane stabilization was calculated (Sadique *et al*, 1989)

Effect on Protein denaturation: Test solution consisting of 1ml of different concentrations of extract/ standard drug, Diclofenac sodium of different concentration 100-600 µg/ml was mixed with 1ml of egg albumin solution (1mM) and incubated at 27 ±1°C for 15 minutes. Denaturation was induced by keeping the reaction mixture at 60°C in a water bath for 10 minutes. After cooling the turbidity was measured spectrophotometrically at 660 nm (Shinde, *et al*, 1999).

Proteinase inhibitory action: The assay was carried out as per the method of (Oyedepo and Femurewa, 1995), with slight modification. In brief, the reaction mixture (2 ml) was containing 0.06 mg Proteinase, 1 ml 20 mM Tris HCl buffer (pH 7.4) and 1 ml test sample/ standard drug, Diclofenac sodium, of different concentration 100-600 µg/ml. The mixture was incubated at 37°C for 5 min and then 1 ml of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 min. 2 ml of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank.

Statistical analysis

The result were express as Mean ± SEM. Statistical analysis was carried out using one

way ANOVA followed by Dunnett's multiple comparison tests

Results and Discussion

The results of anthelmintic activity of EERUL and the standard, Albendazole, are presented in Table 1. Anthelmintic activity was found to be directly proportional to its concentration. Significant activity was seen at the concentration of 100mg/ml with 44.50 and 58 minutes for paralysis and death respectively. The results of membrane stabilization, albumin denaturation and proteinase inhibitory activity of EERUL and Diclofenac sodium are shown in Figure 1.

HRBC Membrane stabilization

Membrane lysis is taken as a measure of *in vitro* anti-inflammatory activity. *In vitro* anti-inflammatory activity of the EERUL were concentration dependent, the maximum protection of 67% was seen at the concentration of 600µg/ ml. All results were compared with standard Diclofenac Sodium which showed 78% protection at the concentration of 600 µg/ ml

Albumin denaturation

The *in vitro* anti-inflammatory effect of EERUL was evaluated against denaturation of egg albumin. The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by EERUL. The percentage of inhibition was found to be 48-85% EERUL and for the standard it was 47 -87% throughout the concentration range of 100 to 600 µg/ml.

Proteinase inhibitory activity

Proteinases have been implicated in arthritic reactions. Lysosomal granules of Neutrophils are known to be a rich source of Proteinase. EERUL exhibited significant antiproteinase activity. It showed maximum

inhibition of 66% at 600µg/ml and that of standard was found to be 73% at the concentration of 600µg/ml.

Tannins are chemically polyphenolic compounds which has anthelmintic activities as they can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and may cause death (Athnasiadou et al; 2001; Waller et al.,1997). They are also known to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation or, binds to the glycoprotein on the cuticle of parasite and cause death. The biochemical structure of nematode surface shows collagen rich extracellular matrix (ECM) in the form of cuticle which forms exoskeleton, and it is critical for viability, The mammalian skin is also made up of, extensively, collagen in the form of fibrous bundles. In leather making industry, vegetable tannins are commonly used in the tanning operation of leather processing that imparts stability to collagen of skin matrix through its reactivity and hence make the collagen molecule aggregate into fibres. This results in the loss of flexibility in the collagen matrix and gain of mechanical property with improved resistance to the thermal (or) microbial/enzymatic attack. Similar kind of reaction is expected to take place between the nematode cuticle (the earth worm), possibly by linking through hydrogen bonding, as proposed in this study. This form of reactivity brings toughness in the skin and hence the worms become immobile and non-functional leading to paralysis followed by death. (Gulnaz and Savitha, 2013). Albendazole acts by binding to the parasite's β -tubulin, inhibiting its polymerization and

impairing glucose uptake and depletion of glycogen stores in test parasite causing death (Ke Min Chen and Shih-Chan Lai, 2007). EERUL exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane (Chou, 1997) and its stabilization indicates that the extract may also well stabilize lysosomal membranes. Stabilization of lysosomal membrane is crucial point in limiting the inflammatory response via inhibiting the release of lysosomal constituents of activated neutrophil. Some of the NSAIDs are known to have membrane stabilization properties which may be contributing to their anti-inflammatory property. Denaturation of proteins is responsible for the cause of inflammation in conditions like rheumatoid arthritis hence by, prevention of protein denaturation may also help in preventing inflammatory conditions. NSAIDS acts in similar way in preventing inflammation (Mizushima *et al.*, 1964). Proteinases are involved in arthritic reactions. The main source of proteinase is the lysosomal granules of neutrophils. Earlier it was reported that leukocytes proteinase play important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors (Das and Chatterjee, 1995). Recent studies have proved that many flavonoids and related polyphenols contributed significantly to the anti-inflammatory activities of many plants (Luo et al., 2002; Okoli and Akah, 2004). Hence, the presence of tannins and flavonoids in EERUL may be contributing for its, anthelmintic, and anti inflammatory activity

Fig 1.

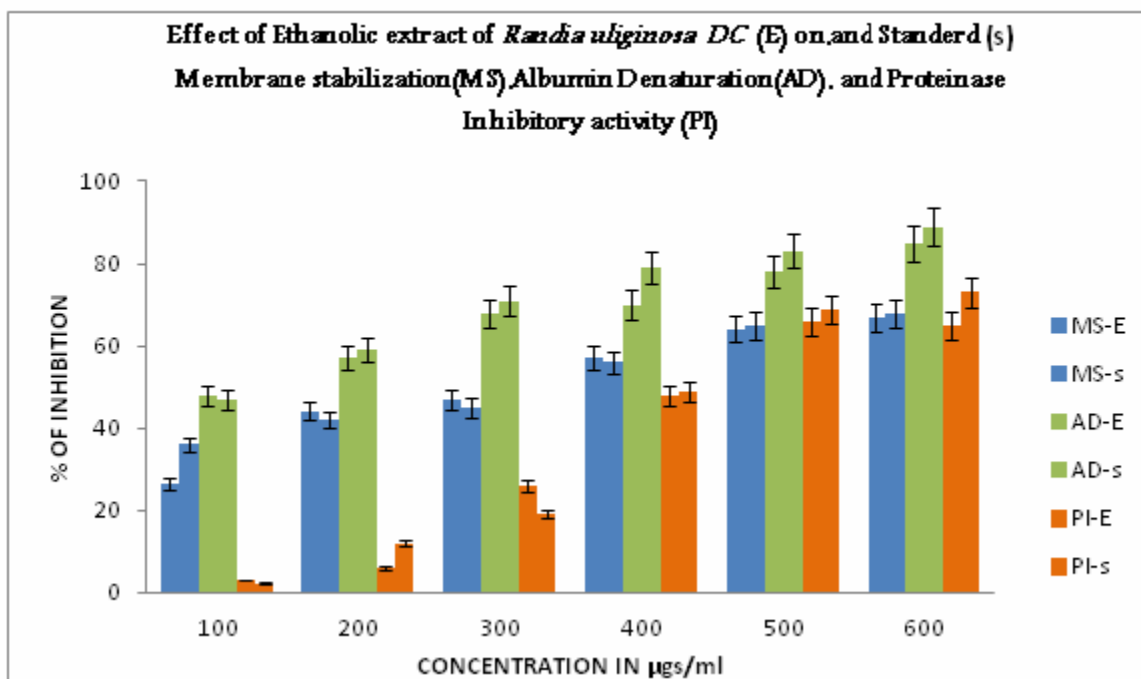


Table.1 Anthelmintic activity of the ethanolic extract of *Randia uliginosa DC* Leaf

S.No	Group	Extract	Dose(mg/ml)	Response	
				Time taken for paralysis (min)	Time taken for death (min)
1	I	Normal control	-----	----	-----
2	II	Negative control	-----	-----	-----
3	III	Standard (Albendazole)	5	33.2±0.006	50.8 ± 0.07
4	IV a	Ethanol	5	71.3 0 ± 0.001	90.70 ± 0.009
	IV b		25	65.38 ± 0.002	80.50 ± 0.001
	IV c		50	45.6 0 ± 0.003	55.54 ± 0.008
	IV d		100	44.50 ± 0.00	58.00 ± 0.001

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References

Ahmadiani, A., Fereidoni, M., Semnianian, S., Kamalinejad, and Saremi, S. 1998. Antinociceptive and anti-

- inflammatory effects of *Sambucus ebulus* rhizome extract in rats. J. Ethnopharm. 61, 229-232.
- Anthnasiadou, S., Kyriazakis, F., Jackson, R. L. 2001. Direct Anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: *in vitro* and *in vivo* studies. Vet. Parasitol. 99, 19.
- Chatterjee, K. D., Parasitology, Protozoology and Helminthology. 1967. In Guha Ray Sree Saraswaty Press Ltd, Calcutta, 6th ed, 196
- Chen, I. N., Chang, C. C., Wang, C. Y., Shyu, Y. T. and Chang, T. L. 2008. Antioxidant and antimicrobial activity of *Zingiberaceae* plants in Taiwan. Plant Food Hum Nutr. 63, 15-20
- Chou, C.T., 1997. The anti-inflammatory effect of *Tripterygium wilfordii* Hook F on adjuvant-induced paw edema in rats and inflammatory mediators release. Phytother Res. 11, 152-154.
- Dash, G. K., Suresh, P., Kar, D. M., Ganpaty S., Panda S. B. Evaluation of *Evolvulus alsinoids* Linn. for anthelmintic and antimicrobial activities. J. Nat. Rem., 2002, 2: 182-5.
- Das, S. N. and Chatterjee, S. Long term toxicity study of ART-400. 1995. Indian. J. Indg. Med. 16, 117-123
- Dey, P., Debnath, P. and Bhakta, T, 2012. Evaluation Of Anthelmintic Activity Of *Molineria Recurvata* Leaf Extracts. Int. Res J Pharm. App Sci. 2, 16-20.
- Fabricant, D. S., Fansworth, N. R., 2001. The value of plants used in traditional medicine for drug discovery. Environ Health Persp. 109, 69-75.
- Farnsworth, N. R., O. Akerele, A. S. Bingel, D. D. Soejarto, and Z.-G. Guo. 1985. Medicinal plants in therapy. Bull. WHO 63, 965-981
- Gulnaz, A. R. and Savitha, G. 2013. Evaluation of Anthelmintic Activity of Different Leaf and Stem extract of *Sida cordata* Burma (brossa) burm.F. Int. J. Curr. Microbiol. App. Sci. 2, 246- 255
- Gulnaz. A. R, Moutawe. A .M. A. E. and Chauhan J. B. 2014. Antimicrobial, antioxidant activities and phytochemical analysis of ethanolic extract of *Randia uliginosa* leaves. Int. J. Curr. Microbiol. App. Sci. 3,
- Idika, I. K., Okonkwo, E. A., Onah, D. N., Ezeh, I. O., Iheagwam, C. N and Nwosu, C. O. 2012. Efficacy of levamisole and ivermectin in the control of bovine parasitic gastroenteritis in the sub-humid savanna zone of southeastern Nigeria. Parasitol. Res. 111, 1683- 7
- Lai, P. K. and Roy, J. 2004. Antimicrobial and chemopreventive properties of herbs and spices. Curr. Med. Chem. 11, 1451-1460.
- Luo, X. D., Basile, M. J. and Kennelly, E. J. 2002. Polyphenolic antioxidants from the fruits of *Chrysophyllum cainito* L.. (star apple). J. Agri. Food Chem. 50, 1379-1382.
- Karale, S. S., Godad, A. P., Yashodhan, B. W. and Suhas, A. S, 2010. Evaluation of *in vitro* anthelmintic activity of *Ceratophyllum demersum* Linn. Indian Drugs. 47, 63-65.
- Ke-Min Chen and Shih-Chan Lai. 2007. Biochemical and pathological evaluation of albendazole/thalidomide co-therapy against eosinophilic meningitis or meningoencephalitis induced by *Angiostrongylus cantonensis*, J. Antimicrob. Chemother. 59, 264-276