



In-vitro Anti-Inflammatory activity of *Digera muricata* Extracts

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*Digera muricata***ABSTRACT**

Inflammation is a process that is generally termed for pain and redness, which includes swelling. But it is defined as a heterogeneous group of processes which result in the pain and increase in vascular circulation and denaturation of protein that results in tissue degeneration. There are many synthetic drugs available to treat and relieve the inflammation. They are potent and have many side effects too. So there is an increased search for alternative means of treatment option. Thereby there was a high focus on the medicinal plants and herbs. *Digera muricata*, which is used to treat many diseases like analgesic activity, antidiabetic activity and anticancer activity too, this plant has proven to possess the antioxidant potential also. So there is no enough work done to verify the inflammatory potential of the plant. This was the primary consideration for this research to select the plant to prove it has anti-inflammatory potential. Having in the account that the ethical problems in the usage of lab animals, the anti-inflammatory potential of the plant was evaluated *in-vitro* using enzyme inhibition assays. The *in-vitro* models of the inflammatory activity are used for testing the anti-inflammatory activity, and the results were not different from *in-vivo* models. The extracts showed a dose based rise in the action, and the plant process a better anti-inflammatory activity than the standard drugs.

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INTRODUCTION

Inflammation is a process that is generally termed for pain and redness, which includes swelling. But

it is defined as a heterogeneous group of processes which result in the pain and increase in vascular circulation and denaturation of protein that results in tissue degeneration. This inflammation may be due to micro-organisms, physiological stress, oxidative stress etc. and result in pain, swelling and redness. There may be seen as an inability to do the normal functioning of the body and its organs (Jain and Bari, 2010). When there is an inflammation in the body, tissue releases cytokines and Pg's and histamines which are called as pain mediators. This will increase blood flow to the site of the injury, which results in the production of chemotaxis. Therefore, tissue repair starts to work (Koster *et al.*, 1959).

There are many synthetic drugs available to treat and relieve the inflammation. They are potent and

Table 1: Anti-inflammatory activity invitro of *Digera muricata*

Treatment	Protein Denaturation Assay		Proteinase inhibition Assay		Lipoxygenase inhibiting Assay	
	Absorbance at 660nm	% Activity	Absorbance at 210nm	% Activity	Absorbance at 234nm	% Activity
Control	0.40±0.07	-	0.39±0.11	-	0.42±0.04	-
DME 100µg/ml	0.28±0.04**	33.12	0.32±0.07**	22.48	0.38±0.05**	10.23
DME 100µg/ml	0.22±0.03**	48.36	0.29±0.02**	28.01	0.34±0.07**	18.41
DME 100µg/ml	0.18±0.05**	58.74	0.26±0.09*	37.39	0.32±0.03**	27.65
DME 100µg/ml	0.15±0.03**	66.58	0.24±0.03**	43.54	0.23±0.08**	49.74
DME 100µg/ml	0.13±0.09**	72.91	0.20±0.05**	54.63	0.17±0.04**	62.86
Standard	0.14±0.02**	69.25	0.19±0.03**	56.82	0.08±0.06**	87.09

Table 2: Anti-inflammatory activity invitro of *Digera muricata*

Treatment	Heat induced hemolysis		Hypotonicity induced Heamolysis	
	Absorbance	% Activity	Absorbance	% Activity
Control	0.32±0.05	-	0.33±0.04	-
DME 100µg/ml	0.26±0.10**	22.67	0.24±0.03**	31.77
DME 100µg/ml	0.23±0.09**	31.24	0.18±0.11**	49.90
DME 100µg/ml	0.21±0.07**	37.51	0.16±0.06**	55.62
DME 100µg/ml	0.19±0.02*	44.73	0.13±0.09**	62.45
DME 100µg/ml	0.16±0.05*	52.06	0.08±0.04**	76.84
Standard	0.11±0.08**	72.92	0.17±0.02**	52.03

have many side effects too. So there is an increased search for alternative means of treatment option. Thereby there was a high focus on the medicinal plants and herbs. (Shiddamallayya *et al.*, 2010). They are serving humans as an alternative treatment option in many diseases like diabetes, hypertension, CV disorders etc. Many of those plants were used to treat inflammations also (Soudahmini *et al.*, 2005).

One of those plants is *Digera muricata*, which is used to treat many diseases like analgesic activity, antidiabetic activity and anticancer activity too (Koster *et al.*, 1959). This plant had been proven to possess the antioxidant potential also. So there is no enough work done to verify the inflammatory potential of the plant. This was the primary consideration for this research to select the plant to prove it

has anti-inflammatory potential. There were many folklore claims of the plant had used to treat many other diseases like diarrhoea, and it as has a cooling effect on the body. Having in the account that the ethical problems in the usage of lab animals, the anti-inflammatory potential of the plant was evaluated *in-vitro* using enzyme inhibition assays.

METHODS

Extraction

The aerial plant parts were collected in November and dried under shade and then ground into a fine powder with a blender. This powder is stored in room temperature for a day to use for further extraction. This powder was used for extraction using methanol using Soxhlet extractor, and the filtrate is

reduced to get the desired consistency of extract, which is like paste consistency using an evaporator and stored the extract in a desiccator. The plant extract was dissolved in distilled water, that the concentration of extracts was maintained at 100, 200, 300, 400 and 500 microgram/ml. This was used for further Assay.

Activity

The anti-inflammatory activity of the plant was assayed *invitro* using enzyme analysis as follows

Protein denaturation inhibition assay

In this method, the heat was used to denaturate the protein as per the method described by Mizushima and Kobayashi (1968). The UV absorption was measured for all the concentrations at 660nm.

Antagonizing proteinase

This method was adopted to test the activity according to the method described by Oyedepo and Femurewa (1995). The UV absorption was measured at 210nm. The percentage inhibition and absorbance were noted according to the method.

Lipoxygenase inhibiting activity

In this method, the extracts were tested from anti-inflammatory activity in different concentrations as planned above. The process was followed as per the procedure by Shinde *et al.* (1999). Lipoxidase enzyme was used as a marker for the inflammatory mediator, and the activity was measured in UV at 234 nm. The percentage of inhibition and absorbance was noted and tabulated.

Membrane stabilization assay

In this method, RBC cells are used to study the stabilization of membrane of cells as there is a similarity of membranes of RBC and cytokines. In this method, a healthy human volunteer was selected who did not use any NSAID in near history for seven days, and his blood sample was withdrawn and centrifuged to separate RBC. The cells were collected and suspended in saline solution.

In this method, hemolysis was measured in two ways.

Haemolysis induced by heating.

The method was adopted to estimate the amount of anti-inflammatory activity by preventing the haemolysis of the RBC *invitro*, which is induced by heat according to the process detailed by Sakat *et al.* (2010). The standard drug used in this method is aspirin.

Haemolysis induced by hypotonicity.

In this method, the extracts were tested by different concentrations, and the haemolysis was

induced using a hypotonic saline solution. The technique was adopted as given by procured by Azeem *et al.* (2010). The percentage inhibition was measured and compared with the standard, Diclofenac sodium.

RESULTS AND DISCUSSION

The anti-inflammatory activity of the plant was investigated *in-vitro* using various models, and the results were tabulated in Table 1 & Table 2. In protein denaturation assay, the extracts showed better activity than the standard drug, aspirin with almost 3-4 per cent better activity. During the inflammation process, the denaturation of protein occurs by the application of heat. It is the most notable causes of inflammation, and the extract showed an increase in activity in a dose based manner (Kim *et al.*, 2004).

In the lipoxygenase method, the extracts at different doses showed a dose-dependent increase in the activity so at 500microgm extracts showed the highest activity, which is less than that of the standard. Lipoxygenase induces the oxidative free radicals and initiates the stress-based inflammation in the body. The extracts, when successfully inhibited the enzymes, means the antioxidant activity of the extract was an added advantage (Sokol *et al.*, 2008).

Proteinase is an enzyme responsible for the inflammation mediation at the lysosomal level. Neutrophils had rich content of the enzyme. Therefore the enzyme inhibition is said to lower the inflammation via the mechanism of the enzyme. The extracts a better activity compared to the standard drug Table 1. The antioxidant activity of the extract might have added advantage in the anti-inflammatory activity in this Assay.

As discussed earlier, membrane stabilization assay was used on RBC as there is a similarity between the lysosome membrane and the RBC. These lysosome breakdowns and releases corrosive enzymes that digest the tissue and cell, which leads to inflammation. The extracts were used to prevent the breakage of the membrane and to stabilize it. So RBC hemolysis was assayed in two methods. In the heat-induced method and hypotonicity method, there was a significant reduction in membrane breakage. The activity was similar and higher than the standard drugs, Aspirin and Diclofenac sodium Table 2 (Alhakmani *et al.*, 2013).

CONCLUSION

The *in-vitro* models of the inflammatory activity are used for testing the anti-inflammatory action, and the results were not different from *invivo* models.

The extracts showed a dose based rise in the activity, and the plant possess a better anti-inflammatory activity than the standard drugs.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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REFERENCES

- Alhakmani, F., Kumar, S., Khan, S. A. 2013. Estimation of total phenolic content, in-vitro antioxidant and anti-inflammatory activity of flowers of *Moringa oleifera*. *Asian Pacific Journal of Tropical Biomedicine*, 3(8):623-627.
- Azeem, A. K., Dilip, C., Prasanth, S. S., Shahima, V. J. H., Sajeev, K., Naseera, C. 2010. Anti-inflammatory activity of the glandular extracts of *Thunus alalunga*. *Asian Pacific Journal of Tropical Medicine*, 3(10):794-796.
- Jain, P. S., Bari, S. B. 2010. Anti-inflammatory Activity of *Abelmoschus manihot* Extracts. *International Journal of Pharmacology*, 6(4):505-509.
- Kim, H. P., Son, K. H., Chang, H. W., Kang, S. S. 2004. Anti-inflammatory plant flavonoids and cellular action mechanisms. *Journal of Pharmacological Sciences*, 96(3):229-245.
- Koster, R., Anderson, M., Beer, E. J. 1959. Acetic acid for analgesic screening. *Federation Proceedings*, 18:412-417.
- Mizushima, Y., Kobayashi, M. 1968. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. *Journal of Pharmacy and Pharmacology*, 20(3):169-173.
- Oyedepo, O. O., Femurewa, A. J. 1995. Anti-protease and membrane stabilizing activities of extracts of *Fagra zanthoxiloides*, *Oxalis subscorpioides* and *Tetrapleura tetraptera*. *International Journal of Pharmacognosy*, 33(1):65-69.
- Sakat, S., Juvekar, A. R., Gambhire, M. N. 2010. In vitro antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. *International Journal of Pharmacy and Pharmacological Sciences*, 2(1):146-155.
- Shiddamallayya, N., Yasmeen, A., Gopakumar, K. 2010. Medico-botanical survey of kumar parvatha kukke subramanya. *Indian Journal of Traditional Knowledge*, 9(1):96-99.
- Shinde, U. A., Kulkarni, K. R., Phadke, A. S., Nair, M. N., Dikshit, V. J., Mungantiwar, M. N., Saraf 1999. Mast cell stabilizing and lipoxygenase inhibitory activity of *Cedrus deodara* (Roxb.) Loud. Wood Oil. *Indian Journal of Experimental Biology*, 37(3):258-261.
- Sokol, H., Pigneur, B., Watterlot, L., Lakhdari, O., Bermudez-Humaran, L. G., Gratadoux, J. J., Blugeon, S., Bridonneau, C., Furet, J. P., Corthier, G., Grangette, C., Vasquez, N., Pochart, P., Trugnan, G., Thomas, G., Blottiere, H. M., Dore, J., Marteau, P., Seksik, P., Langella, P. 2008. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proceedings of the National Academy of Sciences*, 105(43):16731-16736.
- Soudahmini, E., Ganesh, M., Senthil, Panayappa, Madhu, C., Divakar 2005. Herbal remedies of Madugga tribes of Siruvani forest, South India. *Natural Product Radiance*, 4(6):492-501.