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EXPLORING THE ANTI HYLOURINIDASE ACTIVITY OF *GMELINA ARBOREA* USING IN VITRO METHOD

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ABSTRACT

Gmelina arborea is an important medicament in many anti arthritic drugs in ayurvedic system. Arthritis is complex disease which not only affects the joints but also affect the quality of life of patients. Clinical data revealed that increase levels of hyaluronidase contribute significantly to cartilage degradation. We evaluated the cartilage protective action of the plant extract (hydro alcoholic extract *Gmelina arborea*) by examining its effects on the activities of pure hyaluronidase. A simple spectrophotometric method was used to assay Hyaluronidase activity and to detect the potential of extracts. Hydro-alcoholic extracts of *Gmelina arborea* root and bark extract showed strong inhibition of hyaluronidase activity.

Keywords: *Gmelina arborea*, Arthritis, Hyaluronidase

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INTRODUCTION

Arthritis is a common disease of the joints, particularly the large joints. The disease is associated with pain, stiffness, swelling, and loss of physical function of the joint. In India millions of people suffering from the disease. Different forms of arthritis have distinctive symptoms, prognoses, and treatments[1] (**Colburn N, 2012**). Osteoarthritis is the most common form of arthritis, affecting approximately 70– 80% of the population over 50 years of age. Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disease predominantly affecting the joints and periarticular tissue. RA still remains a formidable disease, being capable of producing severe crippling deformities and functional disabilities and cartilage destruction and commonly leads to significant disability, caused by no. of pro inflammatory molecules released by macrophages including reactive oxygen species and ecosanoids such as prostaglandins, leukotrines and cytokines[2] (**Ebringer A, 2012**).

Ayurveda has proved its efficacy in life of humans since ancient Vedic civilization of India. The era of ayurveda and the use of medicinal plants in various disease way back somewhere between 2500- 500 BC[3] (**Samy PR, et al., 2013**). The term ayurveda comes from Sanskrit, two different words "Ayus" meaning life and "Veda" meaning knowledge. Hence, ayurveda is "science of life" that focuses on man and cure of his illness using the knowledge of medicinal plants[4] (**Micozzi MS, 2nd Edition**). The Use of various parts of the plant as well as plant products (phytochemicals), ayurveda works on the principle of maintaining a balance in immune system through the process of immuno-modulation. Till date many plants have been shown to possess significant immuno-modulatory role thus proving to be a potential tool in drug discovery and drug development. The plant, as one of the important sources, still maintains its

original place in the treatment of various diseases, including arthritis, with minimum side effects.

MATERIAL AND METHODS

Bovine testicular hyaluronidase, Hyaluronic acids were obtained from sigma Aldrich, India. Hyaluronidase assays were performed in sterile 96 well plates. The roots and Bark of *G. Arborea* were collected in the month of December, 2014 from Siliguri, West Bengal, India. Roots and bark were identified by botanist.

Preparation of extracts of *Gmelina arborea* root and bark

Aqueous extracts: 100 g. ethanol marc powders were subjected to cold maceration with water in a one litter conical flask for about 7 days at room temperature. The flask was plugged with absorbent cotton and was shaken periodically till complete maceration. The marc was placed in a muslin cloth and the filtrate was concentrated to residue at low temperature. The extracts were subjected to phytochemical investigations by qualitative chemical tests.

Hydro-alcoholic extracts: An appropriate volume of 50% ethanol was added to pre-weighed *Gmelina arborea* powder and the mixture was vortexed. After 12–18 h, distilled water was added to each tube for 1–3 h to obtain a final concentration.

Preparation of petroleum ether, chloroform, methanol and ethanol extracts

The powdered material of *G Arborea* roots and bark were subjected for extractions in an increasing order of polarity using petroleum ether (40-60), chloroform, methanol and ethanol in a soxhlet apparatus for 72 hrs. The different extracts were concentrated by using a rotary flash evaporator and the residues were dried in desiccators. After drying the respective residues were weighed and percentage yield was calculated.

Hyaluronidase assay

Hyaluronidase enzyme was assayed by a spectrophotometric method for the *G. Arborea root and Bark*. The Assay method based on precipitation of HA with cetylpyridinium chloride used for screening of hyaluronidase inhibitors[5] (**Tung, et al 1994**). Enzyme (800 U/ml) and HA substrate (0.40 mg/ml) were incubated at 37°C for 1 h. the Enzyme activity was evaluated by the percentage of undigested HA substrate in the cetylpyridinium chloride precipitate at absorbance 415 nm (A415nm) after the enzyme reaction.

The formula used for calculation is according to (**Venil N Sumantran et al. 2008**)[6]

(i) A415nm value of intact undigested HA substrate was set at 100%.

(ii) % Enzyme activity =

(100%) — {A415nm of HA + enzyme/A415nm of HA x 100}

(iii) Percent digestion of the substrate by an enzyme in the presence of extracts was calculated after correcting for the absorbance of the herbal sample alone at A415nm.

RESULT

The extracts of *G. Arborea* root and Bark extracts and MHP inhibited hyaluronidase activity in a dose-dependent manner. The result depicted in Table No. 1 and Table No. 2 showed that *G. Arborea* root and Bark extracts exhibited complete enzyme inhibition at concentrations of (2.0 mg/ml *G. Arborea* root and Bark extracts) respectively. Thus, the hydro-alcoholic extract *G.Arborea* root extracts was potent as compare to *G.Arborea* Bark extracts with respect to hyaluronidase inhibition. The ethanolic extract also exhibited hyaluronidase inhibition i.e. 38.48% and 42.88 with 200 and 400 mg/kg dosage but minimal inhibition was observed with pet ether and methanolic extracts.

Table 1: Hyaluronidase inhibition by extract of *G.Aeraborea* root

Extracts and combinations	Hyaluronidase inhibition			
	HA*	HA+ Enzyme	EA	% EI
Pet ether extract 200	1.205	0.465	61.42	0.48
Pet ether extract 400	1.125	0.475	57.78	4.11
Methanol extract-200	1.009	0.462	54.21	7.68
Methanol extract-400	0.998	0.487	51.2	12.69
Ethanol extract-200	0.978	0.478	51.12	38.68
Ethanol extract-400	0.968	0.542	44.01	42.88
Aqueous extract-200	0.952	0.782	17.85	44.03
Aqueous extract-400	0.912	0.798	12.5	52.29
Hydro Alcoholic Extract-200	0.912	0.812	10.96	77.03
Hydro Alcoholic Extract-400	0.879	0.859	2.27	85.72
MHP	0.981	0.538	45.16	40.84

*MHP-Marketed Herbal Preparation

Table 2: Hyaluronidase inhibition by extract of *G.Aeraborea* bark

Extracts and combinations	Hyaluronidase inhibition			
	HA*	HA+ Enzyme	EA	% EI
Pet ether extract 200	1.198	0.432	63.94592	0.38
Pet ether extract 400	1.122	0.438	60.96257	3.89
Methanol extract-200	0.997	0.463	53.56068	7.32
Methanol extract-400	0.978	0.467	52.24949	11.52
Ethanol extract-200	0.965	0.454	52.95337	37.12
Ethanol extract-400	0.966	0.532	44.92754	41.48
Aqueous extract-200	0.962	0.778	19.12682	42.45
Aqueous extract-400	0.901	0.774	14.09545	49.89
Hydro Alcoholic Extract-200	0.887	0.822	7.328072	73.32
Hydro Alcoholic Extract-400	0.862	0.852	1.160093	82.34
MHP	0.978	0.545	44.27403	39.78

*MHP-Marketed Herbal Preparation

DISCUSSION

Recent development for the treatment of arthritis is based on specifically to the cartilage degeneration process .Many factors like TNF alpha and interleukins are the important one along with hyalourinidase responsible for cartilage degeneration. The hyaluronidase (HAase) is an endoglycosidase enzyme which controls HA metabolism. In the progression of arthritis the HAases along with other enzymes like HAS play an important role in the rate of HA degradation in the joint there by the lubrication of joints are hindered and arthritic conditions develop.

The present research work revealed the potency of root and bark extracts of *G.Arborea* in hylourinidase inhibition. The findings showed significant inhibition of hylourindase through both hydro alcoholic root and Bark extract.

CONCLUSION

The current research acclaimed the usefulness of hydro alcoholic and aqueous extracts of *Gmelina arborea* roots and bark in the treatment of arthritis. Therefore it should be validated through various animal studies which can provide new direction in the drug discovery process.

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