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IN-VITRO PHARMACOLOGICAL EVALUATION OF ANTI- ULCER AND ANTI-ARTHRITIC ACTIVITY OF *AMARANTHUS SPINOSUS L.*

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ABSTRACT

Amaranthus spinosus L., commonly known as spiny amaranth, was collected, authenticated and dried by the Central National Herbarium of the Botanical Survey of India. The dried whole plant was subjected to extraction using a hydro-alcoholic solvent (70% ethanol and 30% distilled water) through the maceration method. Phytochemical screening of the extract disclosed the existence a number of bioactive components, including alkaloids, carbohydrates, amino acids, flavonoids, glycosides, mucilage, tannins, proteins and terpenoids, all of which are known for their multiple pharmacological properties. In vitro pharmacological investigations were conducted to evaluate the plant's potential therapeutic effects, particularly its anti- ulcer and anti- arthritic activities of the hydro-alcoholic extract when compared to standard drugs used for these conditions. The extract exhibited protective effects on the gastric mucosa, which may contribute to its anti-ulcer activity and anti- inflammatory effects, which may assist alleviate the symptoms of arthritis such as pain and swelling. The observed pharmacological effects are likely due to the synergistic action of multiple phytochemicals existing in the plant, although further studies are required to isolate and identify the specific compounds responsible for these activities. These works suggests that *Amaranthus spinosus L*., may hold promise as a natural therapeutic agent for the treatment of gastric ulcers and inflammatory arthritis.

Keywords: Anti- ulcer, Anti- arthritic, *Amaranthus spinosus L*., in- vitro, hydro-alcoholic

INTRODUCTION:

Herbal drugs are defined as plant substances or herb lore requires the use of complete plants or parts of plants, to cure wound or ailment **[1]**. Herbal therapy, are investigated as phytomedicines of herbal drugs, called for the utilize of shrub fragments (leaves, roots, stem, flower and seeds) for medical/therapeutic purpose. India has a very lengthy, defended and uninterrupted utilization of numerous herbal medicines in the precisely acknowledge different structures of health *viz*. Ayurveda, Yoga, Unani, Siddha, Homeopathy and Naturopathy **[2]**. These organizations have fairly live alongside with Allopathy and are not in 'the domain of obscurity', as stated by Venkat Subramanian. Countless of Indians use herbal drugs frequently, as relish, formula, health foods as well as over-the-counter (OTC) as do- it-yourself or also as drugs prescribed in the non-allopathic systems **[3]**.

An ulcer is a wound that forms on the edge of the stomach, small intestine, or esophagus, often resulting from the decay of the tissue **[4]**. The most common type is peptic ulcer which can be classified into gastric ulcers (in the stomach) and duodenal ulcers (in the upper part of the small intestine). Ulcers can be caused by several factors, including infection with *Helicobactor pylori* bacteria, immoderate use of non-steroidal anti-inflammatory drugs (NSAIDs), stress and certain lifestyle choices such as smoking and alcohol consumption. Symptoms typically include burning stomach pain, bloating, indigestion and nausea. In severe cases, ulcers can lead to complications like bleeding, perforation or obstruction **[5]**.

Arthritis is a broad term encompassing over 100 inflammatory joint untidiness that can cause pain, bulge and rigor in the joints. The most similar categories comprises osteoarthritis (OA) and rheumatoid arthritis (RA) **[6]**. Osteoarthritis is a reverse condition often ensues from depreciation on the joints, leading to cartilage breakdown. It typically affects older adults and is characterised by joint pain that worsens with activity and improves with rest. Rheumatoid arthritis is an auto resistance malady where the immune system falsely strikes to joint matters, causing inflammation. This can lead to significant joint damage if left untreated. RA often influence small scale joints, namely those in the hands and feet, and may also result in systemic symptoms like fatigue and fever. Symptoms of arthritis can vary but commonly similar joint pain, tenderness, swelling and reduced articulation. The condition can significantly impact daily life and mobility, leading to challenges in performing everyday tasks **[7]**.

Amaranthus spinosus L., commonly known as spiny amaranth, has been studied for its potential medicinal properties including its use as an anti ulcer agent. Various phytochemical constituents such as, flavonoids, alkaloids and tannins may contribute to its protective effects on the gastric mucosa **[8]**. The plant may help reduce oxidative stress in the gastric lining, promoting healing. The compounds in *A. spinosus* can potentially decrease inflammation, which is a key factor in ulcer formation. It may enhance the production of mucosal barriers or promote the secretion of gastric mucous, helping to protect the stomach lining. It has been studied for its potential anti-arthritis properties **[9]**. Traditionally used in various cultures for its medicinal benefits, this plant exhibits anti-inflammatory and analgesic effects that may help alleviate arthritis symptoms. Its compounds may inhibit pro-inflammatory cytokines and enzymes involved in joint degradation, thus reducing swelling and pain associated with conditions like rheumatoid arthritis and osteoarthritis **[10]**.

It is an upright prickly yearly plant up to 60 cm high and reproducing from seeds. The stem is fleshy, round, greenish with rigid about 10 mm long. The leaves are 6 to 8 cm long and 2 to 4 cm broad, petioles about 7 cm long. The flowers are small greenish and the fruits a one seeded capsule with shiny, lens-shaped, radish-brown seeds. The roots are pale yellowish in colour. The juice of tuber is used to cure fever, urinary troubles, diarrhoea, stomach disorder etc. **[11]**.

MATERIALS AND METHODS:

Collection and Validation of plant material: Based on the literature consideration, one plant named *Amaranthus spinosus* L. was selected for the work. The whole plant of *Amaranthus spinosus* L. was piled up in the month of January, 2024 from various areas of Haldia, Purba Medinipur Dist., West Bengal and authenticated by experimenter, Central National Herbarium, Botanical Survey of India, Kolkata, West Bengal. Plant Herbarium was produced and conserved in the Department of Pharmacognosy, Haldia institute of Pharmacy, Haldia, Purba Medinipur, West Bengal, India.

Preparation of extract: The selection herb by product plucked out by maceration method. 344 g of seared whole plant fine particles of *Amaranthus spinosus L.* was extracted with 1230 ml ethanol and 526 ml distilled water, then the products are filtered. And then the extracts are seared and aside for more distant use **[12]**.

Identification of phytochemical by preliminary phytochemical tests:

Test for alkaloids: [13]

Mayer's reagent test: Add Mayer's reagent (potassium mercuric iodide solution) to 1 ml of the test solution. The emergence of a cream colored precipitate specifies the presence of alkaloids.

Wagner's reagent test: Add Wagner's reagent (iodine potassium iodide solution) to 1 ml of the test solution. The development of a reddish brown plunge indicates the presence of alkaloids.

Test for Amino acids:

Ninhydrin test: Add 2 ml of Ninhydrin reagent to 1 ml of the test solution and heat in a water bath. The formation of a violet color indicates the presences the presence of proteins.

Test for carbohydrates: [14]

Molisch's test: Add alcoholic naphthol to 2 ml of the test solution, then sincerely layer a few drops of concentrated sulfuric acid adjacent the edges of the test tube. The emergence of a purple to violet color band at the junction confirms the existence of carbohydrates.

Seliwanoff's test (test for ketones): Adjoin crystals of resorcinol and a small amount of concentrated sulfuric acid to a small quantity to the hydro-alcoholic extract, then heat in a water bath. A rose colored formation suggests the presence of ketones.

Test for flavonoids: [15]

Schinoda's test: To 2 ml of the test solution, add a few magnesium turnings, followed by concentrated HCl drop by drop. The appearance of pink, crimson red, or green to blue color after a few minutes indicates the presence of flavonoids.

Zinc hydrochloride test: To the test solution add a mixture of zinc dust and concentrated HCl. A red color develops after a few minutes if flavonoids are present.

Test for glycosides: [16]

Borntrager's test: Boil the test substance with 1 ml of concentrated H_2SO_4 in a test tube for 5 minutes. The hot mixture was filtered out and the filtrate was left for cool down. Shake the cooled filtrate with a same amount of dichloromethane (CH_2Cl_2) or chloroform ($CHCl_3$). Isolate the underneath organic surface and shiver it with half of its cubic measure of dilute ammonia (NH_3). A rose pink to red color will develop in the ammoniacal layer if anthraquinone glycosides are present.

Keller killani's test: take out the drug with chloroform and volatilize the chloroform to dessication. To the residue, add 0.4 ml of glacial acetic acid carrying a clue amount of ferric chloride. Transmit the solution to a small test tube and observe the color change. If cardiac glycosides are present, the acidic layer will turn blue.

Foam test: Place 2 ml of drug in water in a test tube and shake well. If Saponin glycoside are present a stable form will form.

Test for Lignin: The hydroalcoholic extract is treated with concentrated HCl and phloroglucinol solution. The outward form of a pink color confirms the existence of lignin.

Test for mucilage: The hydroalcoholic extract is treated with ruthenium red solution. The outward of a pink color shows the presence of mucilage.

Test for tannins: [17]

$FeCl_3$ test: Add $FeCl_3$ solution to the extract. A blue color shows the presence of hydrolysable tannins, while a green color signifies the presence of condensed tannins.

Test for proteins: [18]

Biuret test: To 1 ml of the test solution, add 2 ml of biuret reagent and heat in a water bath. The appearance of a violet color shows the presence of proteins.

Test for steroids and terpenoids: [19]

Salkowski's test: Add few drops of concentrated sulfuric acid to the extract. A red color in the layer shows the presence of steroids, while a yellow color in the lower layer shows the presence of tri- terpenoids.

In-vitro anti-ulcer activity by determining the acid neutralizing capacity: [20]

Principle: Antacid Neutralizing Capacity (ANC) is the ability to counteract the acids and it is because of any liquefy genus like infirm anions that can receive and neutralize protons. This technique will determine the acid neutralizing capacity by titration with 1N HCl in the middle of the pH range of 4.5 and 3.5 at which the benefaction of hydroxide, carbonate and bicarbonate are neutralized.

Procedure: The Acid Neutralizing Capacity (ANC) value of hydro-alcoholic complete herb extract of *Amaranthus spinosus L.* at different concentrations (100mg, 500mg, 1000mg, 1500mg) was differentiated with the standard antacid Aluminum Hydroxide $[Al(OH_3)]$ + Magnesium Hydroxide $[Mg(OH_2)]$ – 500mg. Take 5 ml of hydro-alcoholic extract of *Amaranthus spinosus L.* and add water to adjust the total cubic measure 70 ml and then combined it for one minute. Subsequently 30ml of 1N HCl was poured into standard and test composition and agitated for 15 min., 2drops of phenolphthalein tincture were added to the mixture and stirred. The surplus HCl was instantly titrated with 0.5N Sodium hydroxide solution drop wise till a pink colour is obtained. The moles of acid neutralizing is figured out by using the formula:

Moles of acid neutralized = (vol. of HCl × Normality of HCl) – (vol. of NaOH × Normality of NaOH)

$$ANC\ per\ gram\ of\ antacid = \frac{moles\ of\ acid\ neutrilized}{grams\ of\ extract\ or\ antacid}$$

In-vitro anti-arthritic activity of hydro-alcoholic whole plant extract of *Amaranthus spinosus L.*:

Bovine Serum Albumin Protein Denaturation Method: [21]

Principle: Protein denaturation refers to the alteration of a protein's secondary and tertiary structures due to external factors like strong acids or bases, concentrated salts, organic solvents, heat. This process is widely recognized as a contributing factor to inflammation in conditions like rheumatoid arthritis. The protective action against such denaturation is a key mechanism by which non- steroidal anti- inflammatory drugs (NSAIDs) exert their anti- arthritic effects.

Method:

The mixture (0.5) comprised of 0.45 of Bovine Serum Albumin (0.5% w/v watered solution) and 0.5ml of test tincture of severous aggregations. Test control tincture (0.5ml) comprised of 0.45ml of Bovine Serum Albumin (0.5% w/v aqueous solution) and 0.05ml of distilled water. Standard solution (0.5ml) consists of 0.45ml of Bovine Serum Albumin (0.5% w/v aqueous solution) and 0.05ml of Diclofenac sodium of various concentrations composing of reagents.

0.5% Bovine Serum Albumin (BSA): Dissolve 500mg of BSA in 100ml water.

Phosphate buffer saline (pH 6.3): Dissolve 8g of Sodium Chloride (NaCl), 0.2 g of Potassium Chloride (KCl), 1.44 g of Disodium Hydrogen Phosphate (Na_2HPO_4), and 0.24 g of Potassium Dihydrogen Phosphate (KH_2PO_4) in 800 ml of distilled water.

1N HCL Preparation: 8.5 ml concentrated HCl diluted in 100 ml of water.

Procedure: 0.05 ml of profuse aggregations (100, 200, 300 µg/ml) of test drugs and standard drug Diclofenac Sodium (100, 200, 300 µg/ml) were chosen independently and 0.45ml (0.5% w/v BSA) stirred. The specimens were incubated at 57˚C for 3 mins. After unheated, add 2.5 ml of Phosphate Buffer to the exceeding tinctures. The UV-Visible Spectrophotometer was used to approximate the absorbance at 255 nm. The control appears 100% protein modification.

The consequences were differentiated with Diclofenac sodium.

The percentage hindrance of protein modification can be deliberated by using the formula.

$$\textbf{Percentage Inhibition} = \frac{Abs.\ control - Abs.sample}{Abs.\ control} \times 100$$

RESULT AND DISCUSSION:

Phytochemical Evaluation: The introductory phytochemical test of hydro-alcoholic extract (whole plant) of *Amaranthus spinosus* L. specifies existence of alkaloids, amino acid, carbohydrate, flavonoids, glycosides, saponins, tannins, proteins, steroids and terpenoids **(Table 1)**.

Results of *in vitro* anti-ulcer activity of whole plant extract of *Amaranthus spinosus*

L. by determining the acid neutralizing capacity:

The hydro-alcoholic whole plan extract of *Amaranthus spinosus L*., were screened for the *in vitro* Anti-Ulcer activity by using Acid Neutralizing Capacity method, where the different concentrations of test (extract) substance 100mg, 500mg, 1000mg and1500mg along with 1N HCl is titrated with 0.5N Sodium hydroxide solution in order to determine the Acid neutralization.

The Acid Neutralizing Capacity (ANC) per gram of antacid ware found to be 106.5, 28.5, 9.75, 5.9 for 100, 500, 1000, 1500 mg concentration of plant extract respectively & 13.2gm for 500mg [$AL(OH)_2$+$Mg(OH)_2$] concentration of standard drug **(Table 2)**.

In vitro Anti-arthritic activity of *Amaranthus spinosus L*.: Bovine Serum albumin Protein Denaturation method:

The Percentage Inhibition ware found by UV Visible Spectrophotometer at 255nm. The percentage inhibitions of test sample (extract) are 44.44, 48.28 and 53.44 for 100, 200 & 300mg concentration respectively &the percentage inhibition of standard drug are 44.82, 50 and 55.17 for 100, 200 and 300mg. IC_{50} value of plant extract is 231.936 and IC_{50} value of standard drug is 223.216 **(Table 3)**.

Table 1: Consequence of introductory phytochemical tests of extract of *Amaranthus spinosus* L. for the existence of numerous compounds

Type of phytochemical constituent	Name of the test	Hydro-alcoholic extract of *Amaranthus spinosus L.*
Test for alkaloids	Mayer's test	Present
	Wagner's test	Present
Test for carbohydrate	Molish's test	Present
	Seliwanoff's test	Present
Test for glycosides	Brontrager's test	Present
	Killer-killani's test	Present
Test for saponins	Foam test	Present
Test for mucilage		Present
Test for tannin	Ferric chloride test	Present
Test for flavonoids	Schinoda's test	Present
	Zinc hydrochloride test	Present
Test for amino acid	Ninhydrin test	Present
Test for protein	Biuret test	Present
Test for lignin		Absent
Test for steroids and terpenoids	Salkowski's test	Present

Table 2: Result of In-vitro anti-ulcer activity by measuring the acid neutralizing capacity

Sl. No.	Concentration	Vol. NaOH consumed (ml)	m. Eq of Acid consumed	ANC per gram of Antacid
1.	100	38.7	10.65	106.5
2.	500	31.5	14.25	28.5
3.	1000	40.5	9.75	9.75
4.	1500	42.3	8.85	5.9
5.	500mg $AL(OH)_2$+$Mg(OH)_2$	46.8	6.6	13.2

Table 3: In-vitro anti-arthritic activity of *Amaranthus spinosus L.* by using bovine serum albumin protein denaturation method

SL no.	Name of the drug	Concentration(mg)	Absorbance	Percentage inhibition
1	Control		0.522 ± 0.001	
2	Test (Extract)	100	0.290 ± 0.002	44.44
		200	0.270 ± 0.002	48.28
		300	0.243 ± .003	53.44
3	Standard	100	0.288± 0.001	44.82
		200	0.261 ± 0.003	50
		300	0.234 ± 0.002	55.17

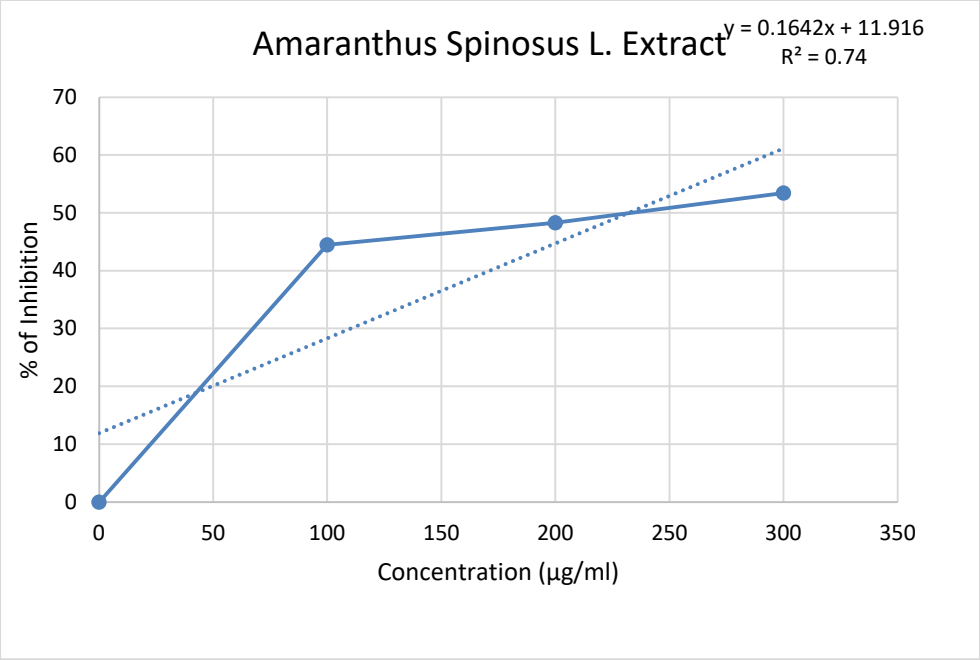

Figure 1: *% inhibition* of *Amaranthus spinosus* L Extract

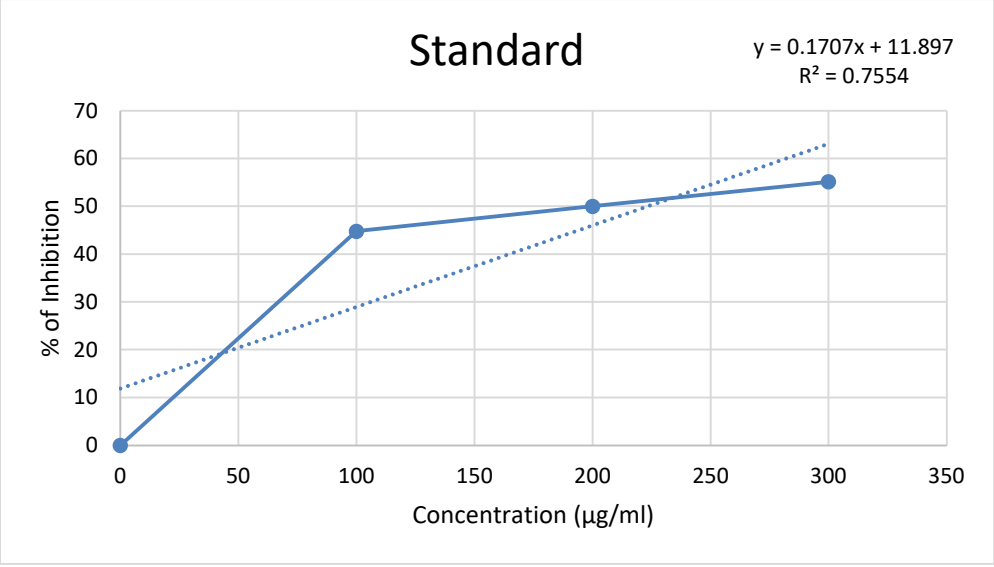

Figure 2: % inhibition of standard drug

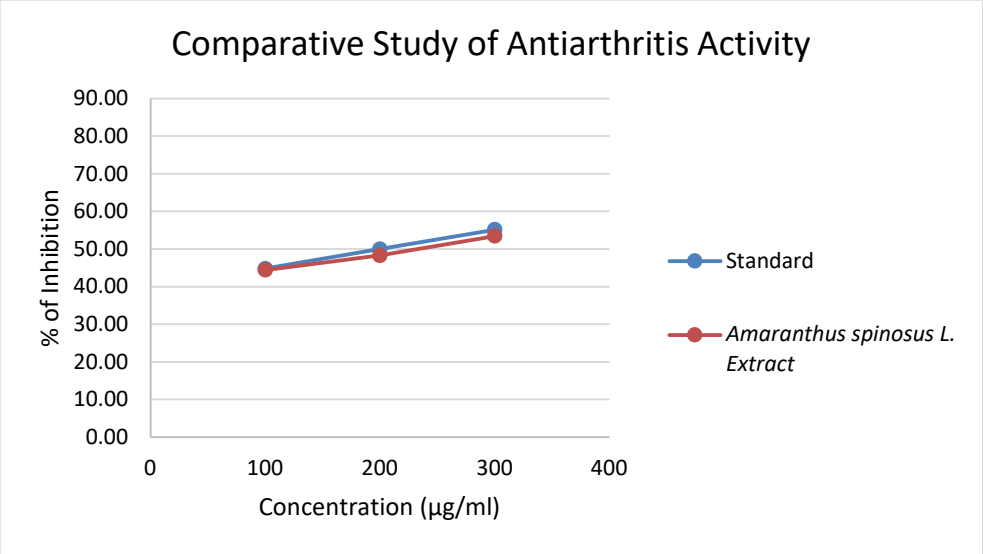

Figure 3: Comparative study of Anti-arthritis activity for test (Extract) and standard sample

CONCLUSION:

The entire herb of *Amaranthus spinosus L.*, was identified, accumulated, substantiated by analyser, Central National Herbarium, Botanical Survey of India, dried and subjected to extraction by maceration method using hydroalcoholic (70% ethanol and 30% distilled water) solvent. The phytochemical screening of whole plant of *Amaranthus spinosus L.*, indicates the existence of alkaloids, carbohydrate, amino acids, flavonoids, glycosides, mucilage, tannins, proteins, terpenoids. The research work was carried out in-vitro method for selected plant and it was concluded that the hydroalcoholic extract of *Amaranthus spinosus L.*, whole plant has significant anti-ulcer and anti-arthritics activity as compared to standard. The pharmacological effect may be occurred due to presence of one or more than one phytoconstituents present in the plant but which phytoconstituents exactly responsible for the above biological activity can be evaluated in further research work.

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