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PHYTOCHEMICAL SCREENING AND PHARMACOLOGICAL EVALUATION OF METHANOL EXTRACT OF AMARANTHUS TRICOLOR L. (STEM)

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1. ABSTRACT

The purpose of this study was to explore the presence of phytochemical constituents and their potential pharmacological activity of Amaranthus tricolor L. Methanol extract of the dry pulverized stem of Amaranthus tricolor obtained by cold extraction method. The stem extract was subjected to a preliminary phytochemical screening by standard methods. Phytochemical analysis revealed alkaloids, glycosides, saponins, phenols, tannins in the stem extract. The methanol extract showed intense thrombolytic activity with 66.3% clot lysis compared to 100µl Streptokinase (Standard), which showed 76% clot lysis. Methanol steam extract 250mg/kg showed 32.7% inhibition of feces and 55% inhibition of diarrhea, and 500mg/kg extract showed 27.9% inhibition of manure and 40% inhibition of diarrhea which Compare to standard [Loperamide] showed 44 % inhibition of feces and 59.97 % inhibition of diarrhea. Methanol steam extract 250 mg/kg reduces 40% pain and at 500 mg/kg reduces 42.5%, which, Compare to the Standard (Diclofenac Na), worked as 77.5% pain induction. All results obtained from the research suggest that the plant's stem has moderately positive Antidiarrheal activity test, Analgesic, and thrombolytic properties. Therefore, considering potential biodiversity, plant ingredients can be widely researched to find their unelected effectiveness and to make use of them as traditional medicines.

Key Words: Thrombolytic activity, Streptokinase, Loperamide, analgesic activity, Diclofenac, Amaranthus tricolor

2. INTRODUCTION

From ancient times until currently, plants are the idea of the many conventional drug systems throughout the globe and continued to furnish human beings with new remedies. an excellent kind of medicative plants, their pure constituents, and natural merchandise from the medicative plants give unlimited opportunities for brand spanking new medication development attributable to the incomparable accessibility of numerous chemical compounds and even have been shown to have useful therapeutic potential [1][2] It is documented that eighty percent of the world's population has belief in ancient medicines notably plant medicine for his or her primary health care [3]. Principally bioactive plant metabolites area unit chargeable for the therapeutic properties of the medicative plants [4]. phenoplast compounds principally flavonoids and phenoplast acids, have many biological properties together with inhibitor, bactericide, malignant neoplasm activities [5]. The inhibitor capability of plant phenolics depends on their concentration [6], number, and position of the hydroxyl radical. These sorts of

compounds area unit chargeable for the neutralization of harmful free radicals. Thanks to the overrun of free radicals and lack of antioxidants a condition called aerophilic stress is developed. Free radical-induced aerophilic harm has long been thought to be the foremost vital reason for several chronic and chronic diseases like polygenic disease, stroke, cancer, coronary-artery disease, and vessel diseases [7]. These venturous conditions are overcome through many plant secondary metabolites together with alkaloids, flavonoids, lignin's, phenoplast compounds, and terpenoids [8].

3. METHOD AND MATERIALS

The Phytochemical screening of a plant can be divided into the following steps:
a) Collection of plant sample and Identification of plant sample
b) Drying of the plant sample
c) Grinding of plant material and preparation of powdered plant material.
d) Preparation of plant sample
e) Extraction of the plant material
f) In-vitro thrombolytic studies and
g) Phytochemical screening

4. COLLECTION OF PLANT MATERIALS

Plant samples Amaranthus tricolor L. were collected from Share-Bangla-Agriculture University located in Dhaka in late July 2019. The plant was identified and confirmed by The National Herbarium; the Government of Bangladesh located in the Mirpur area.

5. DRYING AND GRINDING

The collected plant parts (stem) were separated from undesirable materials or plants or plant parts. They were dried in the sun for one week after cutting into small pieces. Using a suitable grinder, the plant pieces were ground into a coarse powder. The powder was maintained in an airtight container in a cold, dark, and dry location until the analysis began.

6. PREPARATION OF METHANOL EXTRACTION

About 560 gm of powdered sample was taken in a clean, flat-bottomed glass container and soaked in 900 mL of 90% methanol. The container and its contents were sealed and stored for 10 days with intermittent shaking and stirring. The entire mixture was then coarsely filtered using a piece of clean, white cotton material. The water was then filtered using Whatman filter paper.

7. CHEMICALS AND REAGENTS

Diclofenac sodium and Loperamide were purchased from Beacon Pharmaceuticals Ltd. Bangladesh. Streptokinase found from the local market Lazz pharma.

8. PHYTOCHEMICAL SCREENING

Different phytochemical groups such as alkaloids, glycosides, flavonoids, tannins, gums, saponins, steroids were identified by characteristic color change using standard chemical tests. (This information was found from the Ghani A. Practical phytochemistry) [9].

9. THROMBOLYTIC ACTIVITY

Preparation of extract dose: Extract Concentration, Stock solution = 100mg/10ml Streptokinase 1500000 IU/5ml standard, 30000 IU in 100l dose Procedure: The leaves' in vitro clot lysis activity was tested using the technique with slight changes. 5 ml of venous blood was taken from healthy volunteers (n = 3) with no history of smoking, taking lipid-lowering medicines,

oral contraceptive or anticoagulant therapy, and transferred to various pre-weighed sterile microcentrifuge tubes (1 ml/tube) under ethical concerns and aseptic precaution. The micro-centrifuged tubes were incubated at 37°C for 45 minutes. After the clot had formed, the serum was entirely withdrawn from the tubes (without disturbing the clot), and each tube containing a clot was weighed again to measure the weight of the clot (clot weight = weight of clot containing tube – weight of tube alone). 100 l solutions of various extracts, concentration 1 mg/mL, were added to each micro-centrifuge tube containing pre-weighed clot. 100 l of streptokinase was added as a positive control, and 100 l of sterilized distilled water was added as a negative non-thrombolytic control, to the numbered control tubes. The tubes were then re-incubated at 37°C for 90 minutes to check for clot lysis. After incubation, the collected fluid was withdrawn from the tubes and weighed again to determine the weight difference after clot disruption. Finally, the weight difference was calculated, and the result was reported as a percentage of clot lysis using the equation below. % clot lysis = (wt. of lysis clot /wt. of initial clot) 100 [10]

10. ANTIDIARRHEAL ACTIVITY TEST

Diarrhea is defined as feces or any liquid material soiling the absorbent paper placed under the box. The time spent before the first defection was the "latency period". The total fecal count and incubation period of the test group were compared with the positive control group. Anti-diarrheal drugs extend the incubation period and reduce the total fecal count.[11] To prepare suspension of the test samples at the doses of 250 and 500 mg/kg per body weight, 250 and 500mg of samples were measured respectively. The extract was triturated in unidirectional manner. After proper mixing of extract distilled water was added slowly. The final volume was made 10ml. The test animals were randomly chosen and divided into 3 groups which having 3 mice in each. Of the experimental groups.

Group-I is the control group that received only distilled water. Group-II is the positive control group that received the standard antimotility drug, loperamide at a dose of 3mg/kg body weight as an oral suspension. The test group was treated with a suspension of stem extract Amaranthus tricolor L. at the oral dose of 250mg/kg body weight and 500mg/kg body weight. The samples were provided to the mice one hour before the castor oil was administered orally at a dose of.3ml per mouse. Individual animals from each group were housed in separate cages with adsorbent paper bottom and were inspected for the presence of diarrhea every hour for four hours following castor oil delivery. Several feces or any fluid substance that stained the adsorbent paper were counted and recorded for each mouse at each subsequent hour over the course of four hours. New papers were substituted for the old ones at the start of each hour. [12]

11. ANALGESIC ACTIVITY TEST

In the experiment, a total of 20 mice were divided into four groups (each group comprises five mice). Group I served as vehicle control mice received vehicles (1% Tween 80 in saline), Group II served as a standard group and received diclofenac (10 mg/kg i.p) as a standard drug, Group III and Group IV received 200 and 400 mg/kg orally of CF extract respectively. The analgesic effect of the samples was investigated in mice using the acetic acid-induced writhing technique. Writing was elicited in mice by administering 0.1 mL of 1% Acetic Acid intraperitoneally. The extract and vehicle were given orally 30 minutes before the intraperitoneal injection of 1% acetic acid, however diclofenac sodium was given intraperitoneally 15 minutes before the injection of acetic acid. After 5 minutes, the mice noticed an unique spasm of the body known as "writhing," which lasted for the next 10 minutes [13].

12. RESULT

The following table summarizes the phytochemicals present in plant stems:

Table 1: Different chemical group test result

Phytochemical constituents	Result
Alkaloids	+
Carbohydrates	-
Flavonoid	-
Saponins	+
Glycosides	+
Phenols	-

In different chemical group test [+] Indicates Presence and [-] Indicates Absence.

Table 2: Analgesic activity test result

Treatment	Dose	Writhing counting (Mean±SEM)	% of reduction
Group-I [Control]	Water qs	40±0.25	---
Group-II [Standard] Diclofenac Na (inj)	10mg/kg	9±0.16	77.5
Group-III [Extract]	250mg/kg	21±0.12	40
Group-IV [Extract]	500mg/kg	23±0.21	42.5

Data were expressed as Mean±SEM compare to control where diclofenac were as reference standard

Table 3: Antidiarrheal activity by Amaranthus tricolor L.

Group	Dose	Latent Time (min)	Total number of feces (Mean±SEM)	% inhibition of feces	Total number of Diarrheal feces (Mean±SEM)	% inhibition of diarrhea
Group-I [Control] Castor oil	10ml/kg	6	9.67±0.88	--	6.67±0.67	--
Group-II [Standard] Loperamide	3mg/kg	11	5.33±0.88	44%	2.67±0.67	59.97%
Group-III [Extract]	250mg/kg	10	5.5±0.3	32.7%	3±0.0	55%
Group-IV [Extract]	500mg/kg	12	7±1.20	29.7%	4±0.33	40%

Data were expressed as Mean±SEM compare to control where Loperamide were as reference standard

Table 4: Thrombolytic activities on the basis of % clot lysis Amaranthus tricolor L.

Sample	1st clot+tube weight (gm.)	1st clot weight (gm.)	2nd clot + tube weight (gm.)	2nd clot weight	Weight of lysis	% of Lysis
Group-II [Control]	1.70±0.1	0.80±0.2	1.53±0.1	1.48±04	0.22±0.2	27.5%
Group-I [Standard] Streptokinase	1.87±0.2	1.01±0.3	1.11±0.2	0.24±0.4	0.77±0.3	76%
Group-III [Extract]	1.56±0.2	0.83±0.01	1.48±0.1	1.38±0.12	0.55±0.2	66.3%

Data were expressed as Mean±SEM compare to control where Streptokinase were as reference standard

13. DISCUSSION

Phospholipids make arachidonic acid via cyclooxygenase (COX) then it produces prostaglandin for pain sensation. As suggested by peripheral mechanism substances having analgesic activity preferably inhibit the prostaglandin [15]. A natural fibrinolytic agent called plasmin which helps to initiate the breakdown of fibrinogen and fibrin resulting in lysis of a blood clot. The standard SK forms a complex with plasminogen to change plasmin [16]. The use of castor oil to induce diarrhea has been reported in several studies [17,18] The preliminary phytochemical screening results, which indicated positive results for alkaloid, saponins, and glycosides, etc., laid the groundwork for future research. In comparison, the standard (Diclofenac Na) operated as 77.5% pain induction). Methanol stem extract 250mg showed 32.7% inhibition of feces and 55% inhibition of diarrhea. 500mg extract showed 27.9% inhibition of feces and 40% inhibition of diarrhea. In the present study, antidiarrheal activity of the plant extract of Amaranthus tricolor L. was investigated by castor oil-induced diarrhea in mice. Compare to standard [Loperamide] showed 44 % inhibition of feces and 59.97 % inhibition of diarrhea. So, the anti-diarrheal activity of Amaranthus tricolor L. stem extract is moderately positive. Lastly the thrombolytic activity 100 µl Streptokinase (Standard), showed 76% of clot lysis. While distilled water (Control) showed clot lysis of 27.5%. The study of Amaranthus tricolor L. stem extract of Methanol displayed 66.3% of clot lysis. Compare to standard (SK), the result of thrombolytic activity of Amaranthus tricolor L. stem extract is strongly positive.

14. CONCLUSION

In the context of on top of result and discussion it is often aforesaid that the methanolic extract of Amaranthus tricolor L. has analgesic, thrombolytic and antidiarrheal activity.

15. Conflict of Interest

Authors declared no conflict of interest.

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