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RESEARCH ARTICLE

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IN VITRO THROMBOLYTIC AND ANTI-OXIDANT ACTIVITY STUDY OF ABROMA AUGUSTA (ULATKAMBAL)

ABSTRACT

Antioxidants lavishly present in many natural source attributed their capability of slowing or preventing the oxidation of other molecules, helpful in the prevention of many age-related diseases and promotion of health. This study is designed to evaluate the antioxidant properties of the methanolic leaf extracts of *Abroma augusta*. After collection, drying, grinding and extraction, final extract of the plant sample were obtained through filtration followed by concentrated through evaporation in the water bath at 60ºC. DPPH (1,1-diphenyl-2-picryl hydrazyl) aid in the qualitative analysis of the antioxidant activity through color change from deep pink to pale yellow or yellow upon spraying on the chromatogram of the Thin Layer Chromatography on which little amount of plant extract were applied before spraying. Inhibitory concentration 50% (IC_{50}) value is a measure of the concentration of sample required to scavenge 50% of the DPPH free radicals helps in the quantitative study. Thrombolytic activity were utilized with the help of appendrof tube in which blood is kept and plant extract is applied whereas Streptokinase and distill water is used as positive and negative control. Inhibitory conc. 50% of the plant methanolic extract were found to be in the range of 35.87 % - 80.43% at different concentration of the plant extract which also show significant thrombolytic activity at variable range. The above result showed that the plant have good antioxidant and thrombolytic activity and hence support the acclaimed medicinal value of the crop.

Keywords Antioxidant, age related disease, extraction, DPPH, qualitative analysis, thrombolytic activity.

1. INTRODUCTION

Many of the modern day's vital drugs and processed medicines are of plant origin. Medicinal plants contain different remedial agents which may have thrombolytic activity, antimicrobial activity, cytotoxic effect etc. Medicinal plants play a leading role in the treatment of varieties of human diseases from the dusk of human development[1].

Abroma augusta Linn f. (Ambroma) syn. *A. fastuosa* (Family-Sterculiaceae) commonly known as Ulatkambal in Hindi and Devil's cotton in English. *Abroma augusta* has a long history of medicinal use in Ayurvedic system. It is highly possesses in gynecological disorders. It regulates the menstrual flow and also used as abortifacient and anti-fertility agent[2]. *Abroma augusta* have been of medical interest due to their good therapeutic value in folk medicine.

Recent developments in biomedicals point in many diseases to the involvement of free radicals which causes membrane lipid peroxidation leading to cell inactivation. For these reasons, antioxidants are of interest for the treatment of many kinds of cellular degeneration. Antioxidants are compounds that inhibit or delay the oxidation process. There are two basic categories of antioxidant namely synthetic and natural ones. Restriction on the use of synthetic anti-oxidants is being imposed because of their carcinogenicity. Thus, the interest in natural antioxidants has been increased considerably. As resources of natural antioxidants much attention has been paid to plants. The interest in natural antioxidant, especially of plant origin, has greatly increased in recent years[3]. The anti-oxidant activity of the extract of *Abroma augusta* has been observed in many previous studies[2,4].

Like streptokinase many medicinal plants extract can lyses thrombus, a process which breaks blood clots by pharmacological mean. Working with different medicinal plants extract showed that they can lyses thrombus as streptokinase[1].

As a part of our continuing studies of medicinal plants of Bangladesh[5,6] the methanol extracts of leaves of *Abroma augusta* growing in Bangladesh were screened for antioxidant activity and thrombolytic activity.

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2. MATERIALS AND METHOD

2.1. Material

The plants namely *Abroma augusta* are collected from Chittagong. Methanol and ethanol was purchased from Merck India. All other reagents used were of analytical grade.

2.2. Method

2.2.1. Plant samples extraction

The plant leafs of *Abroma augusta* were dried in the sun first then in mechanical dryer at 60 – 70^{0}C, later ground to coarse powder with a mechanical grinder. Then the powdered sample was taken on a glass jar on which methanol (400 ml) was poured up to 1-inch height above the sample surface as it can sufficiently cover the sample surface. To resist the entrance of air, the jar should closed properly with plastic cover and aluminium foil and kept for 3 days. The jar was shaked in several times during the process to get better extraction. After the extraction process the plant extract was filtered with cotton filter fitted and collected in a beaker[7]. The *Abroma augusta* leaf extract was concentrated by evaporating the solvent using a water bath at a temperature of 60^{0}C.

2.2.2. Qualitative analysis

This test was performed to see the presence of antioxidant groups in the plant extract by using DPPH (1,1-diphenyl-2-picryl hydrazyl) which is a stable free radical and neutralized by the antioxidants. DPPH forms deep pink color when it is dissolved in ethanol. Thin Layer Chromatography technique is used here. When DPPH is sprayed on the chromatogram of the plant extract, if it forms pale yellow or yellow color then indicates the presence of antioxidants[7].

$$DPPH\cdot + AH \longrightarrow DPPH + A\cdot$$
$$DPPH + R \longrightarrow DPPH\text{-}R$$

Reddish Purple → Yellow

$DPPH^{\bullet}$ = 1, 1-Diphenyl-2-Picryl Hydrazyl
AH = an antioxidant
$R^{\bullet}$ = a radical species

A fine capillary tube was used as spotter for sample application in the TLC plates. A very little amount of plant extract was taken in a small vial and diluted suitably with ethanol. The sample was spotted in uniform size (about 0.3 cm) on TLC plates. The sample was applied several times in each spot to get better chromatogram. Each spot was dried before applying another volume of solution to the same spot.

The chromatogram was developed by ascending technique. Three solvent systems in the ratio mentioned in table 1 were kept in three jars due to the presence of different groups of compound in the plant extract . The plates were placed in each jar in such a manner that the sample spots were just above the solvent surface. Filter papers were kept into each jar by wetting with respective solvent systems to keep those jars saturated and the jars were closed tightly. The solvents were allowed to move up the plates by capillary action until they have traveled a distance of about 10-15 cm from the point of application of the sample on a 20 cm plate. The plates were then removed from

the jars and dried with a current of air suitably.

2.2.3. Quantitative analysis

DPPH was used to assess the hydrogen donating ability of methanolic extract sample. Stock solution (5 mg/ml) of solvent extract of sample was prepared in respective solvent system. Different dilutions of the stock solution were carried out to obtain concentrations of 1, 5, 10, 20, 40, 60, 80 and 100 µg/ml. In this assay, an equal amount of sample solution (2 ml) was added to an equal amount of 0.1 mM methanolic DPPH solution. The mixture was vortex for few minutes and allowed to stand in dark place at 25 °C for 30 min for reaction to occur. After 30 min of incubation, the absorbance was read against a blank at 517 nm. The radical scavenging activity was expressed as the inhibition percentage (I%) and calculated as per the equation:

I (%) = (Absorbance of the blank – Absorbance of the sample / Absorbance of the blank) x 100

Where blank is the control sample reaction (containing all reagents except the test compound). The concentration of sample required to scavenge 50% DPPH free radical (IC_{50} value) was calculated from the plot of percent inhibition against the concentration of the test samples. All the tests were carried out in triplicate and average of the absorbance was recorded for each time. Ascorbic acid (AA) was used as positive control standard[3].

2.2.4. Thrombolytic study

In vitro thrombolytic activity of the *Abroma augusta* (Ulatkambal) was conducted by using methanolic extract. Streptokinase (SK), a standard clot lysis agent is used as a positive control here and distill water is used as a negative control.

To the commercially available lyophilized SK vial (Polamin Werk GmbH, Herdecke, Germany) of 15, 00,000 I.U., 5 ml sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100 µl (30,000 I.U) was used for in vitro thrombolysis.

100 mg methanoic extract of *Abroma augusta*, was suspended in 10 ml distilled water and the suspension was shaken vigorously on a vortex mixer. The suspension was kept overnight and decanted to remove the soluble supernatant, which was filtered through a paper filter. 100 µl of this aqueous preparation of herbs was added to the appendorf tubes containing the clots to check thrombolytic activity.

Whole blood (4 ml) was drawn from healthy human volunteer without a history of oral contraceptive or anticoagulant therapy and then transferred in eight different pre weighed apperdorf tube (0.5 ml/tube) and incubated at 37°C for 45 minutes. After clot formation, serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube – weight of tube alone).

To each appendorf tube containing pre-weighed clot, 100 µl of aqueous extract of herb *Abroma augusta* was added . As a negative control, 100 µl of distilled water was added to the control tube numbered.

All the tubes were then incubated at 37°C for 90 minutes and observed for clot lysis. After incubation, fluid released was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clotlysis[8].

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$$\text{Percentage of clotlysis} = \frac{\text{Weight of clot after lysis}}{\text{Weight of clot before lysis}} \times 100$$

3. RESULT AND DISCUSSION

3.1. Antioxidant activity

Methanolic extract of *Abroma augusta* exhibited potential antioxidant activity which are shown in the table 2. The color changes (yellow on purple background) on the TLC plates were observed due to the bleaching of DPPH by the resolved bands. The extract scavenged 50% DPPH free radical at the lowest 40 µg/ml inhibitory concentration. These results denote the presence of antioxidant.

The antioxidant activity of *Abroma augusta* has been reported in many previous studies[3, 10], makes the plant popular as additional supplements. To quantify the antioxidant activity one of the widely method is hydrogen donation potential. DPPH is a stable nitrogen-centered free radical was used in the free scavenging study of the extract. Due to reduction by either the process of hydrogen radical or electron donation and subsequent formation of a stable diamagnetic molecule diphenylpicrylhydrazine, the color of ethanolic DPPH solution changes from purple to yellow[9]. The methanol extract can be admitted to be a powerful inhibitor of hydroxyl radicals from the results obtained in the present investigation.

3.2. Thrombolytic activity

The extracts of *Abroma augusta* were assessed for thrombolytic activity and the results are presented in Table 3. Addition of 100-µl Streptokinase, a positive control to the clots along with 90 minutes of incubation at 37°C , showed 86.2% clot lysis. Clots when treated with 100-µl sterile distilled water (negative control) showed only negligible clot lysis (5.2%). The mean difference in percentage of clot lysis between positive and negative control was found to be statistically significant.

After treatment of clots with 100 µl of Abroma augusta, clot lysis 50.1%, 42.9%, 41.6% respectively was obtained respectively. Among these clot lysis, *Abroma augusta* showed significant 50.1% of clotlysis and when compared with the negative control (water) the mean clot lysis % difference was significant. However further research is necessary to find out the thrombolytic activity of the active compound.

4. CONCLUSION

In conclusion, pharmacological evalution of methanolic extract of *A. augusta* reveals that the plant is a potential candidate for future antioxidant and thrombolytic agent and hence can be utilized for prevention and treatment of diseases caused by oxidative stress and also for cardiovascular disease. More studies should be performed on the extract to develop the medicinal and pharmaceutical potentialities.

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6. TABLES

Type	**Solvent system**	**Ratio**
Medium polar	$CHCl_3$: CH_3OH	5:1
Polar	$CHCl_3$: CH_3OH : H_2O	40:10:1
Non-polar	n-Hexane : Acetone	3:1

Table 1 : The solvent system used in (TLC) system is given below –

No.	Sample	Concentration of sample (µg/ml)	Avg. absorbance 517 nm	% inhibition
1	Blank	0.1mM	0.5090	----
2	Methanol extract	5	0.3264	35.87
3	Methanol extract	10	0.3049	40.09
4	Methanol extract	20	0.2954	41.96
5	Methanol extract	40	0.2081	59.12
6	Methanol extract	60	0.1801	64.62
7	Methanol extract	80	0.1074	78.89
8	Methanol extract	100	0.0985	80.65

Table 2: DPPH free radical scavenging activity of methanolic extract of Sample .

No. of tube	Concentration Of negative control, positive control and plant extracts	Weight of clot before lysis (gm)	Weight of clot after lysis (gm)	Difference	Percentage of clot lysis (%)
01	100(Water)	0.121	0.0063	0.115	5.2
02	100(Streptokinas)	0.118	0.016	0.102	86.2
03	100	0.428	0.178	0.25	41.6
04	150	0.471	0.236	0.235	50.1
05	200	0.441	0.189	0.252	42.9
06	250	0.472	0.149	0.323	31.6
07	300	0.474	0.137	0.337	28.9
08	350	0.472	0.164	0.308	34.7
09	400	0.445	0.168	0.277	37.8
10	450	0.454	0.153	0.301	33.7
11	500	0.439	0.113	0.326	25.7

Table 3: Determination of the percentage (%) of clot lysis after adding methanol extracts of herbal plants.

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