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IN VITRO ANTHELMINTHIC AND ANTIOXIDANT ACTIVITY OF ISOLATED COMPOUNDS FROM *ANNONA SQUAMOSA* BARK

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ABSTRACT: Objective: The present investigation of chemical constituents of *Annona squamosa* was undertaken as part of a wider study to find out the pharmacological active constituents present in this plant. So in this study, we investigate the pharmacological evaluation of previously isolated compounds (AS1-AS5) from the bark of *Annona squamosa.* **Methods:** The anthelminthic and antioxidant potential of the isolated compounds (AS-1 to AS-5) from *Annona squamosa* were evaluated for *in-vitro* anthelmintic activity using Indian earthworm *Pheretima posthuma* and DPPH model respectively. **Results:** The *in-vitro* anthelmintic activity results indicated that isolated compounds (AS-4 and AS-5) have good paralysis time comparable with albendazole standard. The antioxidant activity is evaluated for *in-vitro* antioxidant activity using DPPH radical scavenging activity, AS-5 & AS-4 having IC_{50} 65.77 & 92.97 µg/ml revealed potent antioxidant activity comparable to standard ascorbic acid (IC_{50} 43.42 µg/ml). **Conclusion:** This study suggests that bark of *Annona squamosa* have bioactive compounds for a new anthelmintic and antioxidant drug development. Further research on this plants core is needed for the discovery of a potent anthelmintic and antioxidant agent.

INTRODUCTION: Helminth contaminations are among the most widespread infections in humans. According to WHO, only a few drugs are frequently used in the treatment of helminthes infestations in human beings. Anthelmintic resistance is a noteworthy issue for the control of numerous parasitic nematode species and has turned into a noteworthy requirement to domesticated animals creation in many parts of the World.

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Because of the predominance of parasitic contaminations and the created resistance of some anthelmintic medications is presently an encasing zone in the field of research [1]. The family (Annonaceae) is a huge family containing around 135 genera and more than 2500 species conveyed basically in tropical and subtropical regions [2].

The genus name, 'Annona' is from the Latin word 'anon', meaning 'yearly produce', referring to the production of fruits of the various species in this genus. *A. squamosa* has been named botanically from Jamaica [3-5]. *Annona squamosa* L. (Annonaceae), usually known as the custard apple tree is cultivated throughout India, because of its edible nature [6]. The taste of the pulp of the fruit is really sweet because of its higher sugar content of

about 58% of dry mass, and hence it is found clear that the fruit pulp possess a high calorie value [7]. This plant was reputed to contain several medicinal properties. Synthetically, this family is described by the nearness of isoquinoline alkaloids, for the most part aporphines.

As our previous study, phytochemical investigation of the bark of *Annona squamosa* yields five phytoconstituents namely, three known alkaloids anonaine (AS-1) and asimilobine (AS-2), lysicamine (AS-3), together with two unknown constituent 1,2-dihydroxy-7*H*-dibenzo-quinolin-7-one (AS-4) and 1,2,9-trihydroxy-5,6,6a,7-tetrahydro-4*H*-dibenzo-quinoline (AS-5) [8]. The present investigation of chemical constituents of *Annona squamosa* was undertaken as part of a wider study to find out the pharmacological active constituents present in this plant. So in this study, we describe the pharmacological evaluation of previously isolated compounds (AS1-AS5) from the bark of *Annona squamosa.*

MATERIALS AND METHODS: The bark of plant were collected from the herbal garden of A. N. D. College of Pharmacy, Babhnan, Gonda, Uttar Pradesh, India in the month of December and identified by an expert taxonomist in Department of Taxonomy & Pharmacognosy, National Botanical Research Institute, Lucknow. The plant specimens were authenticated (Ref. No NBRI/CIF/ 413/2013). The areal part of plant was shade dried, reduced to coarse powder and stored in airtight container till further use. The extraction and isolation were carried out our previous work [8].

Animal: Healthy adult Indian earthworms *Pheretima postuma*, due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings [9 - 11] were used in this study. All earthworms of approximately equal size of 5-6 cm length and 0.2 - 0.3 cm widths were used. They were collected from the moist place, washed and kept in water.

Drugs: Purchases were as follows. Organic solvents CMC, DMSO from Central Drug House (P) Ltd. (New Delhi) and ethanol from Sd Fine-chem Limited (Mumbai) (all analytical grade). Albendazole from Zentel, GSK Pharmaceuticals Ltd. (Bangalore), 1,1-Diphenyl-2-picrylhydazyl radical (DPPH) from Sigma-Aldrich (New Delhi). Other reagents used were of analytical grade and obtained from different commercial sources.

Procedure:

***In-vitro* Anthelmintic Activity:** All isolated compounds from *Annona Squamosa* (AS-1 to AS-5) plant were investigated for their anthelmintic activity against *Pheretima posthuma* [12]. Six groups of equal size Indian earthworm consisting of six earthworms in each group were taken. Each group was treated with one of the following: vehicle (dimethyl sulfoxide [DMSO]), Albendazole (10 mg/mL) and isolated compounds (AS-1 to AS-5) (10 mg/mL) in DMSO (5.0 mL) and volume was make up to 20 mL by distilled water. Observations were made for the paralysis time and subsequently for death time of the worms.

The mean paralysis and death time for each group were recorded (each reading was taken 6 times). The time taken by the worms to become motionless, was considered as paralysis time, was recorded and the lethal time was also recorded by observing the time taken to become motionless on the application of external stimuli by pricking with pin (**Table 1**). Albendazole (10 mg/ml) was taken as standard drug (**Fig. 1**).

RESULTS AND DISCUSSION: The perusal of the data of **Table 1** revealed that the isolated compound AS-4 at the concentration of 10 mg/mL showed paralysis in 11.56 minutes and death in 37.62 minutes. Similarly AS-1 to AS-3 and AS-5 at the concentration of 10 mg/mL showed paralysis in 14.63, 15.88, 21.44, and 12.07 min and death in 42.39, 45.62, 62.15 and 39.28 min, respectively. This indicates that isolated compounds (AS-4 and AS-5) have good paralysis time comparable with albendazole standard. The predominant effect of albendazole on the worm is to cause a flaccid paralysis that result in the expulsion of the worm by peristalsis.

DPPH-Scavenging Activity: Fig. 2 illustrates a significant decrease in the concentration of DPPH radical due to scavenging ability of the isolated compounds. Ascorbic acid was used as a standard. The compounds tested with this method exhibited marked DPPH free radical scavenging activity in a concentration-dependent manner.

These results indicate that the isolated compounds from *Annona squamosa* (AS-1 to AS-5) plant exhibited potent antioxidant activity. It may be due to the presence of OH groups, which enhance the radical scavenging activity by hydrogen donation. The results indicate that compound AS-5 & AS-4 having IC_{50} 65.77± 0.12 and 92.97± 0.12 µg/ml revealed potent antioxidant activity comparable to standard ascorbic acid (IC_{50} 43.42 ± 0.22 µg /ml) as shown in **Table 2**. Data of percentage inhibition showed that amongst all the test compounds having hydroxyl groups as substituent showed highest activity. This may be due to the available OH group present in AS-5 & AS-4. The isolated compounds have shown good antioxidant effect, amongst all AS-5 has shown excellent activity. Rest of the compounds (AS-1 to AS-4) showed mild-to-moderate antioxidant effect.

TABLE 1: *IN-VITRO* ANTHELMINTIC ACTIVITY OF ISOLATED COMPOUNDS FROM *ANNONA SQUAMOSA* BARK

S. no.	Name of isolated compounds	Concentration (mg/ml)	Paralysis time (min.)	Death time (min.)
1	Albendazole (standard drug)	10	7.78 ± 0.47	31.87 ± 1.24
2	Control	---	----	-----
3	AS-1	10	14.63 ±1.08*	42.39 ±1.04**
4	AS-2	10	15.88 ± 0.32	45.62 ± 0.82*
5	AS-3	10	21.44 ±0.65	62.15 ± 0.29
6	AS-4	10	11.56 ±0.44***	37.62 ±1.02***
7	AS-5	10	12.07 ±0.28**	39.28 ±0.44**

Results expressed as ±SEM of six worms in each group. *p<0.01 and **p<0.001 as compared to control by Dunnet test

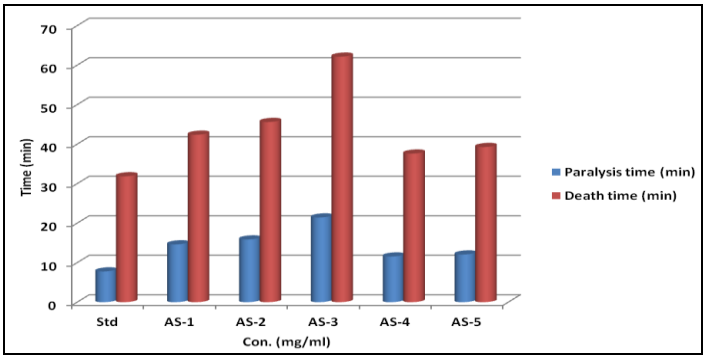

FIG. 1: *IN-VITRO* ANTHELMINTIC ACTIVITY OF ISOLATED COMPOUNDS FROM *ANNONA SQUAMOSA* BARK

***In-vitro* Antioxidant Activity:** DPPH method is the most tremendous, easiest and commonly used method for testing preliminary free radical-scavenging activity. DPPH is a stable free radical containing an odd electron in its structure and usually utilized for detection of the radical scavenging activity in chemical analysis. DPPH is known to abstract labile hydrogen [13 - 16]. DPPH-radical scavenging activity of isolated compounds from *Annona squamosa* (AS-1 to AS-5) plant was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH. Solution of DPPH was prepared and was added to all the isolated compounds (AS-1 to AS-5) at five concentrations (20, 40, 100, 200, 400 µg/ml). Thirty minutes later, the absorbance was deliberate at 517 nm. All the analysis was made with the use of UV-Visible Spectrophotometer (Shimadzu 1700). Absorbance of various concentrations was taken and percentage inhibition was calculated. Lower absorbance of the reaction mixture indicates higher free radical-scavenging activity.

Ascorbic acid was used as a standard antioxidant. IC_{50} (Inhibitory Concentration 50%) value denotes the concentration of sample required to scavenge 50% of the DPPH free radical (**Table 2**). IC_{50} of all isolated compounds was determined from % Inhibition *v/s* concentration graph (**Fig. 2**).

The percentage discoloration was calculated as follows: DPPH radical scavenging activity (%) =

$$[AC_{517} – AE_{517} / AC_{517}] \times 100$$

Where; AC_{517} is absorbance of a DPPH solution without fraction; AE_{517} is the absorbance of the tested compounds with DPPH.

Statistical Analysis: The results of the experiment were expressed as mean ± SEM. For group comparison, analysis of variance followed by Tukey's HSD multiple comparison test with SPSS version 10 was used. The difference among means considered statistically significant when *p*-value was less than 0.05.

TABLE 2: PERCENTAGE INHIBITION OF ISOLATED COMPOUNDS (AS-1 TO AS-5) AT VARIOUS CONCENTRATIONS

S. no.	Con. (µg/ml)	Standard (Ascorbic acid)	AS-1	AS-2	AS- 3	AS-4	AS-5
1	20	24.04 ± 0.0327	12.01 ± 0.1286	11.05± 0.2080	11.01± 0.1208	19.22± 0.1202	21.45± 0.5406
2	40	48.08 ± 0.1280	31.24 ± 0.0704	29.81± 0.2077**	28.41± 0.0224*	39.01± 0.3044	40.23± 0.2076
3	100	68.41± 0.0290	49.01 ± 0.0434**	49.05± 0.1306**	48.44± 0.0820*	52.01± 0.0266	61.01± 0.0456*
4	200	76.44 ± 0.1287	53.09 ± 0.0332**	61.49± 0.0670**	60.25± 0.0772	68.39± 0.1328*	72.45± 0.1206**
5	400	87.66 ± 0.2070	66.95 ± 0.0863***	69.44 ± 0.0904**	68.01± 0.6620	75.11± 0.0376**	78.01± 0.0896**
6	IC_{50}	43.42 ± 0.0506	122 ± 0.1366	108.01± 0.0804	113.19 ± 0.0772	92.97± 0.1206**	65.77± 0.1276***

Data represents mean ± S.E.M. of triplicate analysis, p* <0.05 compared to control

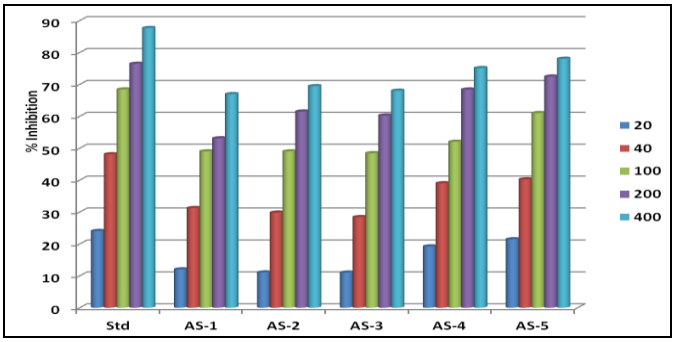

FIG. 2: DPPH SCAVENGING ASSAY OF ISOLATED COMPOUNDS FROM *ANNONA SQUAMOSA* BARK AND COMPARED WITH STANDARD ASCORBIC ACID (% INHIBITION *vs.* CONCENTRATION)

CONCLUSION: This study indicated the potential usefulness of isolated compounds from *Annona squamosa* bark against earthworm infections and provides a rationale for the traditional use of this plant as anthelmintic. Subsequently, in biological screening, the compounds showed potent antioxidant agent. Further research on these plants core is needed for the discovery of a potent anthelmintic and antioxidant agent. Thus we observed that there is enough scope for further study in developing such compounds as a good lead molecule with better pharmacological profile.

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CONFLICT OF INTEREST: None of the author has any conflict of interest in the context of this work.

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