https://doi.org/ 10.48047/AFJBS.6.5.2024. 9368-9380

African Journal of Biological Sciences

Phytochemical Profiling and Evaluation of Antidiabetic Properties of *Annona squamosa* Seed and Bark Extracts

Sabiha Iram[1], Asma Bee[1], Prashant Kumar[1], Shashi Bhooshan Tiwari[1], Shiv Dev Singh[1]*

[1]Department of Pharmacy, MJP Rohilkhand University, Bareilly-243006 Uttar Pradesh, India

Corresponding Author: Dr. Shiv Dev Singh

Department of Pharmacy, MJP Rohilkhand University,

Bareilly-243006 Uttar Pradesh, India

E-mail: shivdev.singh@mjpru.ac.in

Article History
Volume 6, Issue 5, 2024
Received: 15 May 2024
Accepted: 02 Jun 2024
doi: ***10.48047/AFJBS.6.5.2024. 9368-9380***

ABSTRACT

Annona squamosa, known as custard apple, is a small tree from the Annonaceae family, commonly found in tropical and subtropical regions. The present study was designed to investigate the phytochemical properties and antidiabetic potential of Annona squamosa seed and bark extracts. The extracts were prepared using Soxhlet extraction with n-hexane, and their phytochemical composition was analyzed, revealing the presence of alkaloids, cardiac glycosides, saponin glycosides, and flavonoids. FTIR analysis confirmed the presence of bioactive functional groups. Using Wistar albino rats, diabetes was induced via alloxan monohydrate, and the effects of the extracts on blood glucose levels and body weight were evaluated over ten days. The results indicated significant hypoglycemic effects and improved body weight in extract-treated diabetic rats, comparable to the standard drug glibenclamide. Both extracts also improved body weight in diabetic rats. The findings suggest that Annona squamosa extracts have notable antidiabetic properties and potential for developing new antidiabetic treatments. Further research is necessary to fully elucidate the mechanisms underlying these effects and to establish the safety and efficacy of Annona squamosa.

Keywords: Annona squamosa, Seed and bark, Alloxan-induced diabetes, Antidiabetic activity

1 INTRODUCTION

Annona squamosa, commonly referred to as the custard apple, sugar apple, or sweetsop, is a small, densely branched tree belonging to the Annonaceae family. It typically grows to a height of 5 to 7 meters and thrives in hot climates, adapting well to various soil types. It is widely found in India, West Indies, America, Egypt, Brazil, and other sub-tropical countries[1,2]. It is a plant native to the deep forests of the Indian subcontinent, has now been domesticated and is being cultivated systematically. The development of various hybrid varieties has not only increased its yield but also enhanced its therapeutic properties[3]. Today, the cultivation of Annona species has expanded and they are thriving crops in numerous tropical and subtropical countries[4.] The seeds of custard apples germinate within three weeks at temperatures between 18°C to 25°C[5]. This plant exhibits diverse activities, such as anti-cancer and anti-tumor activity[6, 7, 8], analgesic and anti-inflammatory activity[9,10,11], anti-oxidant activity[12, 13], antidiabetic activity[14, 15], anti-bacterial activity[16, 17, 18], pesticidal/insecticidal activity[19, 20].Additionally, its seeds serve as effective insecticides, and the oil derived from them acts as a repellent against pests such as aphids, white mealy bugs, grasshoppers, caterpillars, and plant hoppers[5]. The main focus of this study was directed towards the phytochemical and antidiabetic evaluations of extracts from *Annona squamosa* seed and bark. In the present study the extract has been characterized by qualitative and quantitative estimation methods by FTIR analysis.

2 MATERIAL AND METHODS

2.1 Chemicals and instrumentation

All chemicals used were of analytical grade: n-Hexane (Merck, Mumbai, India), Alloxan monohydrate (Spectrochem, Mumbai, India), Glibenclamide (Emcure Pharmaceuticals, Pune, India), Normal saline, Glucose, Distilled water, Soxhlet apparatus, ACCU-CHEK Instant S blood glucometer.

2.2 Collection and authentication of the plant

Annona squamosa were collected from MJP Rohilkhand University, Bareilly (UP) campus and authenticated by Dr. Alok Shrivastava, Department of Plant Science, MJP Rohilkhand University, Bareilly (UP) India.

2.3 Preparation of plant extracts

Seeds and bark flakes were washed, air-dried and grinded. To prevent any moisture absorption, the prepared seed and bark samples were stored in airtight containers until further analysis. Dried

powder from *Annona squamosa* seeds and bark were subjected to Soxhlet extraction separately using n-hexane for 12-24 hours. The extract was concentrated through a rotary vaccum evaporator. It was kept in air-tight containers and stored in a desiccator for further phytochemical and pharmacological evaluation.

2.4 Phytochemical identification of *Annona squamosa* extracts

The preliminary phytochemical identification or the extracts of *Annona squamosa* was conducted by standard chemical tests for the presence and absence of flavonoids, tannins, cardiac glycosides, saponin glycosides, steroids. FTIR spectral analysis was conceded from the *Annona squamosa* seed oil to detect the functional groups of secondary metabolites those were present in sample.

2.5 Experimental animals

Wistar albino rats (8–10 weeks) of either sex were used in the present study. Before and during the experiment, rats were fed with standard diet. After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 15 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived of food and water for 16 hours ad libitum. Ethical clearance was obtained from Institutional Animal Ethics Committee (IAEC), Department of Pharmacy, M.J.P Rohilkhand University, Bareilly (Reg. No. 1884/GO/Re/S/16/CPCSEA).

2.6 Experimental protocol

The study involved five groups of six rats each. Group I, the normal control, received distilled water. Group II, the diabetic control, was treated with a single dose of alloxan monohydrate (150 mg/kg, i.p) to induce diabetes. Group III received glibenclamide (5 mg/kg, p.o) for 10 days as a standard treatment. Groups IV and V were treated with *Annona squamosa* seed extract and bark extract (300 mg/kg, p.o) respectively, for 10 days. Diabetes induction followed Ahmed et al.[21], and an oral glucose tolerance test was performed following Joy et al.[22] The rats fasted for 24 hours prior to alloxan injection, which was freshly prepared in normal saline. Rats with blood glucose levels above 140 mg/dl were considered diabetic. Treatments were administered orally once daily for 10 days using feeding cannulas. Blood glucose levels and body weights were measured on days 1, 3, 7, and 10 using an ACCU-CHEK Instant S blood glucometer and a weighing scale, respectively.

3 RESULT

3.1 Phytochemical identification

Table 1: Phytochemical identification of seeds and bark of *Annona squamosa*

Phytochemical Test	**Seed Extract**	**Bark Extract**
Alkaloids	+	+
Cardiac Glycosides	+	+
Saponin Glycosides	+	+
Steroids	+	-
Tannins	-	+
Flavonoids	+	+

The IR spectrum of *Annona squamosa* seed oil is shown in Fig.1. Various peaks observed were found to be concordant with the functional groups present in the structure of *Annona squamosa* seed oil. FT-IR spectroscopy is a fast, non-destructive, and reliable method for confirming the purity of a procured drug. The wave number values for each peak have been mentioned below and found to be similar to the reported data Pathak *et al.*[23].

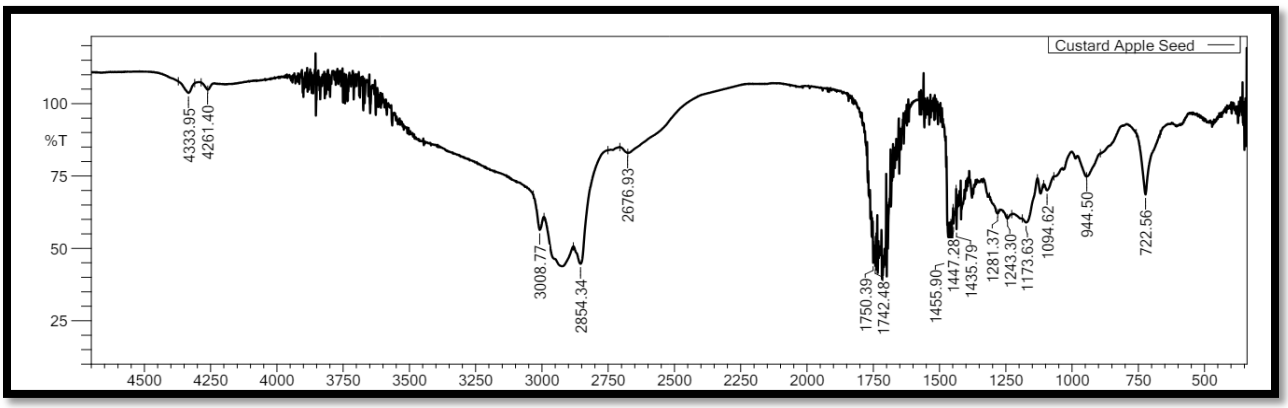

Fig 1:FTIR spectrum of *Annona squamosa* seed.

IR Spectra (KBr, cm^{-1}) 3008.77 (=C-H stretch, Dimer OH, Aromatic-H stretch); 2854.34 (C-H stretch, -CH2, Dimer OH); 1750.39 & 1742.48 (C=O stretch doublet, C=O stretch); 1455.9, 1447.28 (C-C stretch, -CH3, -CH2); 1173.63 (C-F stretch, C=S, P-H, P=O, C-O stretch)

3.2 Effect of *Annona squamosa* seed and bark extract on alloxan-induced diabetic rats

The effect of *Annona squamosa* seed and bark extract on glucose tolerance test are shown in Figure 2 and Table 3. The administration of *Annona squamosa* improved the glucose tolerance in the fasted normal rats. Extract also showed significant hypoglycemic effect after 90 minutes of treatment. The blood glucose levels were 200.1 ± 3.68mg/dl in *Annona squamosa* seed extract, 223.00 ± 4.56 mg/ dl in *Annona squamosa* bark extract treated rats on day 0 (Table 4). In the glibenclamide-treated group, the blood glucose was 215.68 ± 4.93mg/dl on day 0. It may be noted in the above table that seed extract-treated rats showed a significant decrease in blood glucose compared to bark extract-treated rats after a daily dose of 300 mg/kg. These results align with Sangala et al., indicating a reduction in blood glucose levels with *Annona squamosa* extracts[14].After 10 days, the body weight of normal, standard and *Annona squamosa* extracts treated rats increased significantly, whereas diabetic control rats lost weight (Table 5). *Annona squamosa* treatment significantly improved body weight in alloxan-treated rats, though not dose-dependently. Glibenclamide treatment also significantly increased body weight compared to diabetic control rats, showing a greater effect than *Annona squamosa* treatments.

Table 3: Effect of *Annona squamosa* extract on oral glucose tolerance in normal and alloxan-induced diabetic rats

Groups	**Groups treatment blood sugar level**				
	Fasting	**30 min**	**60 min**	**90 min**	**120 min**
Control Group	76.5±0.5	148.46±0.2	172.5±0.5	121.3±1.52	85.66±2.08
Diabetic Group	249.53 ± 0.5$^{\$}$	327.8±0.72$^{\$}$	369.43±1.69$^{\$}$	310.6±1.52$^{\$}$	307.66±1.52$^{\$}$
Standard Group (Glibenclamide)	76.66±1.52	156.6±1.5	185.86±1.80	121.33±1.52	86.34±1.52
Test Group (*Annona squamosa* seed extract)	72.66±1.57*	151.5±1.32*	175.26±1.61*	123.2±0.72*	84.16±0.76*
Test Group (*Annona squamosa* bark extract)	70.6±1.21$^{\#}$	145.2±1.70$^{\#}$	172.27±1.10$^{\#}$	119.67±0.577$^{\#}$	83.567±1.50$^{\#}$

Data indicates as mean ± S.D, two-way ANOVA followed by Tukey's post-hoc test.$^{\$}$p<0.05 vs. control group, *p<0.05 vs. test group of seed extract and $^{\#}$p<0.05 vs. test group of bark extract.

Table 4: Effect of treatment of *Annona squamosa* extracts on blood glucose level in alloxan induced diabetic rat.

Groups	**Blood glucose level (mg/dl)**			
	Day 0	**Day 3**	**Day 7**	**Day 10**
Control group	94.96 ± 1.55	89.81 ± 2.07	84.08 ± 4.00	78.96 ± 4.28
Diabetic Control Group	208.5 ± 2.61$^{\$}$	211.83 ± 6.05$^{\$}$	212.33 ± 3.93$^{\$}$	215.17 ± 4.54$^{\$}$
Standard Group (Glibenclamide)	215.68 ± 4.93	207 ± 3.74	156.67 ± 3.14	128.33 ± 2.73
Test Group (*Annona squamosa* seed extract)	200.1 ± 3.68*	185.33 ± 5.5*	154.67 ± 5.35*	141.83 ± 2.32*
Test Group (*Annona squamosa* bark extract)	223.00 ± 4.56$^{\#}$	217.17 ± 3.43$^{\#}$	204.17 ± 3.76$^{\#}$	185.17 ± 3.66$^{\#}$

Data indicates as mean ± S.D, two-way ANOVA followed by Tukey's post-hoc test.$^{\$}$p<0.05 vs. control group, *p<0.05 vs. test group of seed extract and $^{\#}$p<0.05 vs. test group of bark extract.

Table 5: Effect of aqueous extract of *Annona squamosa* extract on body weight in alloxan-induced diabetic rats

Groups	**Change in body weight (g)**			
	Day 0	**Day 3**	**Day 07**	**Day 10**
Control group	133.5 ± 0.5	135.96 ± 00.9	138.67 ± 0.41	139.36 ± 1.34
Diabetic Control Group	138.467 ± 0.5$^{\$}$	135.96 ± 0.25$^{\$}$	130.93 ± 0.64$^{\$}$	128.8 ± 0.26$^{\$}$
Standard Group (Glibenclamide)	138.81 ± 0.93	140.73 ± 0.64	143.16 ± 2.05	145.67 ± 1.52
Test Group (*Annona squamosa* seed extract)	128.5 ± 0.79$^{\#}$	131.16 ± 1.05$^{\#}$	136..56 ± 1.72$^{\#}$	140.3 ± 0.75$^{\#}$
Test Group (*Annona squamosa* bark extract)	126.067 ± 0.30*	129.3 ± 0.6*	133.56 ± 1.20*	137.5 ± 1.32*

Data indicates as mean ± S.D, two-way ANOVA followed by Tukey's post-hoc test.$^{\$}$p<0.05 vs. control group, *p<0.05 vs. test group of seed extract and $^{\#}$p<0.05 vs. test group of bark extract.

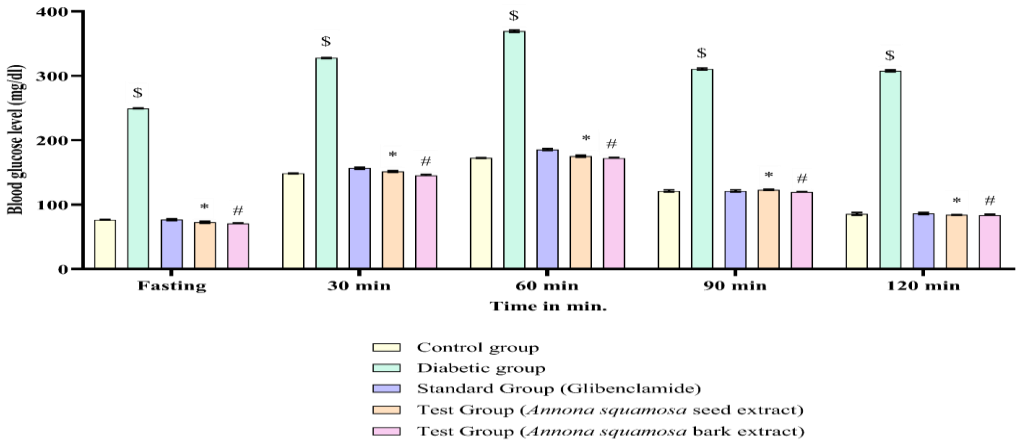

Fig 2: Effect of *Annona squamosa* extract on oral glucose tolerance in normal and alloxan-induced diabetic rats

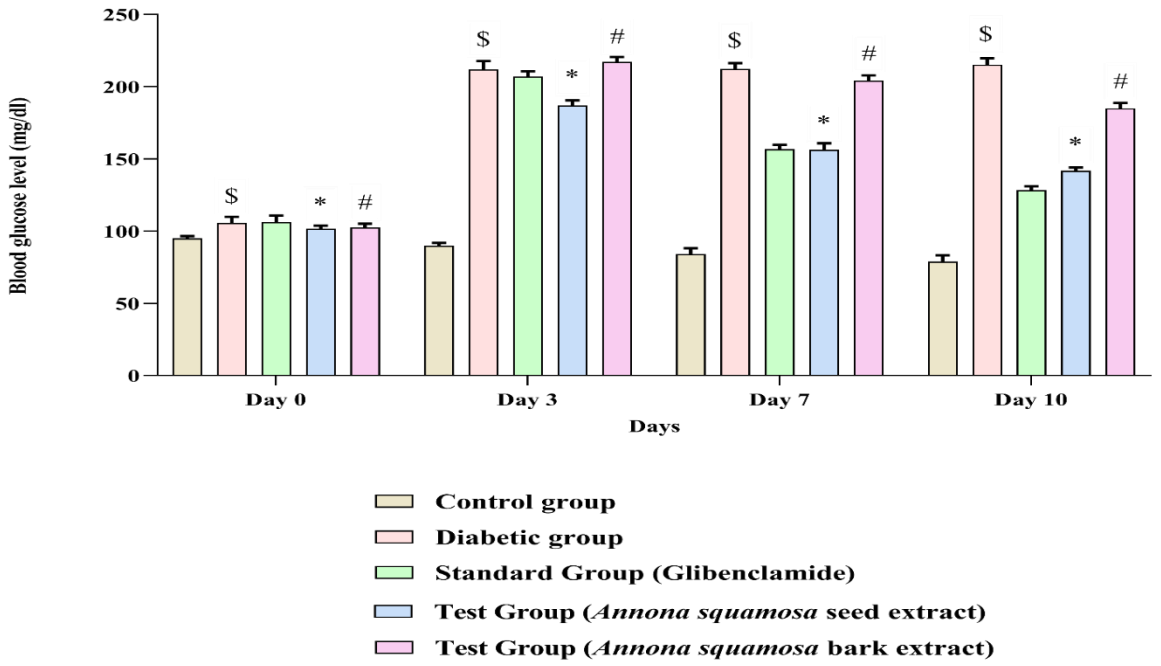

Fig 3: Effect of treatment of *Annona squamosa* extracts on blood glucose level in alloxan induced diabetic rat

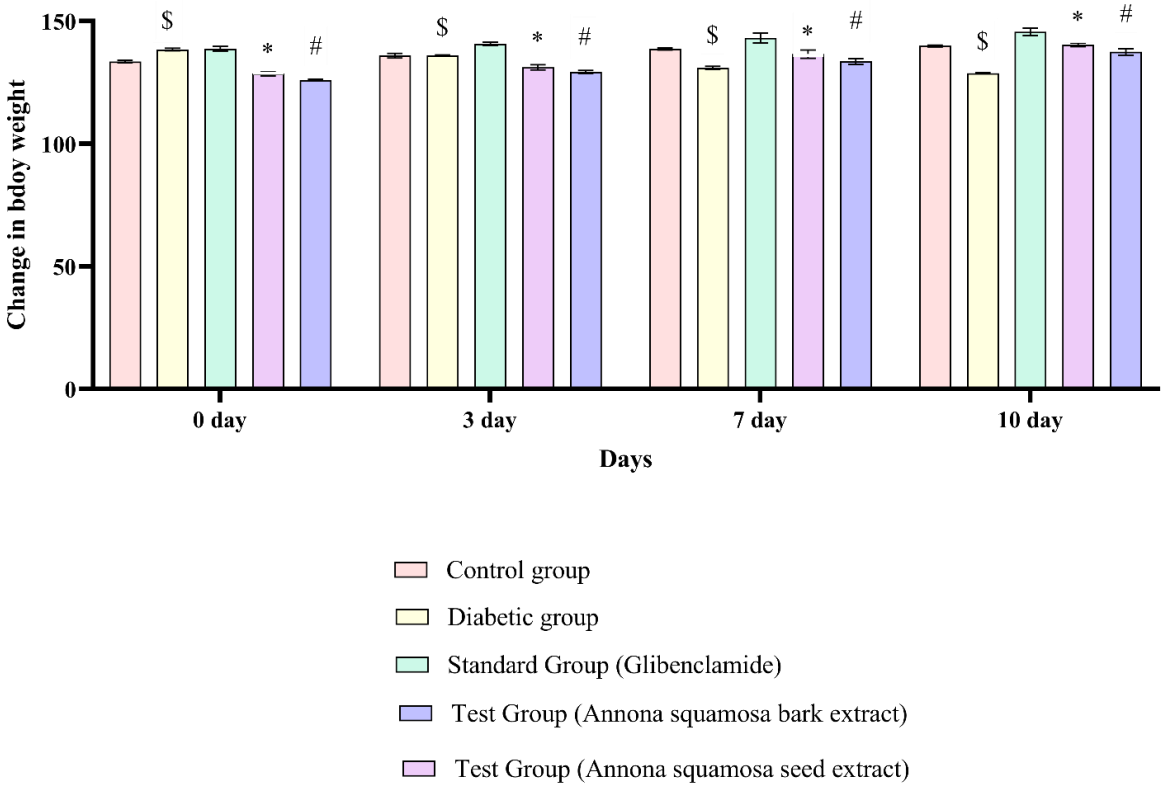

Fig. 4: Effect of *Annona squamosa*on body weight in alloxan induced diabetes in rats.

4 DISCUSSION

The phytochemical screening of *Annona squamosa* seed and bark extracts revealed the presence of various bioactive compounds. Both the seed and bark extracts tested positive for alkaloids, cardiac glycosides, saponin glycosides, and flavonoids. However, steroids were present only in the seed extract, and tannins were found only in the bark extract. These findings suggest that *Annona squamosa* contains various phytochemicals that might contribute to its diverse biological activities.The FT-IR analysis of *Annona squamosa* seed oil revealed several characteristic peaks corresponding to functional groups typically found in bioactive compounds. Peaks observed at 3008.77 cm^{-1}, 2854.34 cm^{-1}, and 1750.39 cm^{-1} indicate the presence of alkenes, carboxylic acids, and ketones, respectively. The presence of these functional groups aligns with the phytochemical screening results, further confirming the rich chemical diversity of *Annona squamosa* seeds.In the experimental design, alloxan-induced diabetic rats treated with *Annona squamosa* seed showed a significant decrease in blood glucose levels as compared to bark extracts over a 10-day period. On day 10, the blood glucose levels in seed extract-treated rats dropped from 200.1 ± 3.68 mg/dl to 141.83 ± 2.32 mg/dl, while bark extract-treated rats showed a reduction from 223.00 ± 4.56 mg/dl to 185.17 ± 3.66 mg/dl. These reductions were comparable to the standard antidiabetic drug, glibenclamide, which brought down blood glucose levels to 128.33 ± 2.73 mg/dl on day 10.The administration of *Annona squamosa* extracts also had a beneficial effect on the body weight of diabetic rats. While the diabetic control group experienced a significant decrease in body weight, rats treated with *Annona squamosa* seed and bark extracts showed an increase in body weight over the 10-day period. This improvement in body weight suggests a general amelioration of diabetic symptoms and an overall improvement in health status. The body weight of seed extract-treated rats increased from 163.17 ± 2.19 g on day 0 to 171.09 ± 0.83 g on day 10, and bark extract-treated rats increased from 158.52 ± 0.59 g to 171.14 ± 0.79 g, which is a significant recovery from the weight loss observed in untreated diabetic rats.

5 CONCLUSION

The study provides substantial evidence for the antidiabetic potential of *Annona squamosa* seed and bark extracts. The presence of bioactive compounds such as alkaloids, flavonoids, and saponin glycosides in the extracts supports their use in traditional medicine and highlights their potential for developing novel antidiabetic therapies. The significant reduction in blood glucose

levels and improvement in body weight in diabetic rats treated with *Annona squamosa* extracts underscore the plant's therapeutic promise. However, further research is necessary to fully elucidate the mechanisms underlying these effects and to establish the safety and efficacy of *Annona squamosa*.

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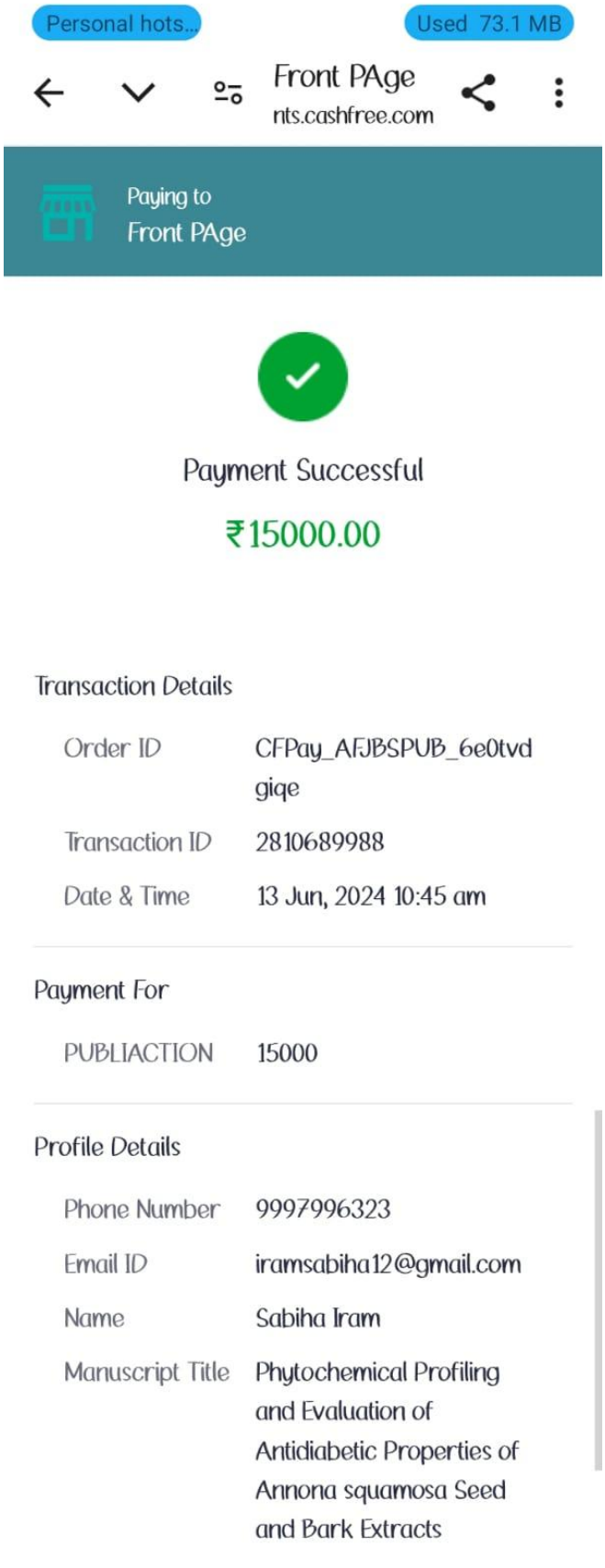
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