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Research

Assessment of the Pharmacognostic Phytochemicals and Antidiabetic properties of the flowers of *Acacia nilotica Linn.*

Ashutosh Singh[1]*, Vivek Gupta[2], Shivam Raikwar[3], Gyan Singh[4]

[1]*PG, Research scholar, Faculty of Pharmacy, PK University, Thanra, Shivpuri, M.P, India*
[2]*Professor, Faculty of Pharmacy, PK University, Thanra, Shivpuri, M.P, India*
[3]*Assistant Professor, Faculty of Pharmacy, PK University, Thanra, Shivpuri, M.P, India*
[4]*Associate Professor, Faculty of Pharmacy, PK University, Thanra, Shivpuri, M.P, India*

Corresponding Author:
Ashutosh Singh

Email:
ashusingh9169@gmail.com

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Abstract:

Objective: The quality criteria for herbal medicines are based on an accurate scientific description of the source material. Herbal resources are commonly acquired, processed, and released into the market without completing the necessary safety and toxicological testing, despite their immense potential as natural remedies and their substantial financial worth. This led to an attempt to assess phytochemical and toxicological characteristics in the flowers of the popular herbal remedy A. nilotica linn, including heavy metals, aflatoxins, total microbial load, and pesticide residues. **Method:** The procedures recommended by AOAC and ASTA were followed in order to determine the analysis of heavy metals, aflatoxins, microbiological load, and pesticide residues. Thin layer chromatography was used to separate the chemical components of the medication. **Result and conclusion:** Although pesticidal residues, heavy metals, aflatoxins, and the total microbial load varied, the experiment's results demonstrated that they were all below permitted limits. A phytochemical examination of A. nilotica revealed that the former lacked phytoconstituents known as catechins. Therefore, in order to ensure the safety and efficacy of Indian medicinal plants and increase their adoption on a worldwide scale, it is essential that these qualities be assessed in all crude medications prior to further processing. The study's identification of unique IDs for the particular crude medicine will aid in the detection and prevention of adulterated raw drugs.

Keywords: A. nilotica flowers, Phytochemical, microbiological load, TLC and Catechin

1. INTRODUCTION

Herbal medicines have been used in medicine for thousands of years, and they are especially prized as an easily available and reasonably priced kind of medical care. Traditional herbs and herbal products have long been regarded as safe, non-toxic, and even mild due to their natural state. Because medicinal plants are susceptible to insects and diseases in the field and during storage, much like other crops, pesticides are commonly used to protect them. Actually, complaints of tainted crude medical plants and their products have been increasing.[1,2] This has raised questions about the professionalism of practitioners, the effectiveness, safety, and quality of their treatment plans, as well as the market's supply of natural and herbal goods. Organic farming is only feasible on a small scale due to the high expense of pesticide-free cultivation, and there are not enough wild raw materials to fulfill the demands of the herbal medication industry. Large-scale medical plant production is required due to the steadily rising

demand for these plants, which cannot be achieved without the usage of pesticides. Because of their toxicity and environmental durability, organochlorine pesticides (OCPs) and common pesticide contamination often receive the most attention.[3,4].

Similarly, secondary metabolites known as aflatoxins (AFs) are produced by the Aspergillus fungus, particularly flavus and parasiticus. [5] Of the four naturally occurring AFs (designated B1, B2, G1, and G2), AFB1 is the most common and dangerous. Numerous studies have examined the mutagenic, immunosuppressive, and carcinogenic effects of aflatoxin on a range of animals.[6] Aflatoxin levels in the daily diet are closely linked to human liver cancer, according to epidemiological study.[7] Furthermore, previous studies show that AFs do not decompose at the temperature of boiling water while preparing a drink, and that cooking at home with a microwave or a conventional gas oven does not reduce aflatoxins' levels. [9] Therefore, controlling the amount of aflatoxins in food requires a simple, quantitative testing method. In 2002, European rules placed maximum limitations on spices (Capsicum spp., Piper spp., Myristica fragrans, Zingiber officinale, and Curcuma longa) (AFB15 g/kg; total AFs 10 g/kg) (02/472/EC). To ensure the quality of the preparations prepared from traditional herbs, it is crucial to employ modern techniques and suitable standards.

Standardizing the raw materials utilized is essential to maintaining quality control in the herbal industry. Many analytical parameters, such as physico-chemical constants, element estimations, heavy metals, microbiological contamination, aflatoxins, and pesticide residue, need to be tested as part of a quality check. The World Health Organization [10] has emphasized certain quality standards and proposed certain processes for the assessment and development of standard herbal products.

Members of the Leguminosae family include Acacia nilotica. It is a very large tree with numerous fissures and tall, straight, white, extremely thorny spines. Globule heads are present in the golden, fragrant axillary flowers of A. nilotica [11]. The components of the tree are used to cure skin problems, diabetes, and leucorrhea, according to ethnomedical literature. They are also used as anti-diarrheal, anti-dysenteric, and antidiabetic drugs. Stem bark is used as an astringent to cure toothaches, ulcers, and wound healing. It is also used to treat diarrhea, avoid snake bites, and treat toothaches. This work aimed to investigate the phytochemical assessment of A. nilotica and its flowers, aflatoxins, microbiological load, and heavy metal pesticide residues [12–14].

MATERIALS AND METHOD

Parameters such as moisture content, total ash, water soluble ash, and acid insoluble ash were calculated using the methods suggested by WHO recommendations (Anonymous, 1998; Anonymous, 1996). Additionally, the proportion of water-soluble and alcohol-soluble extractive was calculated [15].

The following first phytochemical assays were performed after the A. nilotica shadow-dried flowers were independently ground into powder.

1.1 Ash value

The total ash content of the crude medication is the inorganic residue that remains after burning. It represents both the naturally occurring inorganic salts in the drug and the inorganic material from external sources [16].

To determine the ash value, a dry, pre-weighed silica crucible holding exactly 2 g of the powdered sample was fired in a muffle furnace for 6 hours at a temperature of no more than 450° C. The igniting procedure was repeated until the weight stayed constant. The proportion of ash was calculated in respect to the air-dried sample.

1.2 Acid Soluble Ash value

The whole ash was boiled in 25 milliliters of 1N hydrochloric acid for five minutes, and then the mixture was filtered using Whatmann ashless filter paper. The residue was washed with hot water, burnt, and then allowed to cool in desiccators before being weighed. The amount of acid-insoluble ash was calculated in relation to the air-dried sample. [17–18]

1.3 Acid Soluble Ash value

It was filtered through ash-less filter paper after the full quantity of ash was boiled for five minutes with 25 milliliters of water. The residue was washed with hot water, then burned for 15 minutes at a temperature of no more than 450° C, cooled, and weighed. This weight was subtracted from the weight of the ash; the weight difference represents the water-soluble ash. The percentage of water-soluble ash in the air-dried sample was calculated. [19]

1.4 Alcohol soluble extractive

About 5 g of coarsely powdered material was macerated with 100 ml of 95% alcohol in a closed flask for 24 hours. The mixture was stirred

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frequently for the first 6 hours and then allowed to stand for 18 hours. It was then rapidly filtered so that no alcohol was lost. 25 ml of the filtrate was pipetted out and allowed to evaporate on a water bath in a 100 ml beaker that had been previously weighed. In a hot air oven, the extract was kept at 105ºC until its weight didn't change. The percentage of extractive that is soluble in alcohol was calculated using the air-dried sample as a reference. [20]

1.5 Water soluble extractive

5 g of coarsely powdered material and 100 ml of distilled water were macerated for 24 hours in a sealed flask, with frequent stirring for the first 6 hours before the mixture was allowed to stand for 18 hours. It was then rapidly filtered so that no alcohol was lost. 25 ml of the filtrate was pipetted out and allowed to evaporate on a water bath in a 100 ml beaker that had been previously weighed. In a hot air oven, the extract was kept at 105ºC until its weight didn't change. The amount of water-soluble extractive was calculated using the air-dried sample as a reference.[21]

1.6 Loss on Drying:

After being precisely weighed on a tarred dish, two grams of the powdered leaves were baked for an hour at 105° C. After cooling in a desiccator, it was weighed once again. The amount of dried powder that was obtained and tabulated was used to compute the drying loss.[22-24]

2. PHYTOCHEMICAL EVALUATION

Preliminary study of the flower powder of AN and AL

S.No	Content	Powder
1	Nature of the powder	AN
2	Colour	Coarse
3	Odour	Yellow
4	Taste	Aromatic

2.1 Extraction procedure

Preparation of the Hydro alcoholic extract of the flowers of AN:

The soxhlet apparatus was used to extract 70% ethanol from the shade-dried and powdered flowers of A. nilotica (3 kg). Reduced pressure was used to concentrate the hydroalcoholic extract. After being vacuum-dried, a dry brown residue was produced. [25–27]

Qualitative chemical test for AN and AL

Chemical tests	***AN* Alcoholic extract**
STEROLS	
a) Salkowski test	+
b) Liebermann Burchard's test	+
TERPENOIDS	+
CARBOHYDRATES	
a) Molisch's test	+
b) Fehling's test	+
FLAVONOIDS	
a) Shinoda test	+
b) Alkali test	+
PROTEINS	
a) Millon's test	-
b) Biuret test	-
ALKALOIDS	
a) Mayer'sreagent	+
b) Dragendorff's reagent	+
c) Wagner's reagent	+
SAPONINS	+
GLYCOSIDES	+
TANNINS	+

2.2 TLC and HPTLC fingerprinting analysis

TLC analysis

The alcoholic extracts of AN flowers included gallic acid, quercetin, and luteol, according to the TLC findings. The simultaneous measurement of quercetin and gallic acid in AN flowers using the same TLC technique has never been reported before. The retention factors (Rf) of the two spots, as determined by chromatography in a thin-layer of

silica gel, are 0.22, 0.36, and 0.70, respectively, and they match the standards. [28]

Gallic acid Quercetin Lupeol

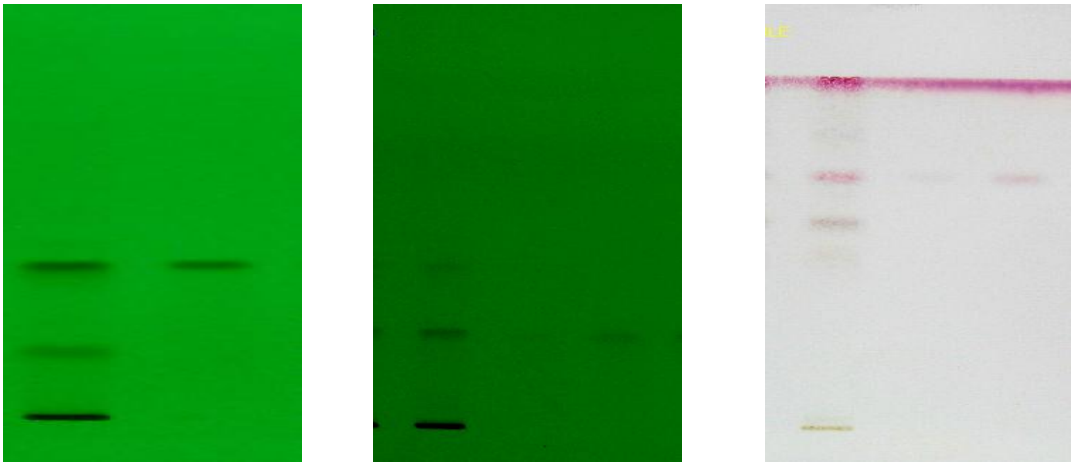

Figure 1. TLC of AN flower

Chromatographic conditions

Chromatography was carried out using a 20 x 20 cm, 0.2 mm thick HPTLC silica gel 60F254 plate. Using an automated TLC applicator Linomat V with N2 flow (CAMAG, Switzerland), the sample and standards were placed to the plate as 6 mm broad bands, with a 13 mm gap between two bands and 10 mm from the bottom. These bands were the same for all studies. [29]

Detection of Gallic acid and Quercetin

Each HPTLC plate was developed to a height of about 8 cm using a CAMAG twin trough glass tank that had been presaturated with the mobile phase Toluene-Ethyl Acetate-Formic Acid (6:4:0.8) for an hour. The HPTLC runs were conducted in a lab setting with 50% relative humidity and 25 ± 5ºC. Following development, the plate was taken out and allowed to dry. UV light at 254 and 366 nm was used to view the spots (UV cabinet, CAMAG, Switzerland). [30]

Detection of Lupeol

Each HPTLC plate was developed to a height of about 8 cm using a CAMAG twin trough glass tank that had been pre-saturated with the mobile phase Toluene-ethyl acetate (7:3) for an hour. The HPTLC runs were conducted in a lab setting with 50% relative humidity and 25 ± 5 ºC. Following development, the plate was taken out and allowed to dry. UV light at 254 and 366 nm was used to view the spots (UV cabinet, CAMAG, Switzerland). Vanillin in sulfuric acid reagent was used for visualization, and it was heated to 105 ˚C till color appeared. [31–32]

Quantification

Under the following circumstances, a computer running Wincats software version 1.3.4 and a CAMAG TLC scanner 3 were used to quantify gallic acid, quercetin, and luteol: 6 x 0.45 mm slit width, UV (Deuterium lamp) wavelengths of 254 nm, 280 nm, and 538 nm, absorption-reflection detection mode. [33]

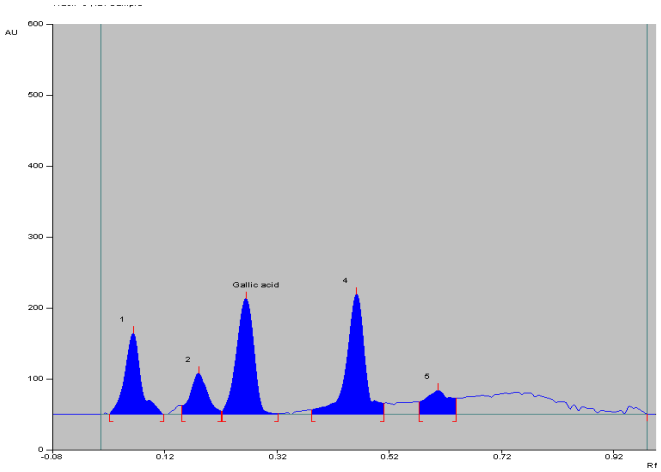

Figure 2 – HPTLC fingerprinting of AN containing Gallic acid

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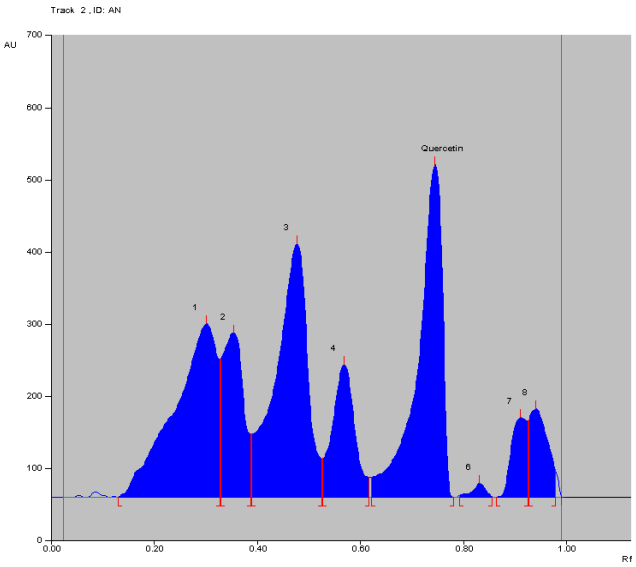

Figure 3 – HPTLC fingerprinting of AN containing Quercetin

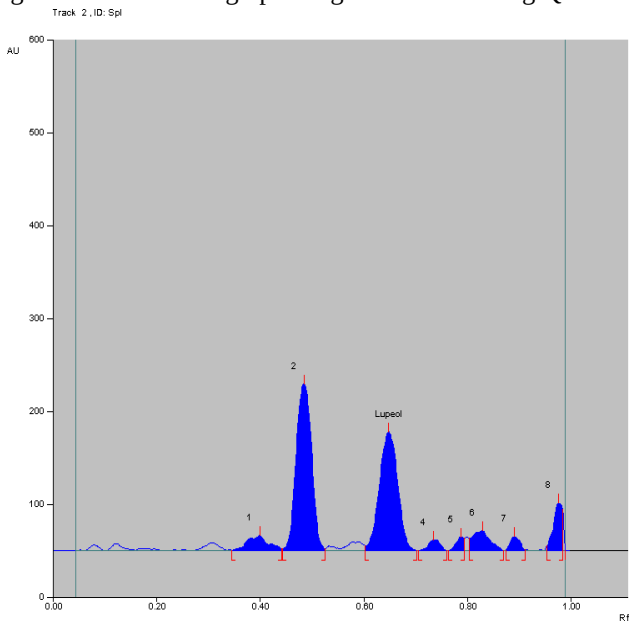

Figure 4 – HPTLC fingerprinting of AN containing Lupeol

3. TOXICOLOGICAL EVALUATION

Experimental design

Every rat was examined for any indications of clinical harm, such as changes in respiratory patterns, body coloration, locomotion patterns and types, severe involuntary muscle contractions or seizures of voluntary muscle contractions, loss of reflexes, and other symptoms, first at the first, second, fourth, and sixth hours, and then once daily for 14 days.

Group	Treatment
I	For 14 days, the normal control animals were given 5 millilitres of distilled water per kilogramme of body weight.
II	The animals received hydro alcoholic extract of AN at dose of 2000 mg/kg body weight for 14 days

Collection of blood, pancreas and kidney

All of the rats received ether at the end of the research to produce anesthesia so that blood could be drawn. To measure hematological parameters, we used a capillary tube to draw blood from the retroorbital plexus and then put it into sterile, dry EDTA vials. Blood was drawn in heparinized tubes for biochemical examination. Within five minutes after collection, the heparinized blood was centrifuged for ten minutes at 2500 rpm. A dry and sanitized sample container was filled with plasma.[34]

Following blood collection, the kidney and pancreas were immediately removed and carefully

cleaned with ice-cold physiological saline. As soon as they were removed from the animal, they were then preserved in 10% formalin.

Estimation of Biochemical Parameters

The levels of biochemical indicators such as SGOT, SGPT, ALP, and total bilirubin were measured using the Humazym MUV test kits. According to Dacie and Lewis (1991), the superior Neubauer counting chambers were used to measure the WBC. The Cyameth-haemoglobin method used to measure hemoglobin (Hb) concentration is explained by Dacie and Lewis (1991).

Groups	Days			
	0	21	28	41
Group I	170.2±9.7	202.8±8.6	207.8±7.2	NA
Group II	172.0±6.0	193.1±6.1	196.6±6.5	NA
Group III	172.5±6.9	198.7±8.2	202.5±7.3	NA
Group IV	167.4±6.0	197.4±6.5	202.2±7.6	NA
Group V	168.0±4.3	195.4±6.7	200.2±7.0	235.8±7.4
Group VI	160.0±6.9	215.1±7.1	233.3±7.1	NA
Group VII	153.4±6.6	209.8±6.7	226.8±6.8	NA
Group VIII	156.3±6.9	212.1±6.3	230.5±6.1	NA
Group IX	163.5±5.8	212.5±8.4	229.5±8.8	265.1±8.1

4. Antidiabetic evaluation

Rats that were fasting and had not eaten or drunk for eighteen hours were used for the oral glucose tolerance test. Rats were split into eight groups of six, and each group received hydroalcoholic extracts of AN at dosages of 250, 500, and 1000 mg/kg, standard insulin at 5 IU/kg by intraperitoneal injection, and water (0.9% w/v NaCl). Half an hour after the extracts were provided, sugar (2 g/kg) was given. A glucose oxidase–peroxidase reaction strip and a glucometer (Accu-check sensor) were used to measure the concentrations of glucose.[35]

Experimental Design

The animal kingdom is made up of eight groups of animals. Group I rats' blood glucose levels were normal. STZ was given to the remaining seven groups in order to cause diabetes. To obtain the hydroalcoholic extract of AN flowers, gastric washing was used.

For 21 days, each group of animals follows the previously specified treatment strategy. Animals without food or water were lanced in the tail to draw blood on the first, seventh, fourteenth, and twenty-first days. The fasting blood glucose level was measured using test strips (One Touch Horizon Sensor test meter) and a professional glucometer. The distal part of the tail vein was used to draw blood.

Collection of blood, pancreas and kidney

All of the rats received ether at the end of the trial to create anesthesia so that blood could be drawn. Using a capillary tube, blood samples were drawn from each rat's retroorbital plexus and placed in sterile, dry EDTA vials for hematological examination. Heparinized tubes were used to collect blood for biochemical examination. Within five minutes after collection, the heparinized blood was centrifuged for ten minutes at 2500 rpm. The sample jar was dry and clean before the serum was put inside.[37]

Estimation of Biochemical parameter

In an autoanalyzer using Ecoline kits from E. Merck in Mumbai, India, we measured blood lipid profile using standard protocols, serum glucose, and plasma insulin (measured using the ELISA approach using a Boehringer Mannheim GmbH kit from Werk Penzberg, Germany).

Figure 5: showed the effect of AN hydro alcoholic extract on oral glucose tolerance test (OGTT) in normal rats

The values are expressed as mean ± SEM. n=6 animals in each group Statistical significant test for comparison was done by ANOVA, followed by Tukey test. The blood glucose values of groups are compared with normal control animals, values ***p<0.05.

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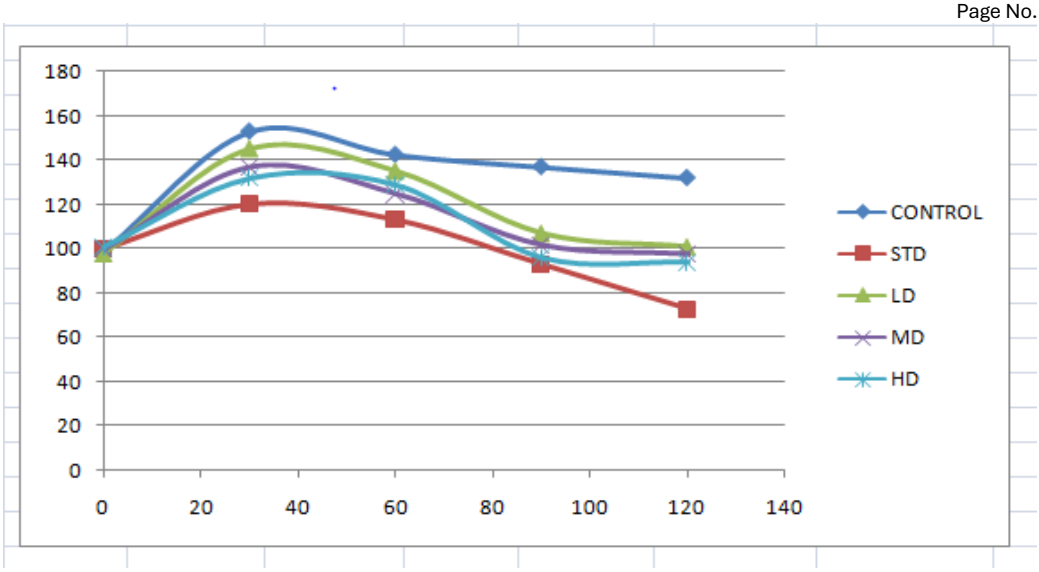

The rats' blood glucose levels significantly increased after 30 minutes of receiving a glucose load (2g/kg) in the oral glucose tolerance test. Diabetes was not present in the rats. Within 30 minutes of receiving insulin (5 IU/kg i.p.) the rats' glucose tolerance significantly improved. However, it dropped 120 minutes after the glucose infusion.

AN hydroalcoholic extracts dramatically improved the non-diabetic rats' OGTT glucose consumption. According to the results of the OGTT, the hydroalcoholic extracts AN were successfully administered to stop blood glucose levels from rising without causing hypoglycemia.

The hydroalcoholic extract of AN showed the highest glucose tolerance at 1000 mg/kg. The result might be explained by the insulin response, which was earlier delayed, returning to normal. Numerous medicinal plants, including Cassia auriculata (Latha and Pari 2003) and Elaeodendron glaucum (Debapriya et al. 2011), are claimed to have similar benefits in this case.

The changes in blood glucose levels brought on by AN after 0, 7, 14, and 21 days are shown in Table 16 for both experimental and healthy animals. Groups IV to VIII had considerably ($p<0.001$) lower blood glucose levels than groups II and III. From day 14 onward, it became clear that the end of day 21 saw the biggest drop in the serum blood glucose level.

Rats that were part of the experiment and those who were not are shown in Table 17 with their total cholesterol, HDL, LDL, and VLDL values. Compared to group I, group II's serum levels of T.CHO, TGL, LDL, and VLDL were significantly higher ($p<0.001$). When groups IV through VIII were given hydroalcoholic extracts AN and AL, their blood levels of T.CHO, TGL, LDL, and VLDL were significantly ($p<0.001$) lower at the end of the trial than those of group II. The levels of T.CHO, TGL, and VLDL were lower in groups III and VIII.

Group II's HDL levels were significantly lower than those of group I ($p<0.001$). When groups IV through VIII were administered hydroalcoholic extracts AN their HDL levels at the end of the study were significantly ($p<0.001$) higher than those of group II. In group VIII, the HDL levels have changed very little compared to group III.

CONCLUSION

The present study provides information in respect of their identification, physicochemical characters and chemical constituents which may be useful in standardization of herbal drugs of folk medicinal practice of present era and enrichment of Ayurvedic pharmacopoeia. The developed HPTLC method is an attractive alternative for the quantitative determination of quercetin, gallic acid and lupeol in methanolic extract of flower of AN with regard to the simplicity, accuracy and selectivity. This method could be widely applied directly for routine analysis and quality assurance of related extracts and drugs. The Chromatographic analysis of the petroleum ether extract of AN revealed the presence of phytoconstituents belonging to the type acids, esters, alcohols, ethers, etc. Thus, the medicinal plant AN was found to possess significant phytoconstituents. The presence of such a variety of phytochemicals may be attributed to the medicinal characteristics of the plant AN.

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