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PHARMACOGNOSTICAL, PHYTOCHEMICAL EVALUATION OF *ACACIA NILOTICA* AND *ACACIA LEUCOPHLOEA WILLD* FLOWERS

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ABSTRACT

Objective: A precise scientific characterization of the raw material serves as the foundation for the quality standards for herbal medications. Despite their enormous potential as natural medications and their significant commercial value, herbal resources are frequently sourced, processed, and introduced to the market without undergoing the required safety and toxicological testing. Based on this, an attempt was made to evaluate phytochemical and toxicological parameters such as heavy metals, aflatoxins, total microbial load, and pesticide residues in the flowers of the well-known herbal medication A. nilotica and A. leucophloea.

Method: To ascertain the analysis of heavy metals, aflatoxins, microbiological load, and pesticide residues, the protocols suggested by AOAC and ASTA were adhered to. The drug's chemical components were separated using a thin layer chromatographic method.

Result and conclusion: The experiment's findings showed that while pesticidal residues, heavy metals, aflatoxins, and the overall microbial load varied, they were all within the allowed ranges. When comparing the blooms of A. leucophloea to those of A. nilotica, a phytochemical analysis showed that the former lack catechin phytoconstituents. Therefore, it is imperative that these characteristics be evaluated in all crude drugs before they are processed further in order to guarantee the safety and effectiveness of Indian medicinal plants and improve their acceptance on a global scale. The study identified distinct identities for the specific crude drug, which will help detect and prevent raw drug adulterations.

KEYWORDS: A. leucophloea, A. nilotica flowers, Phytochemical, TLC and Catechin.

1. INTRODUCTION

For thousands of years, herbal remedies have been utilized in medicine, and they are particularly valued as an accessible and affordable source of healthcare. Because of their natural nature, traditional herbs and herbal products have been seen as gentle, non-toxic, and even harmless. Pesticides are frequently employed to protect medicinal plants because, like other crops, they are vulnerable to insects and illnesses both in the field and during storage. In actuality, there have been more and more reports of contaminated crude medicinal plants and their products.[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, Aspergillus fungus, especially flavus and parasiticus, create secondary metabolites called aflatoxins (AFs).[5] AFB1 is the most prevalent and harmful of the four naturally occurring AFs, which are labeled B1, B2, G1, and G2. Aflatoxin's mutagenic, immunosuppressive, and carcinogenic effects on a variety of animals have been well studied.[6] As epidemiological research, there is a close correlation between levels of aflatoxins in the daily diet and human liver cancer.[7] Additionally, prior research indicates that home cooking using a microwave or a traditional gas oven does not lower aflatoxins' levels, and that while

making a drink, AFs do not break down at the temperature of boiling water.[9] A straightforward and quantitative analytical technique is therefore crucial for regulating the amount of aflatoxins in food. Spices (Capsicum spp., Piper spp., Myristica fragrans, Zingiber officinale, and Curcuma longa) are subject to maximum restrictions imposed by European regulations in 2002 (AFB15 g/kg; total AFs 10 g/kg) (02/472/EC). Therefore, it is essential to use contemporary methods and appropriate standards to guarantee the quality of the preparations made from traditional herbs.

One crucial element in ensuring quality control in the herbal sector is standardizing the raw materials used. As part of a quality check, a number of analytical parameters, including physico-chemical constants, element estimates, heavy metals, microbiological contamination, aflatoxins, and pesticide residue, must be measured. The World Health Organization[10] has suggested specific procedures for the evaluation and creation of standard herbal products and has highlighted certain quality requirements.

Acacia leucophloea and Acacia nilotica are members of the Leguminosae family. It is a somewhat big tree with many cracks and long, straight, white spines that are highly prickly. The yellow, fragrant axillary blooms of A. nilotica have globule heads. The creamy yellow, fragrant axillary blooms of A. leucophloea have globule heads.[11] According to ethnomedical literature, the tree's components are used to treat leucorrhea, diabetes, and skin conditions. Additionally, they are utilized as antidiabetic, antidiarrheal, and antidysenteric medications. As an astringent, stem bark is used to treat toothaches, ulcers, and wound healing, as well as to prevent snake bites, treat dysentery, and treat toothaches. The investigation of heavy metal pesticide residues, aflatoxins, microbiological load, and phytochemical evaluation of A. nilotica and A. leucophloea flowers was attempted in this work.[12-14]

MATERIALS AND METHOD

The procedures recommended as per WHO guidelines (Anonymous, 1998; Anonymous, 1996) were followed to calculate the parameters like moisture content, total ash, water soluble ash and acid insoluble ash. The percentage of alcohol soluble and water soluble extractive was also determined.[15]

The shadow dried flowers of A.nilotica and A.leucophloea were powdered separately then subjected to the following preliminary phytochemical tests.

1.1 Ash value

The inorganic residue left over after burning is the crude drug's total ash content. It stands for both the inorganic stuff from outside sources and the inorganic salts that are naturally present in the medication.[16]

A dry, pre-weighed silica crucible containing precisely 2 g of the powdered sample was ignited for 6 hours at a temperature of no more than 450° C in a muffle furnace to measure the ash value. Until the weight remained steady, the igniting process was repeated. In relation to the air-dried sample, the percentage of ash was computed.

1.2 Acid Soluble Ash value

After five minutes of boiling the whole ash in 25 milliliters of 1N hydrochloric acid, the mixture was filtered using Whatmann ashless filter paper. After being cleaned with hot water, the residue was burned, allowed to cool in desiccators, and then weighed. In relation to the air-dried sample, the proportion of acid-insoluble ash was computed.[17-18]

1.3 Acid Soluble Ash value

After boiling the entire amount of ash for five minutes with twenty-five milliliters of water, it was filtered through ash-less filter paper. After being cleaned with hot water, the residue was cooled and weighed after being ignited for 15 minutes at a temperature of no more than 450° C. This weight was deducted from the ash's weight; the water-soluble ash is represented by the weight difference. In relation to the air-dried sample, the proportion of water-soluble ash was computed.[19]

1.4 Alcohol soluble extractive

In a closed flask, about 5 g of coarsely ground material was macerated with 100 ml of 95% alcohol for 24 hours, stirring regularly for the first 6 hours before being left to stand for 18 hours. After that, it was quickly filtered without losing any alcohol. In a 100 ml beaker that had been previously weighed, 25 ml of the filtrate was pipetted out and dried by evaporation on a water bath. The extract was maintained at 105ºC in a hot air oven until its weight remained constant. Using the air-dried sample as a reference, the proportion of alcohol-soluble extractive was determined.[20]

1.5 Water soluble extractive

In a sealed flask, 5 g of coarsely ground material was macerated with 100 ml of distilled water for 24 hours, stirring regularly for the first 6 hours before being left to stand for 18 hours. After that, it was quickly filtered without losing any alcohol. In a 100 ml beaker that had been previously weighed, 25 ml of the filtrate was pipetted out and dried by evaporation on a water bath. The extract was maintained at 105ºC in a hot air oven until its weight remained constant. Using the air-dried sample as a reference, the proportion of water-soluble extractive was determined.[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	
		AN	AL
1	Nature of the powder	Coarse	Coarse
2	Colour	Yellow	Light Yellow
3	Odour	Aromatic	Aromatic
4	Taste	Astringent	Astringent

2.1 Extraction procedure

Preparation of the Hydro alcoholic extract of the flowers of AN and AL

The shade dried and powdered flowers of A.nilotica and A.leucophloea (3kg) were extracted with 70% ethanol by using soxhlet apparatus. The Hydro alcoholic extract was concentrated under reduced pressure. A dried brown residue was obtained and dried in vacuum.[25-27]

Qualitative chemical test for AN and AL

Chemical tests	*AN* Alcoholic extract	*AL* 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 TLC results showed the presence of Gallic acid, Quercetin and Lupeol in the alcoholic extracts of AN and AL flowers. There have been no previous reports of the simultaneous analysis of Gallic acid and Quercetin in AN and AL flowers using the same TLC method. According to chromatography in a thin-layer of silica gel, the retention factors (Rf) of the two spots are 0.22, 0.36 and 0.70 respectively, which are also the same as the standards.[28]

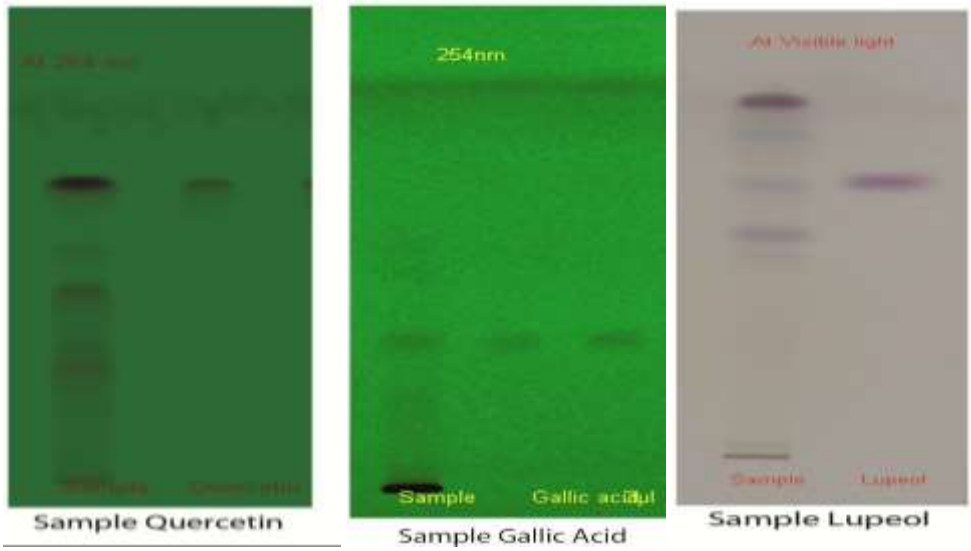
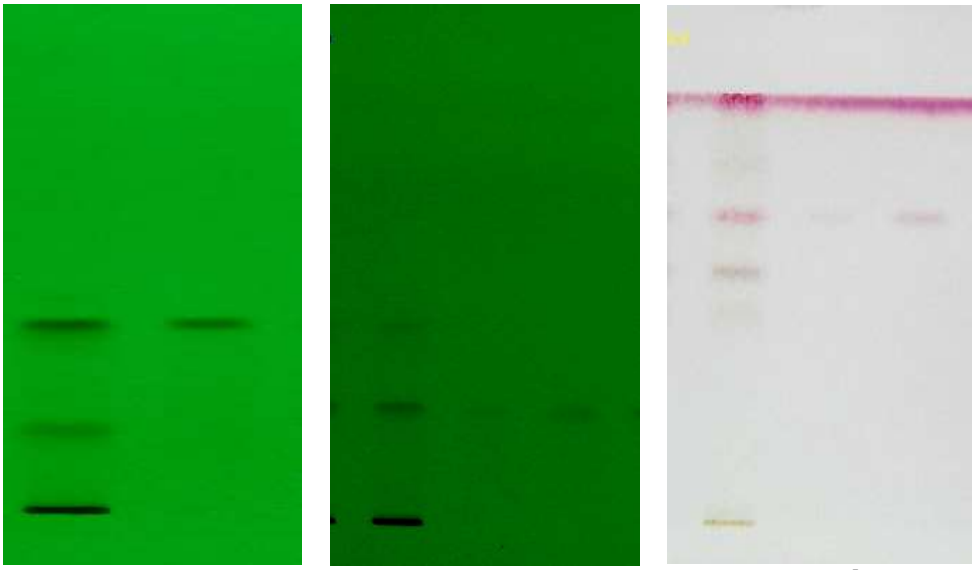


Figure 3: TLC of AL flower.



Gallic acid **Quercetin** **Lupeol**

Figure 4: TLC of AN flower.

Chromatographic conditions

Chromatography was performed on a HPTLC silica gel 60F254, 20 X 20 cm with 0.2 mm thickness plate. Sample and standards were applied to the plate as 6 mm wide bands with an automatic TLC applicator Linomat V with N2 flow (CAMAG, Switzerland), 10 mm from the bottom and 13 mm space between two bands were identical for all the analyses performed.[29]

Detection of Gallic acid and Quercetin

The HPTLC Plates were developed using a CAMAG twin trough glass tank which was presaturated with the mobile phase Toluene - ethyl acetate - Formic acid (6:4:0.8) for 1 hour and each plate was developed to a height of about 8 cm. The HPTLC runs were in laboratory conditions of 25 ± 5ºC and 50% relative humidity. After development the plate was withdrawn and dried .Spots were visualized in UV light at 254 nm and 366 nm (UV cabinet, CAMAG, Switzerland).[30]

Detection of Lupeol

The HPTLC Plates were developed using a CAMAG twin trough glass tank which was pre saturated with the mobile phase Toluene-ethyl acetate (7:3) for 1 hour and each plate was developed to a height of about 8 cm. The HPTLC runs were in laboratory conditions of 25 ± 5 ºC and 50 % relative humidity. After development the plate was withdrawn and dried .Spots were visualized in UV light at 254 nm, 366 nm (UV cabinet, CAMAG, Switzerland). Visualization was carried out with vanillin in sulphuric acid reagent, heated at 105 ˚C till colour appears.[31-32]

Quantification

Gallic acid, Quercetin and Lupeol was quantified with CAMAG TLC scanner 3 equipped with Wincats software version 1.3.4 and computer under the following condition: Slit width 6 X 0.45 mm, wavelength 254 nm, 280 nm and 538 nm of UV (Deuterium lamp), absorption –reflection detection mode.[33]

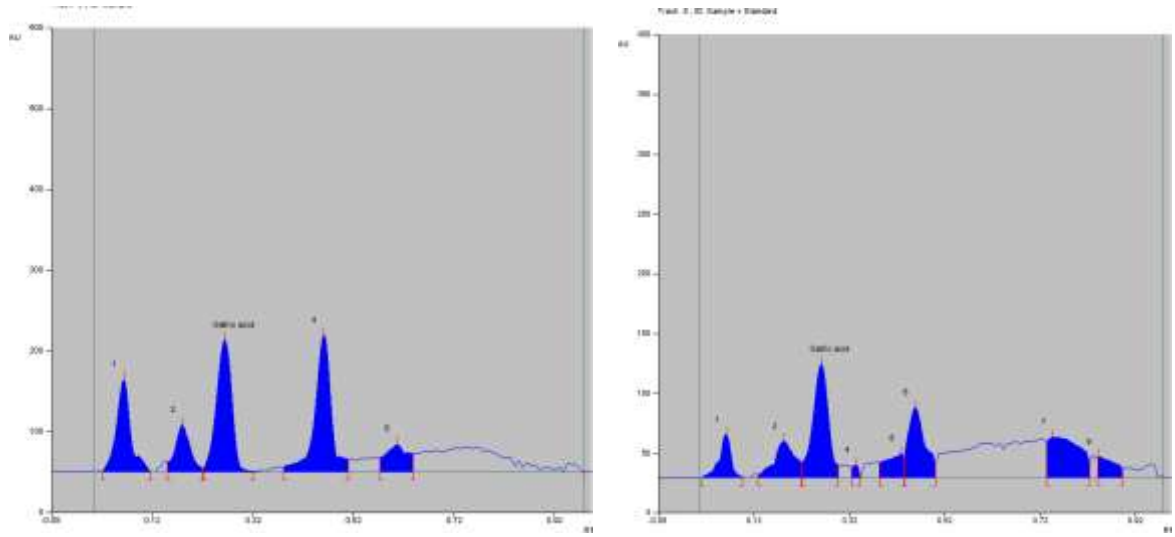


Figure 5: HPTLC fingerprinting of AN containing Gallic acid and AL containing Gallic acid.

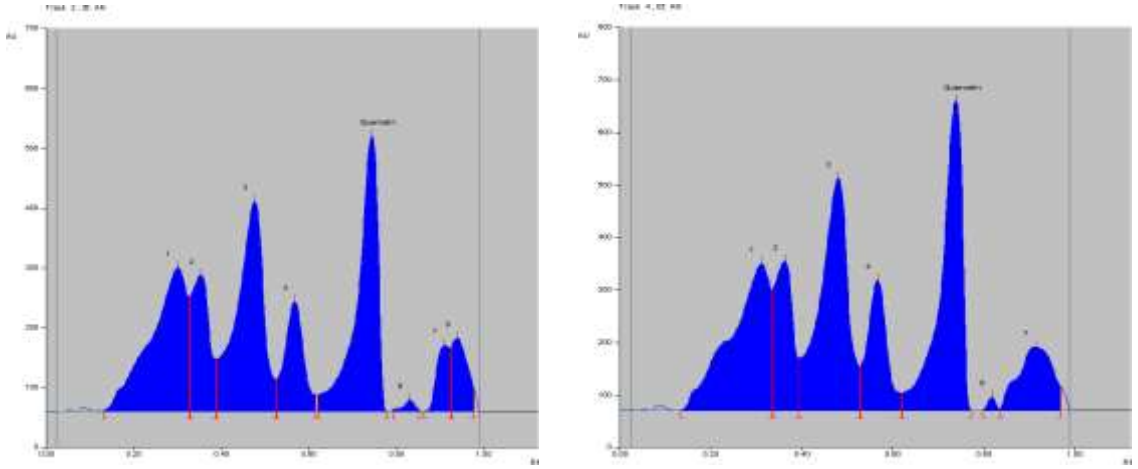


Figure 6: HPTLC fingerprinting of AN containing Quercetin and AL containing Quercetin.

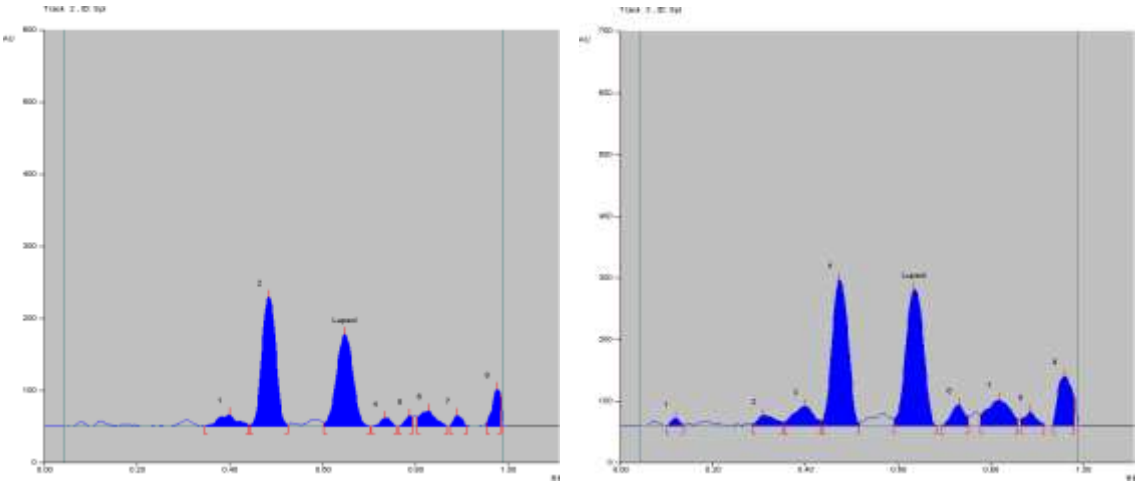


Figure 7: HPTLC fingerprinting of AN containing Lupeol and AN containing Lupeol.

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 and AL 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 GCMS analysis of the petroleum ether extract of AN and AL revealed the presence of phytoconstituents belonging to the type acids, esters, alcohols, ethers, etc. Thus, the medicinal plant AN and AL 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 and AL.

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