RESEARCH PAPER

# LC-MS/MS Metabolomics and Pharmaco-Chemical Characterization of Acacia arabica (Lam.) Willd Seeds

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## ABSTRACT

This study reported the metabolomics and pharmaco-chemical characterisation of Acacia arabica (Lam.) Willd seeds based on microscopic, proximate analysis, phytochemical assessment, Atomic Absorption Spectroscopy (AAS), UV-vis Spectrophotometer, Fourier Transform Infrared Spectroscopy (FTIR), liquid chromatography and mass spectrometry (LC-MS/MS). Powder microscopy revealed that Arabica seed powder free from foreign matter, and it contains lignified cell walls. Proximate analysis revealed that the seeds of Arabica contain more carbohydrate 84.48± 0.80% and dry matter (94.0 ±0.60%), and few amounts of protein (6.80 ± 0.50), fiber (4.40 ± 0.10%) ash (4.32 ± 1.20%) and fat (2.2 ± 0.80%). The preliminary phytochemical screening revealed that methanolic extract have proteins, phenolics, tannins, flavonoids, glycosides, fat and oils. And the seed powder did not reveal the presence of any heavy metal contamination. Total twenty chemical constituents were identified with the help of accurate molecular weights, fragmentation pathways. The phyto-steroids are major chemical constituents present in the seed extract. Asparacosin A, Galphimin B, Cabralealactone, Cathasterone, and Stigmastan-4,22, -diene-6-one are belonging to this group. The chemical compositions of the investigated samples might be responsible for their medicinal values. The computed studies on its molecular structure revealed that most constituents in the methanolic extracts had good drug-likeness and bioavailability for oral administration. Therefore, this pharmaco-chemical profiling can be useful for quality determination as well as pharmacological properties of Acacia seeds.

**Keywords**: Arabica Seed*,* Pharmaco-Chemical, Heavy Metal, LC/MS, Computational

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## INTRODUCTION

Even today, a large portion of the Indian population relies on the Indian system of medicine - Ayurveda and the use of medicinal plants is one of the oldest healthcare systems and has gained considerable recognition globally [1]. About 1178 medicinal plant species are involved in the all-India trade. Of these, 242 medicinal plants are consumed at a rate of more than 100 metric tons per year, and a significant amount of foreign exchange is earned through the export of medicinal plants to other countries [2]. Moreover, medicinal plant extracts are a rich source of biologically active compounds, and their use is limited due to their poor solubility, stability, bioavailability, and absorption, which leads to reduced its biological activity [3]. Recently, liposomes have been introduced as versatile assemblies to overcome these limitations of medicinal plant extracts, and their demand has increased in international trade within the nutraceutical [4] cosmetic,[5], and pharmaceutical industries [6]. The increase in the use of medicinal plants has led to various forms of misuse and adulteration of the products [7], which has led to frustration for consumers and producers and in some cases fatal consequences [8]. Consequently, it is imperative to evaluate the safety, efficacy, and quality of medicinal plants. The integration of contemporary scientific technologies with traditional knowledge is essential for standardisation and quality control in the herbal sector [9,10]. Therefore, there are several integrated approaches for the quality assurance of commercially important Indian medicinal plants are reported in the literature [11–13].

In India, *Acacia* species are often referred to as "Babool". *Vachelia nilotica*, formerly known as *Acacia arabica*, is a medium-sized tree belonging to the Fabaceae family, which has been widely used in traditional medicine [14]. Different medicinal properties have been demonstrated from various parts of the plant, including the fruits, leaves, bark, and flowers [15]. The plant has been reported to possess significant antibacterial [16], chemoprotective [17], immunomodulatory (Shalaan et al. 2024), antidiabetic [19], and anti-inflammatory [20] activities. Moreover, several clinical studies have confirmed its therapeutic potential [21–27]. The medicinal properties are mainly attributed to its phytochemicals and bioactive components. The major phytochemicals found in this plant are tannins, flavonoids, alkaloids, saponins, glycosides, terpenoids, steroids, volatile oils, and carbohydrates [15]. Studies on *A. arabica* seeds have rarely been explored, especially nano-engineered silver nanoparticles from *A. arabica* seeds have been reported to have potent anti-cancer properties [14] and the methanolic extract has shown good antiviral and antibacterial activities [28]. Moreover, the seeds are a rich source of minerals, protein, fats, and oils. Linoleic acid and oleic acids are identified as the major fatty acid components [29]. In addition, gallic acid, m-gallic, acid, m-gallic dimer, ellagic acid, leucocyanidin, and the more common 3, 4,7-

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tetrahydroxy flavones were found in the seeds [15]. To avoid substitution or adulteration, it is important to understand the pharmaco-chemical profile of seeds obtained from *A. arabica*. However, quality parameters directly affect its pharmacological activity and efficacy. New and innovative methods are now available for the standardization of herbal medicines. However, in this study, we focus on establishing the pharmaco-chemical profiling of seeds obtained from *A. arabica* tree through various spectrometric and chromatographic methods such as atomic absorption spectroscopy (AAS), UV-vis spectrophotometer (UV-vis), Fourier transform infrared spectroscopy (FTIR), and Liquid chromatography/mass spectrometry (LC/MS). In addition, we investigated the computed analysis on selected biomolecules.

## MATERIALS AND METHODS

### Seed collection and identification

The arabica seeds for this study were collected from the Rural area of Kuppam, Andhra Pradesh India. This area is classified as Aw by Koppen and Geiger (Coordinates: 12.75°N 78.37°E). The average annual temperature is 22.3 °C. The collected plant and seed were authenticated from Botanical Survey of India, Bangalore, Karnataka with botanical voucher No.33708.

### Proximate analysis

The proximate content of the *Arabica* seed powder was determined by adopting the official method of analysis by AOAC methods [30]. The moisture and ash were determined from the seed powder using weight difference method. Crude fibre was estimated from the extracts by loss in weight on ignition of dried residue following digestion of fat free samples. Crude fat was determined by extracting the samples with petroleum ether in a Soxhlet apparatus. Kjeldahl method used to determines crude protein. Whereas, total carbohydrate was calculated using this Eqn., total carbohydrate =100 − (% ash+% moisture+% crude fibre+% crude protein). All the proximate values were reported in percentages.

### Heavy metal analysis

The test solutions of *Arabica* seed were prepared through a standard acid digestion method in a micro-oven system (from Anton paar, USA) and $HNO_3$ and $H_2O_2$ (3:1 v/v) used as a digestion mixture. The test solutions atomic measurements are carried out with PerkinElmer-AAS coupled with Flame Photometer. The electrode less discharge lamp used for Cd, Pb, Hg and As analyses are used as a light source to provide specific wavelength of the elements to be determined. High purity (99.999%) Acetylene and Nitrous oxide are used to provide constant thermal energy for atomization process and Argon gas used for carrier gas removal purposes for Graphite furnace.

### Preparation of methanolic extract and phytochemical screening

About 50 g of the powdered seed was carefully weighed and loaded into a thimble (made up with Whatman's filter paper) and loaded into Soxhlet extractor. The seed powder was subjected to methanolic extraction under controlled temperature of 60-80ºC. After the successful extraction cycle, the extract was then concentrated in a rotary evaporator at about 40ºC. Finally, the semi-liquid extract was subjected to air drying to give dried extracts for further analysis. The methanolic seed extract was subjected to determine the presence of phytoconstituents described by Harborne [31].

### LC-MS/MS analysis

High-resolution MS/MS analysis was performed with *Aarabica* seed extract using the Thermo Scientific mass spectrometry system (Thermo Fisher Scientific, Waltham, MA, USA). The capillary column was used for separation of phytoconstituents. 0.1% of formic acid in water is used as mobile phase A, 0.1% of formic acid in methanol and acetonitrile as mobile phase B1 and B2 respectively. This gradient elution allows efficient separation of compounds with a wide range of polarities. The flow rate of elute was 0.30 ml min−1. The eluent from the chromatographic column successively enters the ESI interface ion trap mass analyzer. Mass spectrometer equipped with an H- ESI ion source was used for the MS analysis. The positive ion and negative ion potentials were set at 3.5 kV and 3.0 kV respectively. The mass range was 100-1500 m/z. The vaporizer temperature was set to 300 ◦C.

### Computational study based on Lipinski criteria

The physicochemical properties of chemical constituent identified from LC/MS were analysed with Swiss-Target-Prediction web tools [32]. The selection of these services was based on their ability to provide relevant physicochemical data for small molecule drug candidates such as molecular mass (D< 500), Partition coefficient (Log P ≤ 5), hydrogen bonding acceptor (HBA≤ 5), hydrogen bonding donor (HBD≤ 10), and Topological polar surface area (TPSA< 140 Å²**)**. The compounds structures were transformed into simplified molecular-input line entry specification (SMILES) format and then uploaded into selected web services.

### Statistical analysis

The mean ± SD was used to express the results. SPSS version 17.0 was used for the statistical analysis of the data. At <0.05, based on a Tukey post-hoc test, the mean values were considered statistically significant.

## RESULTS

### Proximate and heavy metal analysis

The seeds of *A. arabica* are dark brown to yellowish in color, with a thick and lignified seed coat. Powder microscopy revealed no presence of foreign matter, and the powder was found to have lignified cell walls and elliptical cells (Fig. 1). The proximate analysis of *A. arabica* seed powder is depicted in Table 1. The dry matter of seed powder was 94.0 ±0.60%, indicating a moisture content of 6±0.60%. The powder contains only 0.34 ±0.20 foreign matters. Which demonstrated that the seed was free of contamination. The crude protein and fiber contents were 6.80 ± 0.50 and 4.40 ± 0.10%, respectively. Moreover, the seed had a fat content of 2.2 ± 0.80%. The total ash content, 4.32 ± 1.20%, was found in *A. arabica* seed powder. Analysis of heavy metals, including arsenic (As), mercury (Hg), lead (Pb), and cadmium (Cd), indicated that the seed powder was devoid of heavy metal contaminants (Table 1). Finally, the total carbohydrate was calculated as 84.48%.

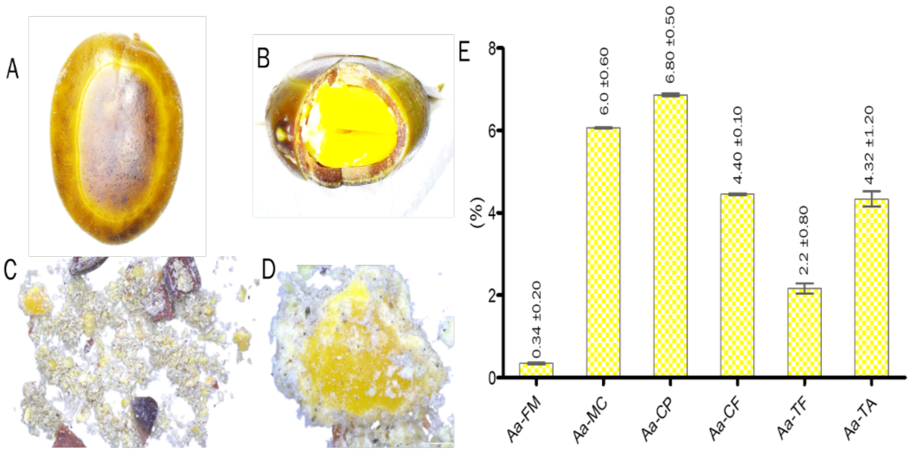


**Fig. 1 Microscopic images of *A. arabica* seed powder and proximate analysis. [A]- Microscopic image of single seed; [B]- Seed coat thick and lignified; [C]- Lignified cell walls and elliptical seeds (storage area of secondary metabolites); [D]- Elliptical seed found in yellowish in colour; [E]- Graph representing proximate analysis of seed powder of *A. arabica* (Lam.) Willd. Aa-FM- Foreign Matter; Aa-MC-Moisture content; Aa-CP- Crude Protein; Aa-CF- Crude fiber; Aa-TF-Total fat content; and Aa-TA- Total ash content.**

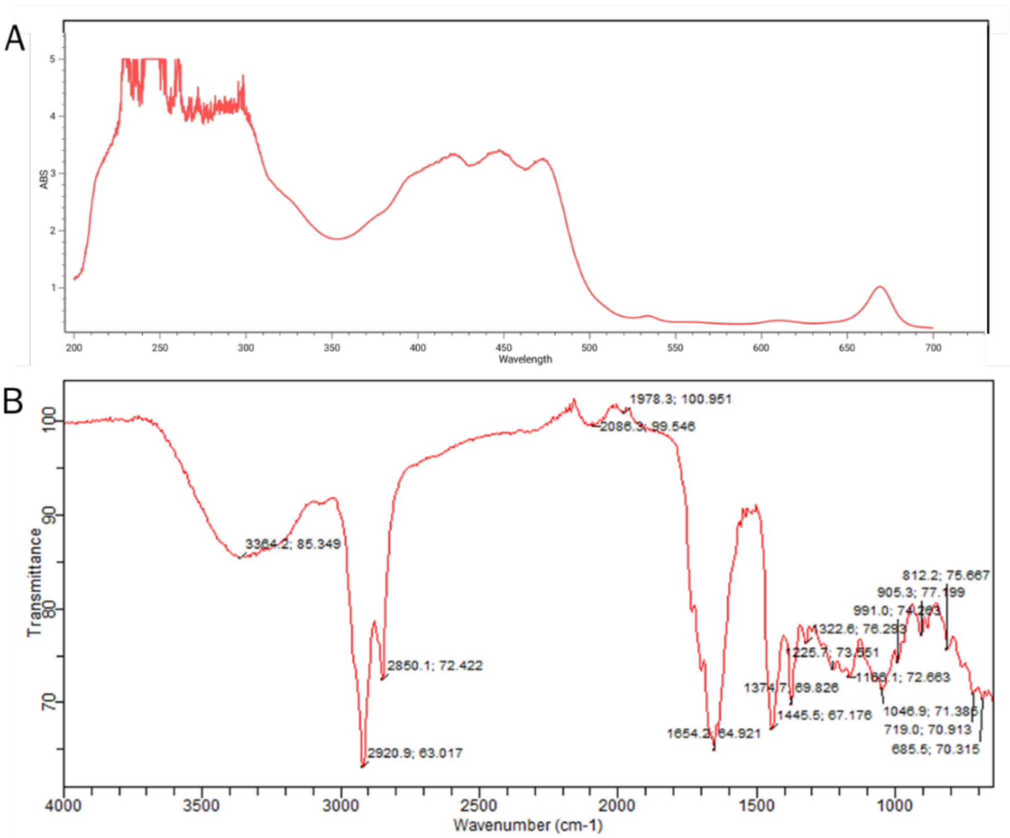


**Fig 2. [A] Ultraviolet (UV)-visible spectral and [B] Fourier Transform Infrared (FTIR) profiling of methanolic seed extract of *Acacia arabica* (Lam.) Willd.**

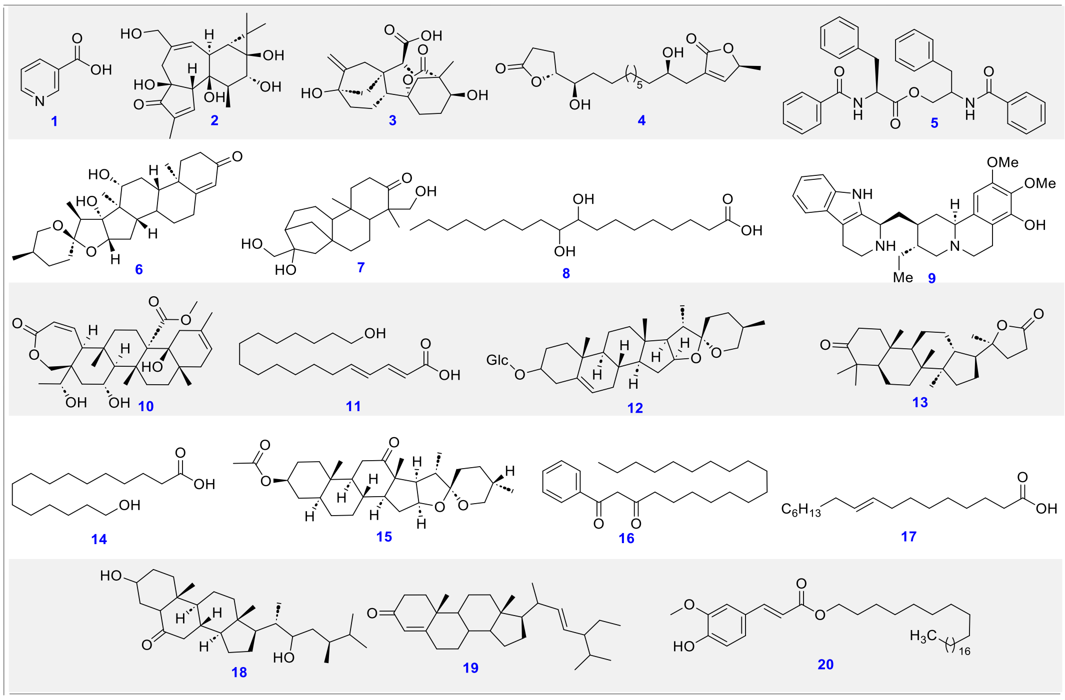

**Fig. 3 Phyto-constituents from the methanolic seed extract of *Acacia arabica* (Lam.) Willd. 1. Nicotinic acid; 2. 4β-Phorbol; 3. Gibberellin A1; 4. Rollicosin; 5. Auranamide; 6. Asparacosin A; 7. Calliterpenone; 8. Dihydroxystearic acid; 9. Alangimarckine; 10. Galphimin B; 11. Hydroxyoctadecadienoic acid; 12. Disogluside; 13. Cabralealactone; 14. Juniperic acid; 15. Hecogenin acetate; 16. Stearoylbenzoylmethane; 17. Elaidic acid; 18.Cathasterone; 19. Stigmastan-4,22-diene-6-one and 20. Hexacosyl (E)-ferulate.**

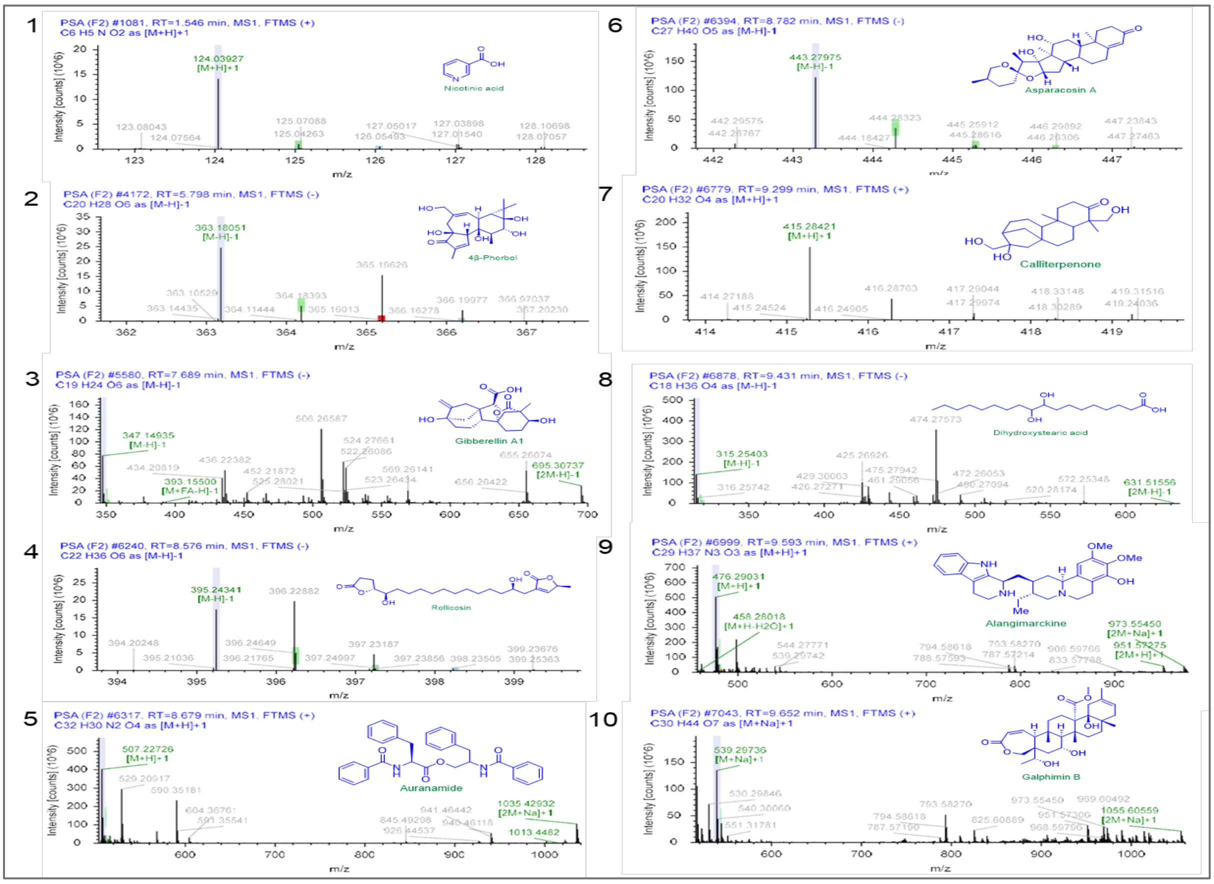


**Fig 4. Mass spectra of phyto-constituents (1-10) were identified in the methanolic seed extract of *Acacia arabica* (Lam.) Willd.**

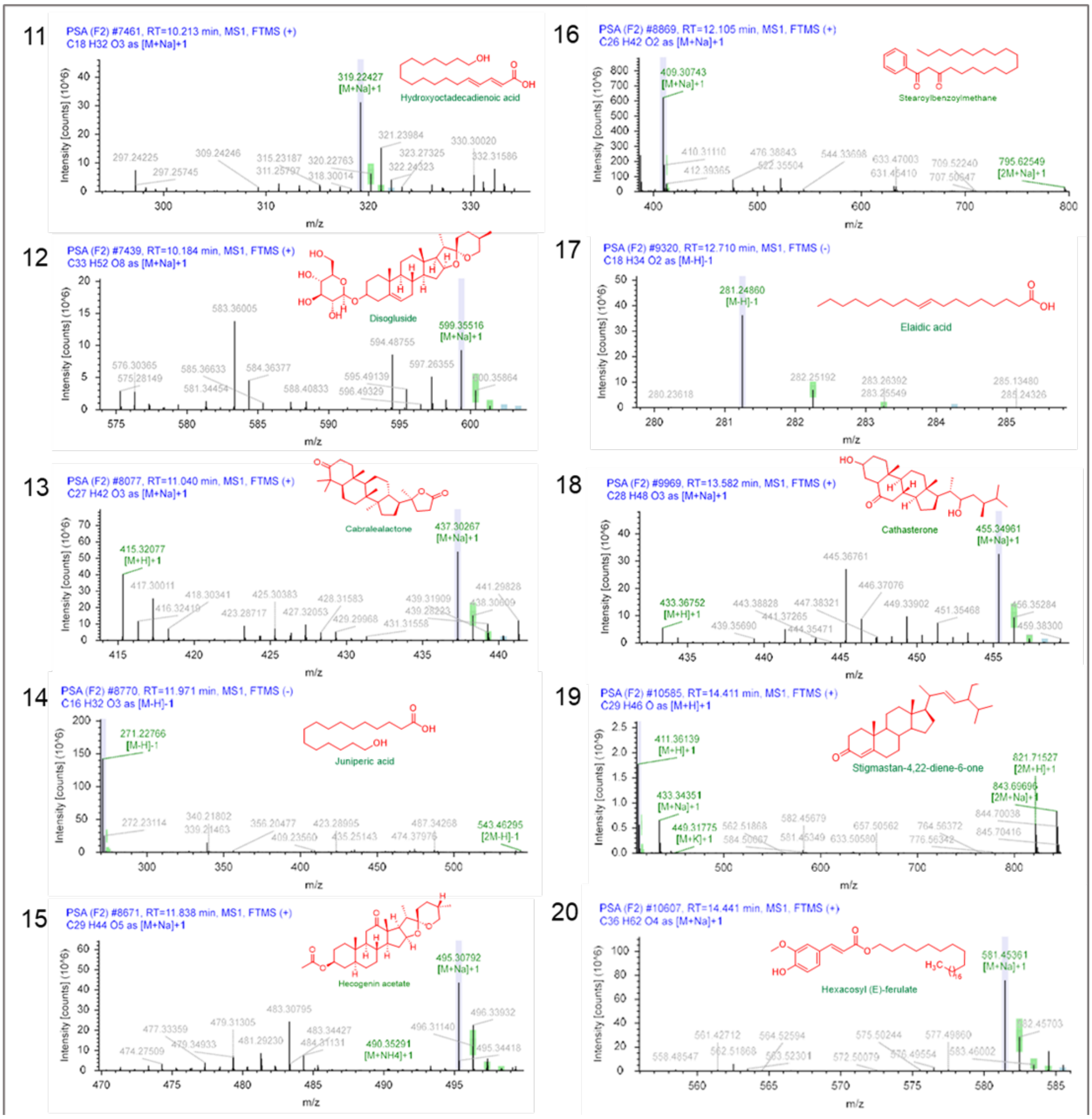


**Fig 5. Mass spectra of phyto-constituents (11-20) were identified in the methanolic seed extract of *Acacia arabica* (Lam.) Willd.**

**Table 1. Proximate and heavy metal analysis of seed powder of *Acacia arabica* (Lam.) Willd**

| No. | Proximate/Heavy metal analysis | Results |
|---|---|---|
| 1 | Dry Matter (Aa-DM) | 94.0 ±0.60 |
| 2 | Foreign Matter (Aa-FM) | 0.34 ±0.20 |
| 3 | Crude Protein (Aa-CP) | 6.80 ±0.50 |
| 4 | Crude Fiber (Aa-CF) | 4.40 ±0.10 |
| 5 | Total Fat (Aa-TF) | 2.2 ±0.80 |
| 6 | Total Ash (Aa-TA) | 4.32 ±1.20 |
| 7 | Moisture Content (Aa-MC) | 6.0 ±0.60 |
| 8 | Heavy Metals (As, Hg, Pb and Cd) | BDL |

Key: BDL- Below detection limit, As – Arsenic, Hg- Mercury, Pb- Lead, and Cd- Cadmium.

**Table 2. Preliminary phytochemical analysis of methanolic seed extract of *Acacia arabica* (Lam.) Willd.**

| No. | Metabolites | Methanolic extracts |
|---|---|---|
| 1 | Alkaloids | -Ve |
| 2 | Proteins | +Ve |
| 3 | Phenolics | +Ve |
| 4 | Tannins | +Ve |
| 5 | Flavonoids | +Ve |
| 6 | Glycosides | +Ve |
| 7 | Terpenes | -Ve |
| 8 | Saponins | -Ve |
| 9 | Carbohydrate | +Ve |
| 10 | Fat and oils | +Ve |

Key: (-): Absence, (+):- Present,

**Table 3. Fourier Transform Infrared profiling of methanolic seed extract of *Acacia arabica* (Lam.) Willd.**

| No. | Peak ranges (cm−1) | Peak values (cm−1) | Vibration/bond | Specific functional group | Chemical compound |
|---|---|---|---|---|---|
| 1 | 3600–3200 | 3364.2 | O–H stretch | Alcohols, phenols | Aromatic |
| 2 | 3000–2850 | 2850.1 | C–H stretch | Alkanes | Aliphatic |
| 3 | 2270–1940 | 1978.3 | C≡C stretch | Alkynes | Aliphatic |
| 4 | 1680–1620 | 1654.2 | C≡C stretch | Alkynes | Aliphatic |
| 5 | 1500–1340 | 1445.5 | C-H bending | Alkanes | Aliphatic |
| 6 | 1470-1380 | 1374.0 | O-H bending | Phenols/Tertiary alcohols | Aromatic |
| 7 | 1000–1300 | 1166.1 | C-O stretching | Esters/Carboxylic acids | Aliphatic |

**Table 4. LC/MS profiling and computed properties (Lipinski criteria) of chemical constituents identified in the methanolic seed extract of *Acacia arabica* (Lam.) Willd.**

| No | Chemical constituents | RT (min) | Mole. Formula | Calc. MW (< 500 D) | Log P (< 5) | Rotatable Bonds | HBA (≤ 5) | HBD (≤ 10) | TPSA (< 140 Å²) | Lipinski Alert |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Nicotinic acid | 1.54 | $C_6H_5NO_2$ | 123.0319 | 0.86 | 1 | 3 | 1 | 50.19 | 0 |
| 2 | 4β-Phorbol | 5.79 | $C_{20}H_{28}O_6$ | 364.1877 | 2.47 | 1 | 6 | 5 | 118.22 | 0 |
| 3 | Gibberellin A1 | 7.68 | $C_{19}H_{24}O_6$ | 348.1567 | 1.74 | 1 | 6 | 3 | 104.06 | 0 |
| 4 | Rollicosin | 8.58 | $C_{22}H_{36}O_6$ | 396.2506 | 4.14 | 14 | 6 | 2 | 93.06 | 0 |
| 5 | Auranamide | 8.67 | $C_{32}H_{30}N_2O_4$ | 506.2203 | 3.93 | 14 | 4 | 2 | 84.50 | 1 |
| 6 | Asparacosin A | 8.78 | $C_{27}H_{40}O_5$ | 444.2870 | 4.09 | 0 | 5 | 2 | 75.99 | 0 |
| 7 | Calliterpenone | 9.29 | $C_{20}H_{32}O_4$ | 414.2769 | 2.27 | 2 | 4 | 3 | 77.76 | 0 |
| 8 | Dihydroxystearic acid | 9.42 | $C_{18}H_{36}O_4$ | 316.2613 | 3.76 | 16 | 4 | 3 | 77.76 | 0 |
| 9 | Alangimarckine | 9.59 | $C_{29}H_{37}N_3O_3$ | 475.2830 | 4.25 | 5 | 5 | 3 | 69.75 | 0 |
| 10 | Galphimin B | 9.66 | $C_{30}H_{44}O_7$ | 516.3081 | 3.16 | 3 | 7 | 3 | 113.29 | 2 |
| 11 | Hydroxyoctadecadienoic acid | 10.17 | $C_{18}H_{32}O_3$ | 296.2350 | 3.78 | 15 | 3 | 2 | 57.53 | 0 |
| 12 | Disogluside | 10.18 | $C_{33}H_{52}O_8$ | 576.3659 | 4.52 | 3 | 8 | 4 | 117.84 | 2 |
| 13 | Cabralealactone | 11.04 | $C_{27}H_{42}O_3$ | 414.3134 | 3.86 | 1 | 3 | 0 | 43.37 | 0 |
| 14 | Hecogenin acetate | 11.85 | $C_{29}H_{44}O_5$ | 472.3186 | 4.28 | 2 | 5 | 0 | 61.83 | 0 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 15 | Juniperic acid | 11.96 | $C_{16}H_{32}O_3$ | 272.2349 | 3.39 | 15 | 3 | 2 | 57.53 | 0 |
| 16 | Stearoylbenzoylmethane | 12.11 | $C_{26}H_{42}O_2$ | 386.3182 | 5.20 | 19 | 2 | 0 | 34.14 | 1 |
| 17 | Elaidic acid | 12.71 | $C_{18}H_{34}O_2$ | 282.2558 | 4.27 | 15 | 2 | 2 | 37.30 | 0 |
| 18 | Cathasterone | 13.59 | $C_{28}H_{48}O_3$ | 432.36036 | 4.18 | 5 | 3 | 2 | 57.53 | 0 |
| 19 | Stigmastan-4,22, -diene-6-one | 14.41 | $C_{29}H_{46}O$ | 410.3540 | 4.96 | 5 | 1 | 0 | 17.07 | 0 |
| 20 | Hexacosyl (E)-ferulate | 14.44 | $C_{36}H_{62}O_4$ | 558.4644 | 8.32 | 29 | 4 | 1 | 55.76 | 2 |

**LC/MS-** Liquid chromatography/mass spectrometry**;** **RT**- Retention time**;** **D**- Dalton; **Log P**- Partition coefficient; **HBA**- hydrogen bonding acceptor; **HBD**- hydrogen bonding donor, and **TPSA**- Topological polar surface area
.

**Preliminary phytochemical analysis**

The phytochemical screening of methanolic extracts of *Acacia arabica* (Lam.) Willd seed were collected from Rayalaseema region of Southern Eastern Ghats, Andhra Pradesh, India. The methanolic extracted material was subjected to phytochemical screening in order to identify the various secondary metabolites such as alkaloids, proteins. phenolics, tannins, flavonoids, glycosides, triterpenoids, saponins, and carbohydrate, the details are depicted in Table 2. The results of the methanolic extract revealed that proteins, phenolics, tannins, flavonoids, glycosides, fat and oils were present, but it did not reveal the existence of alkaloids, carbohydrates, terpenes, and saponins.

**Ultraviolet (UV)-visible and FTIR spectral profiling**

The UV-Vis profile of methanolic extract of *A. arabica* was taken at the wavelength of 200 nm to 700 nm. The 400-600 nm range in UV-Vis spectra identifying several phytochemicals such as carotenoids (400–450 nm), terpenoids (400–550 nm), and tannins (350–500 nm). The profile showed the characteristic peaks in between 400-500 nm range with the absorption of 0.9-3.2. Which indicate that the methanolic seed extract was rich in carotenoids, terpenoids and or tannins. The Fig. 2A shows the absorption spectrum of methanolic seed extract. The appearance of several peaks in the region from 200 to 400 nm is a clear indication of the presence of flavonoids, phenols, and other aromatic molecules. Further, FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The FTIR spectral analysis of methanolic extract detected the presence of functional groups in different frequency ranges (Fig. 2B). Among these eight peaks were found to be more prominent. The details are described in table 3.

**LC/MS profiling and drug likeness properties**

LC/MS profiling of methanolic seed extract of *A. arabica* was reveals the fatty acids and phytosterols are major contents (Table 4). Total seven compounds such as Asparacosin A, Galphimin B, Disogluside, Cabralealactone, Cathasterone, and Stigmastan-4,22, -diene-6-one was found be with divergent structural diversity belongs to steroid class (Fig. 3). The components such as dihydroxystearic acid, hydroxyoctadecadienoic acid, Juniperic acid, and elaidic acid are belongs to the class of fatty acids. The mass spectra and structure of chemical constituents were depicted in Fig. 4 and 5. The computed properties (Lipinski criteria) of the identified chemical components are also predicted and depicted in Table 4. Only five compounds violated the Lipinski rule in terms of molecular weight (< 500 D), lipophilicity (logP < 5), and number of hydrogen bonding acceptors (HBA≤5). Fifteen compounds passed the Lipinski criteria and had good drug-likeness and bioavailability for oral administration.

**DISCUSSION**

India is known for its traditional medicinal systems—Ayurveda and a large number of peoples even today depend on herbal remedy for various ailments. *Acacia* genus is of great and varied economic importance, yielding edible seeds and valuable timber and gum. *A. arabica* is a medium-sized tree belonging to the Fabaceae family, which has been widely used as traditional medicine. Its bark is used as an astringent/tanning agent, a gum for digestive/respiratory problems, and the leaves/nuts are commonly used for livestock/blood purification purposes. In the literature, scientific studies on the seed are very limited [33]. From 5-7 years, the *Arabica* tree produces a large number of seeds. A mature tree can produce pods containing about 18 kg of seeds. On average, large-scale plantations produce 8-10 tons of pods per hectare [34]. The alcoholic extracts obtained from *Arabica* pods have been shown anti-HIV-PR effects [15]. And, *Arabica* seed extract has demonstrated their potent antimalarial [35], anti-viral, and anti-bacterial properties [28]. Recently, we found that silver-nanoparticles (AgNPs) obtained from *Arabica* seed extract exhibit potent anticancer properties [14]. As a result, more research is needed to demonstrate the therapeutic properties of *Arabica* seeds. Therefore, it is important part to establish quality control parameters for the seeds.

Numerous physical, chemical, and geographical factors that affect the quality of raw materials [36]. This study began by establishing heavy metal analysis, as heavy metals are mentioned to be toxic even at low concentrations, affecting the normal growth of plant, especially in altering concentrations of secondary metabolites. This disruption may compromise the overall quality and efficacy of plant. [37] Interestingly, the heavy metal analysis revealed that

*Arabica* seed are free from toxic metals. Further, proximate parameters such as moisture, ash, crude protein, fat, and fibre contents of the seed were established asper AOAC methods.[30] The dry matter content of the seed powder was identified as 94.0 ±0.60%, which indicating that the moisture content of seed is 6±0.60%. The higher content of moisture (>10%) leads to microbial contamination [38]. Moreover, the seed free from foreign matters. The crude protein and fiber contents were identified as 6.80 ± 0.50 and 4.40 ± 0.10%, respectively. Moreover, the seed have of 2.2 ± 0.80% of fatty matters. Additionally, the total ash content of 4.32 ± 1.20% was found in *A. arabica* seed powder. Mathew et al.,[39] reported the proximate profile of *A. arabica* seed purchased from local markets in Minna, Nigeria, and the seed powder contained 6.67% moisture content, 2.80% ash, 6.53% crude fibre, 30.95% crude protein and 29.72% carbohydrate. Compare to our study, seed powder had high content of crude fatty matters 23.33%. Similarly, Bwai and co-workers [40] reported that 15.8% of crude lipid content in fruit of *A. arabica* Differences in nutritional composition may directly affect their therapeutic potential. Yang et al. reported that environmental factors significantly influence the metabolic profile of *Lycium barbarum* seeds collected from different regions in China. Therefore, a comparative study on the therapeutic properties of *Arabica* seeds is needed. The proximate profile of several medicinally important seeds such as *Nigella sativa*,[41] *Piliostigma thonningii,*[42] *Moringa oleifera,*[43] *Jatropha curcas,*[44]*Millettia ferruginea,*[45] has been reported in the literature. This data will be helpful for quality standardization or control of *Arabica* seed.

The therapeutic performance of herbal drugs can change significantly batch to batch production due to variation in the quality of the raw materials in term of active component present in it.[46] Adulteration using closely related plant species is a common issue in this field. It is quite challenging to determine the quality by visual inspection because of its physical resemblance. Reduced therapeutic effects and significant health hazards for consumers may result from this kind of approach.[47] DNA barcoding is a preferred method for medicinal plant authenticity and has detected adulteration in several high-value species.[48] However, there are limitations within genetic profiling, and in the accessibility of such technology in basic laboratory settings. In another way identification of key chemical biomarkers can used for the authentication hyphenated analytical techniques [49]. The UV-visible and FTIR profiling gave an idea about the metabolic profile of possible class of compounds (via functional groups) present in the plant. LC/MS, LC/HRMS and LC/NMR are powerful analytical techniques, in which liquid chromatography coupled with different detectors such as mass spectrometry, high-resolution mass spectrometry, and nuclear magnetic resonance, which help to separate, and identify the individual bioactive present in the herbal materials. In this study, several phyto-constituents were identified from the methanolic seed extracts of *Arabica* with the help of accurate molecular weights and fragmentation pathways given by LC-MS/MS. The seed extract is rich in fatty acids and phytosterols. The components such as dihydroxystearic acid, hydroxyoctadecadienoic acid, Juniperic acid, and elaidic acid are belongs to the class of fatty acids and the presence of different reactive chemical groups and stereochemistry of fatty acid chain contributes to the bioactivity [50]. Notably, (*S*)-hydroxyoctadecadienoic acid is a linoleic acid metabolite, reported to have anticancer activity via halting mTOR cells [51]. Phytosterols play an important role in the development of fiber, and seeds usually contain high amounts of fiber, which is mainly found in the seed coat (skin) and the cell walls of the seed. Fiber is mainly composed of indigestible polysaccharides such as cellulose, hemicellulose, and lignin. Our microscopic examination revealed that the seed coat was thick and lignified, and the total fiber content of the seed was estimated to be 4.40%. The phytosteroids such as Asparacosin-A, Galphimin-B, Disogluside, Cabralialactone, Hecogenin acetate, Cathesterone, and Stigmastan-4,22, and -dien-6-one are identified in the seed extract of *Arabica*. Among this most of the phytosteroids that have been reported to have several therapeutic potentials [52–55]. Remarkably, Asparacosin-A is a spirostanol derivative, which belongs to the class of sterol lipids showed anti-nociceptive and anti-inflammatory properties [52]. Among the twenty phyto-constituents from LC-MS, only five constituents were did not comes under the criteria of Lipinski rule of five in terms of molecular mass (< 500 D), lipophilicity (logP < 5), and number of hydrogen bonding acceptors (HBA≤5). However, the most of phyto-constituents have been reported to have potent medicinal properties. Therefore, these computed results were useful for further *In silico* studies and the pharmacochemical results for quality control.

## CONCLUSION

*Acacia arabica* is one of the most pharmacologically diverse medicinal plants. Scientific studies on *Arabica* seeds are rarely explored. This investigation evaluated and identified the pharmaco-chemical profile and LC-MS metabolomics of *Arabica* seeds. The analysis revealed a significant presence of fatty acids and phytosteroids in the ethanol extract. In addition, computational studies revealed that fifteen compounds passed Lipinski's rule of five and some compounds reported from the literature have good anti-cancer and anti-inflammatory properties. This indicates the pharmacological importance of *Acacia* seeds. Pharmaco-chemical profiling of *Acacia* seeds was carried out on the basis of heavy metal, proximate, phytochemical and spectrometric analysis, the results revealed that the seeds were free from heavy metal contamination. Notably, the crude protein and fiber contents were 6.80 and 4.40, respectively. The total ash content was observed to be 4.32%. The preliminary phytochemical examination revealed that the seeds contained protein, phenolics, tannins, flavonoids, carbohydrates, glycosides, fat and oils. These findings were supported by UV-visible and FTIR profiling. Therefore, this pharmaco-chemical profiling can be useful for quality determination of *Acacia* seeds.

**Conflict of Interest**

The authors declare no conflict of interest.

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