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RESEARCH ARTICLE

Phytochemical Profiling and Antioxidant Activity of Acacia Nilotica and Quercus Infectoria

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Abstract: This study investigates the phytochemical composition and antioxidant potential of Acacia nilotica and Quercus infectoria leaf extracts, traditionally used in Ayurvedic and Unani systems for neuroprotection and inflammation. Shade-dried leaves were subjected to physicochemical analysis (loss on drying, ash values, extractive values, and pH), followed by phytochemical screening of aqueous and ethanolic extracts. Profiling methods included HPTLC for phenolics and GC-MS for volatile and quantifiable bioactives. Antioxidant activities were assessed using DPPH and FRAP assays. Phytochemical screening revealed the presence of flavonoids, tannins, phenolic acids, steroids, and saponins. Acacia nilotica showed higher aqueous extractive value (19.35%) than Quercus infectoria (15.12%) and a slightly acidic pH of 5.54. HPLC revealed high concentrations of ferulic acid (5451.04 μg/mL), chlorogenic acid (4572.26 μg/mL), and quercetin (3733.37 μg/mL). DPPH scavenging was 76.38 ± 2.99 μM/g for Acacia and 67.94 ± 3.95 μM/g for Quercus, while FRAP values were 2002.39 ± 10.88 and 1739.50 ± 11.94 μM Fe(II)/g, respectively. HPTLC confirmed betulinic acid (Rf = 0.31 ± 0.001) and β-sitosterol (Rf = 0.41 ± 0.001) in Acacia nilotica. Both extracts demonstrated potent antioxidant profiles and high levels of neuroprotective compounds. Acacia nilotica exhibited superior extractive yield and radical scavenging efficacy, supporting its therapeutic relevance in combating oxidative stress and neurodegeneration.

Keywords: Neuroprotection, phytoconstituents, oxidative markers, quercetin, extract.

INTRODUCTION

Plants have been used in every corner of the world as a vital link in health care from ancient times to modern times [1]. In ancient medical systems successful on their curative properties, including those of Ayurveda and Unani, Siddha and TCM [2]. In the past decade, a resurgence of interest in herbal medicine has arisen from numerous sources. With rising concerns about synthetic drug side effects, increasing demand for natural health remedies not to mention the still unsatisfied search for new physiological active plant chemicals and further research into their ways of use continue even to this day is aimed at finding plant-based medicines worldwide as well [3]. In recent years this ancient knowledge has gained a new lease on life and is being actively investigated by the scientific community in relation to the growing need for plant-based therapies, particularly in chronic progressive diseases such as neurodegenerative disorders [4].

Acacia nilotica, commonly known as Babul, the Gum Arabic tree, or Egyptian thorn, is a medium-sized thorny tree native to Africa, the Middle East and the Indian subcontinent [5]. It has a long history of usage in traditional medicine systems to treat many types of illness. Conventional medicine has used the plant's bark, leaves, brains and gum to cure illness of all sorts; including, though not limited to: dysentery, coughs, leprosy ulcers, TB skin problems as shown by [6]. Studies of the phytochemical constituents of Acacia nilotica have shown it to contain secondary metabolites such as tannins, flavonoids, saponins, alkaloids, terpenoids, glycosides, polyphenols, and volatile oil [7]. Its pharmacological activities are expected to be greatly influenced by these phytoconstituents. Thus, *A. nilotica* is rich in tannins, which are powerful antioxidants and antibacterial agents [8]. Quercetin and kaempferol are two kinds of flavonoids that have been shown to have anti-inflammatory, anticancer effects respectively. Moreover, this plant contains agents which influence oxidative stress and cellular inflammation. These processes are central to many chronic disorders [9]. These include substances such as gallic, ferulic and catechin

It is well-known that *Quercus infectoria*, or the Fagaceae family's Aleppo oak, has medical effects. As a medicinal herb, it has long been employed in Ayurvedic and Unani medicine and numerous other therapeutic practices. Its galls are famous for being so rich in bioactive substances. There is a wide range of pharmacological actions area exhibited by *Quercus infectoria* [10]. The plant possesses qualities that help with wound healing, neuroprotection, inflammation and gastrointestinal health. Gallic acid, ellagic acid, hydrolysable tannins, flavonoids, syringic acid, sitosterol, amentoflavone, isocryptomerin, hexamethyl ether, methyl betulate, methyloleanate and hexagalloyl glucose are among the bioactive chemical constituents of *Quercus infectoria* var galls [11].

In this context, the current study centers on the application of established analytical techniques to physiochemical and phytochemical characterization of

leaf extracts from *Acacia nilotica* and *Quercus infectoria*. Physiochemical parameters such as loss on drying, ash values, extractive values and pH are basic criteria for quality assessment of plant materials. Phytochemical screening provides preliminary information on what types of bioactive compounds are present. To further validate the analysis, HPTLC fingerprint profiling was used for exact profiling and selective detection of key phytoconstituents such as phenolic compounds, flavonoids and sterols. GC-MS was used for identification of volatile molecules and confirmation of major phytochemicals present. The combination of free radical scavenging assays– such as DPPH and FRAP with in vivo outcomes– helps to link biochemical antioxidant capacity as a function with functional and structural neuroprotection.

MATERIAL AND METHODS

Physiochemical Analysis Methodology

The leaves of *Acacia nilotica and Quercus infectoria* leaves were powdered for physiochemical parameter determination employing standard procedures [12]. To determine water content, a sample of the powdered material is placed in an oven at 105°C until its weight is constant. The percentage yield was calculated based on the dry weight, which was obtained by macerating a known amount of dried plant material in water, ethanol, and ether for 24 hrs respectively, then filtering the liquid under reduced pressure to remove all but solid material that remains on changing temperature from 25°C up to 85°C. Aqueous, alcoholic and ethereal extractive values were then confirmed through filtration plus evaporation until they became crystalline solids (by means of drying in vacuo). Samples of plant powder were fixed in muffle furnaces and incinerated at 550°C until white ash had formed to determine total inorganic content; this also measured total ash content. The acid-insoluble ash was gotten by boiling the whole ash with water-hydrochloric acid, filtering, and then burning out what remained- it was thus possible to weigh this last. The water-soluble ash stood amount of insoluble residue measured the whole ash, after boiling with water, distilling off the liquid and igniting residue that remained [13]. The pH of a 1% solution made from the plant powder was measured in water at 25°C. using calibrated digital pH meter at room temperature.

Extraction

To conduct further analysis, the leaves of *Acacia nilotica* and *Quercus infectoria* were collected, shade-dried, and then powdered. The physical and chemical standards of the powder were appraised for loss on drying, extractive value (water soluble, alcohol soluble, ether soluble). It was then burned completely into either white or pale gray ash turned a clay pink colorless after it had been warmed and then cooled [14]. For phytochemical screening, aqueous and ethanol extracts were prepared and subjected to qualitative tests to detect carbohydrates. Ninhydrin test was conducted to detect amino acids at this stage in the research Biuret, Xanthoproteic, Millon tests were performed for proteins and saponins (Borntrager's test). Phenolic compounds, steroids (Salkowski test), and tannins are assessed using ferric chloride, lead acetate, and potassium dichromate tests.

HPTLC analysis

For the purposes of HPTLC conducted to assess phenolic constituents, 50 mg of the extract was dissolved in 1 mL of ethanol [15]. It was carried out on the supernatant obtained by centrifuging the solution. By means of a Hamilton syringe and CAMAG LINOMAT 5, 2.5 µL aliquots were pipetted onto 5 cm x 10 cm Silica gel 60F254 plates. The mobile phase was a mixture of 2.3:3.5:1.2:0.8 ethyl acetate, toluene, formic acid and methanol. The plates were developed with a TLC twin trough chamber, dried with hot air at 80°C and then examined using a CAMAG REPROSTAR 3 under white as well as UV 254 nm light. After scanning at 254 nm peak densitograms were obtained. A well characterized HPTLC method was used in order to quantify betulinic acid and β-sitosterol, the mobile phase being chloroform, methanol, and glacial acetic acid at a ratio of 97:2:1 (v/v/v). Standard solutions were used to determine the presence of stigmasterol in petroleum ether extracts and at different concentrations. After UV illumination at 366 nm, derivatization was carried out. GC-MS was used for phytoconstituents of volatile nature in methanolic extracts. Additionally, the HPTLC chromatograms of the extracts were also compared with those of reference substances such as gallic acid and lupeol. Solvent fractions of plant material were subjected to UV absorption spectra and chromatographic fingerprinting to identify and authenticate phytochemical constituents [16].

Antioxidant activity

DPPH free radical scavenging method

DPPH stable free radical assay to evaluate the antioxidant quality of individual compounds or extracts from plants [17]. This assay was developed by Aoshima et al. in 2004 [18] The oxidative ability of a sample was measured by the procedure. In brief, 2.9 mL of DPPH reagent (0.1 mM in methanol) were aggressively mixed with 100 µl of sample extract or standard. Before the reaction mixture was placed in the dark at room temperature for 30 minutes, the decoloration of DPPH was measured along a blank at 517 nm by ultraviolet-visible (UV-Vis) spectrophotometer. Linear calibration curves were generated with R2=0.9998, and results were expressed in Trolox equivalent antioxidant capacity per gram of dried sample.

Ferric Reducing Ability (FRAP) Test

A 100 mm ferric chloride (FeCl3) solution, 10 mm TPTZ (2,4, 6-tripydridyl-s-triazine) in 40 mM hydrochloric acid (HCl) and 300 mM acetate buffer (3.1 g sodium acetate trihydrate and 16 ml acetic acid) at a pH of 3.6 were used in this study25 milliliters of acetate buffer, 2.5 milliliters of FeCl3, and 2.5 milliliters of TPTZ were combined to make up the working solution. To heat the

solution, it was raised to 37°C. The extract and the control were each given 10 microliters of a solution. Each was then combined with 300 solution of the FRAP working solution to carry out the experiment. In the end, a wavelength of 593 nm was used to determine the absorbance of the blue colored ferrous tripyridyl triazine complex [19].

RESULTS AND DISCUSSION

Physiochemical analysis

The physicochemical analysis of *Acacia* bringing leaf extract gives important data about its quality, purity and stability (Table 1). The loss on drying was 7.23%, and that told us there's an air of moderation in this plant material [20]. The aqueous extractive was 15.12%, reporting upon the water-soluble active constituents' solubility glycosides, sugars and some alkaloids. The alcoholic extractive was 11.23%, indicating that the present alcohol-soluble compounds may be tannins, flavonoids or other certain phenolic substances. The ether extractive was just 5.23%, reflecting a small content of non-polar materials in its product since this represents percentage by mass. Inferior ash originates from plant tissue, and inorganic ash of non-plant origin together constitute the proportion of inorganic residue remaining after combustion, i.e., 3.29% of total ash content. Acid-insoluble ash was 0.95%, being a subset of total ash indicating the amount of silica in the sample, often as a contaminant such as sand or dirt. The water-soluble ash was 2.18%, indicating those inorganic substances which dissolve in water, and therefore the availability of minerals for plants. Lastly, a 1% aqueous solution of the extract established pH as 6.25, which is slightly acidic but still safe for use in herb that suggests compatibility with organic systems and stability in a homogeneous solution.

Table 1 Physiochemical analysis of plant extract

S. No.	Test	*Acacia nilotica*	*Quercus infectoria*
1	Loss on drying (%)	8.14	7.23
2	Aqueous Extractive Value (%)	19.35	15.12
3	Alcoholic Extractive Value (%)	14.35	11.23
5	Ether Extractive Value (%)	5.49	5.23
6	Total Ash (%)	6.15	3.29
7	Acid Insoluble Ash (%)	2.12	0.95
8	Water Soluble Ash (%)	5.59	2.18
9	pH	5.54	6.25

Phytochemical analysis

The phytochemical screening of *Acacia nilotica* extracts revealed the presence of a wide array of bioactive constituents with variation across solvent types (Table 2). The aqueous extract tested positive for carbohydrates (Molish and Benedict tests), alkaloids (Dragendorff), amino acids (Ninhydrine), proteins (Biuret), saponins (Borntrager's), phenolic compounds, steroids (Salkowski), and tannins ($FeCl_3$ and Lead acetate), indicating a hydrophilic profile rich in secondary metabolites. In contrast, the ethanol extract showed limited positivity—detecting carbohydrates (Fehling), alkaloids (Wagner's), amino acids, proteins (Xanthoproteic), phenolics, and tannins—suggesting selective solubility of certain compounds. Notably, both extracts lacked response to Hager's and Millon tests for alkaloids and proteins, and potassium dichromate failed to confirm tannins.

The phytochemical analysis of *Quercus infectoria* extracts reveals a diverse presence of secondary metabolites with solvent-dependent variability (Table 3). The aqueous extract proved richer, showing positive results for carbohydrates (Molish and Benedict tests), alkaloids (Dragendorff), amino acids, proteins (Biuret), saponins, phenolic compounds, steroids, and tannins—indicating a complex mixture of bioactive hydrophilic compounds. In contrast, the ethanolic extract identified fewer constituents but did detect specific compounds like carbohydrates (Fehling), alkaloids (Wagner's), proteins (Xanthoproteic), and phenolics, highlighting its role in extracting slightly more lipophilic or structurally distinct metabolites. Notably, both extracts responded positively to tannin tests ($FeCl_3$ and Lead acetate), confirming their abundance and likely contribution to antioxidant and astringent activity.

Table 2 Phytochemical analysis of Acacia nilotica extract

Name of Test	Aqueous Extract	Ethanol Extract
Carbohydrate		
Molish test	+ ve	- ve
Benedict test	+ ve	- ve
Fehling test	- ve	+ ve
Alkaloids		
Dragendorff test	+ ve	- ve

Wagner's test	- ve	+ ve
Hager's test	- ve	- ve
Amino acid		
Ninhydrine	+ ve	+ ve
Protein		
Biuret test	+ ve	- ve
Xenthoprotic test	- ve	+ ve
Millon test	- ve	- ve
Saponin		
Borntrager's test	+ ve	- ve
Phenolic compound		
Phenolic test	+ ve	+ ve
Steroids		
Salkowaski	+ ve	- ve
Tannins		
FeCl3	+ ve	+ ve
Lead acetate	+ ve	+ ve
Pot. Dichromate	- ve	- ve

Table 3 Phytochemical analysis of *Quercus infectoria* extract

Name of Test	**Aqueous Extract**	**Ethanol Extract**
Carbohydrate		
Molish test	+ ve	- ve
Benedict test	+ ve	- ve
Fehling test	- ve	+ ve
Alkaloids		
Dragendorff test	+ ve	- ve
Wagner's test	- ve	+ ve
Hager's test	- ve	- ve
Amino acid		
Ninhydrine	+ ve	+ ve
Protein		
Biuret test	+ ve	- ve
Xenthoprotic test	- ve	+ ve
Millon test	- ve	- ve
Saponin		
Borntrager's test	+ ve	- ve
Phenolic compound		
Phenolic test	+ ve	+ ve
Steroids		
Salkowaski	+ ve	- ve
Tannins		
FeCl3	+ ve	+ ve
Lead acetate	+ ve	+ ve
Pot. Dichromate	- ve	- ve

HPTLC Analysis

Figure 1 illustrates the High-Performance Thin Layer Chromatography (HPTLC) profiling of phenolic constituents present in the ethyl acetate (EA) extracts of *Acacia nilotica* leaves. In panel (a), the TLC plate, viewed under UV light at 254 nm, shows distinct dark bands—each corresponding to a separated phenolic compound. These bands visually confirm the presence of multiple phenolic constituents and help determine their respective retention factor (Rf) values. Panel (b) is a densitogram that quantifies those visual bands, plotting intensity against Rf values. The graph displays nine distinct peaks—five major and four minor—indicating the relative abundance and diversity of phenolic compounds in the extract. The presence of multiple high-intensity peaks suggests a rich phenolic profile, which aligns with the strong antioxidant and therapeutic potential attributed to *Acacia nilotica*. This chromatographic fingerprint is key in standardizing and validating the extract for further pharmacological applications.

With various solvents, the ideal mobile phase for examining betulinic acid β-sitosterol in LAHE was determined as chloroform, methanol and glacial acetic acid (97:2:1) [22]. From the figure 2A. Betulinic acid and β-sitosterol are both characterized by intense sharp peaks with an Rf of 0.31 ± 0.001, and 0.41 ± 0.001 respectively (Figure 2). From Figure 2B we can see that Using the present method betulinic acid β-sitosterol typical substances in LAHE are well separated. Figure 2C reports that the bands of the extract of LAHE, Figure 2D as shown in this paper matched those for betulinic acid and β-sitosterol (Figure). After derivatization under 366 nm, the HPTLC chromatoplate containing stigmasterol and petroleum ether extracts of Babul leaves (S1, S2, S3, S4, S5, B6, B7) was used [23]. S1-S5 standard stigmasterol concentrations in ng/spot range from 50 to 250 ng/spot (Figure 3). Figure 4 and 5 showing HPTLC chromatogram of sample containing gallic acid and lupeol, respectively.

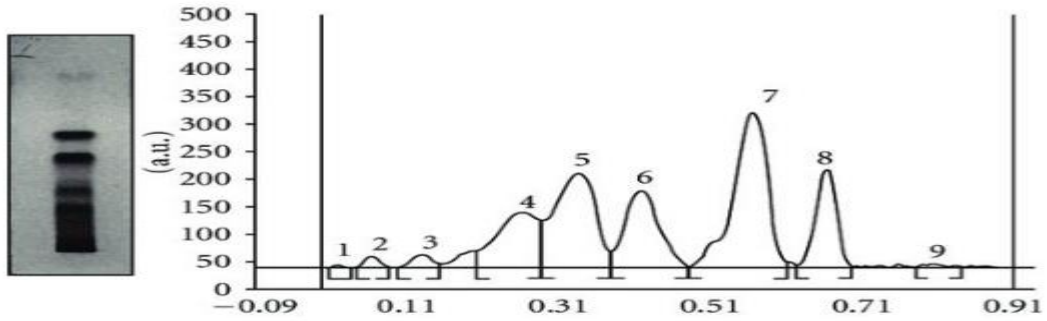

Figure 1 HPTLC Leaves of *Acacia nilotica* (L.)

HPTLC chromatogram displaying phenolic components in EA extracts derived from A. nilotica leaves. (a) TLC of phenolic compounds after exposure to 254 nm. (b) Densitogram of phenolic compounds showing four minor and five major peaks.

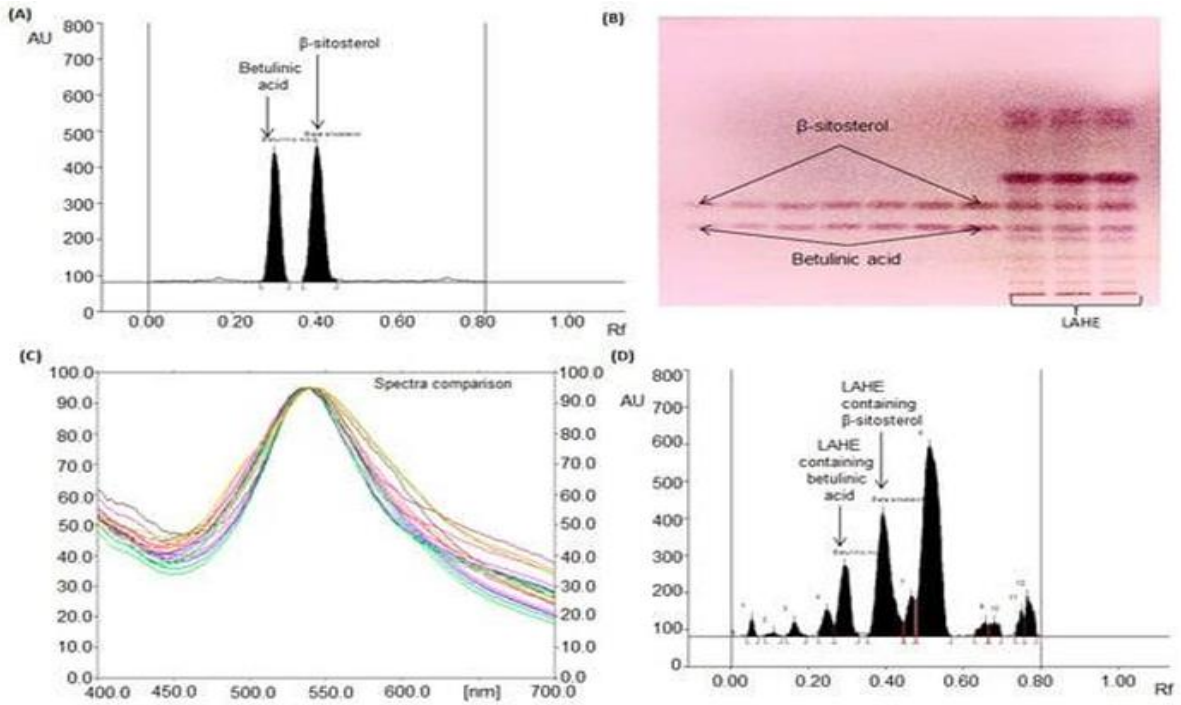

Figure 2 Betulinic Acid and β-Sitosterol in LAHE at the Same Time using a Verified HPTLC Technique

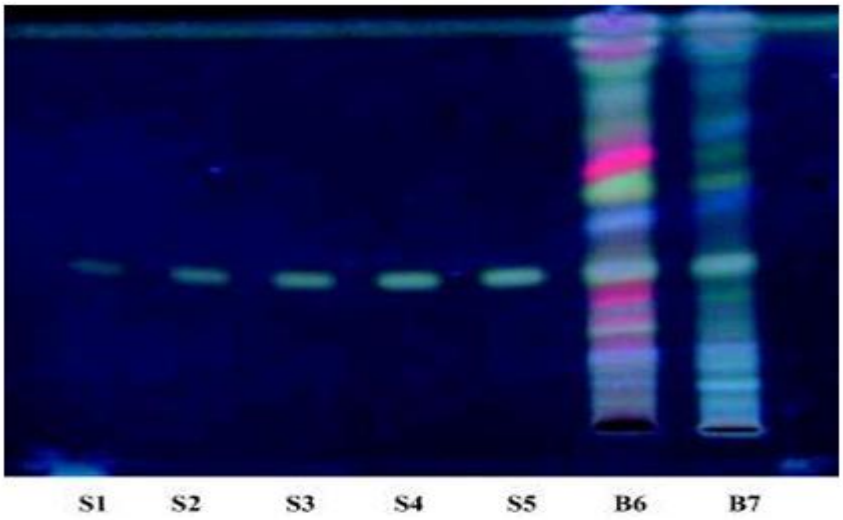

Figure 3 HPTLC chromatoplate containing stigmasterol and petroleum ether extracts of Babul leaves (S1, S2, S3, S4, S5, B6, B7)

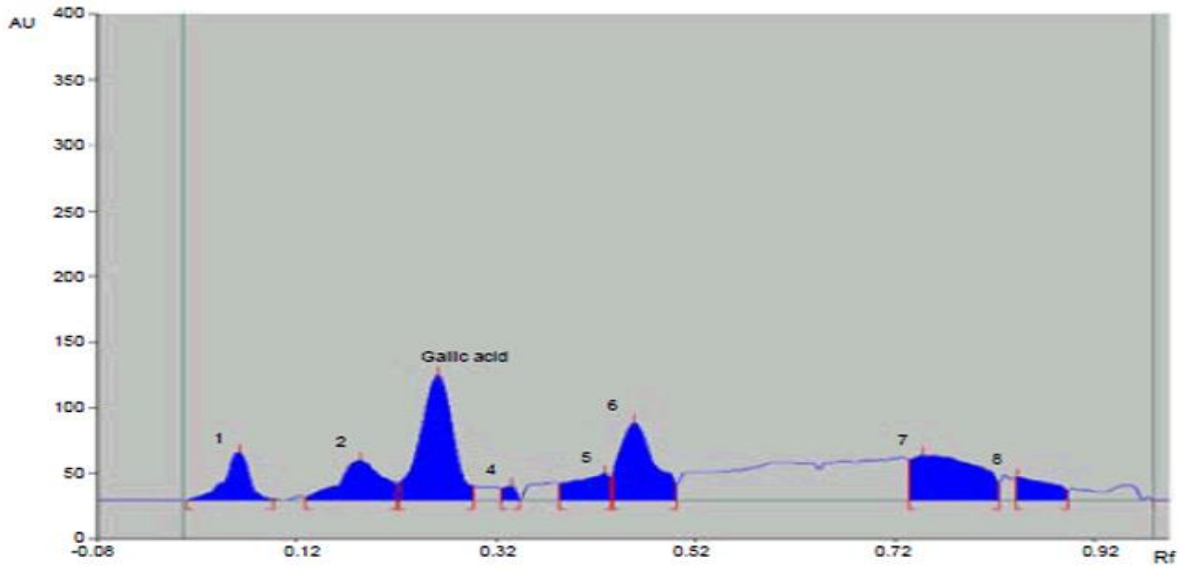

Figure 4 HPTLC chromatogram of sample containing Gallic acid

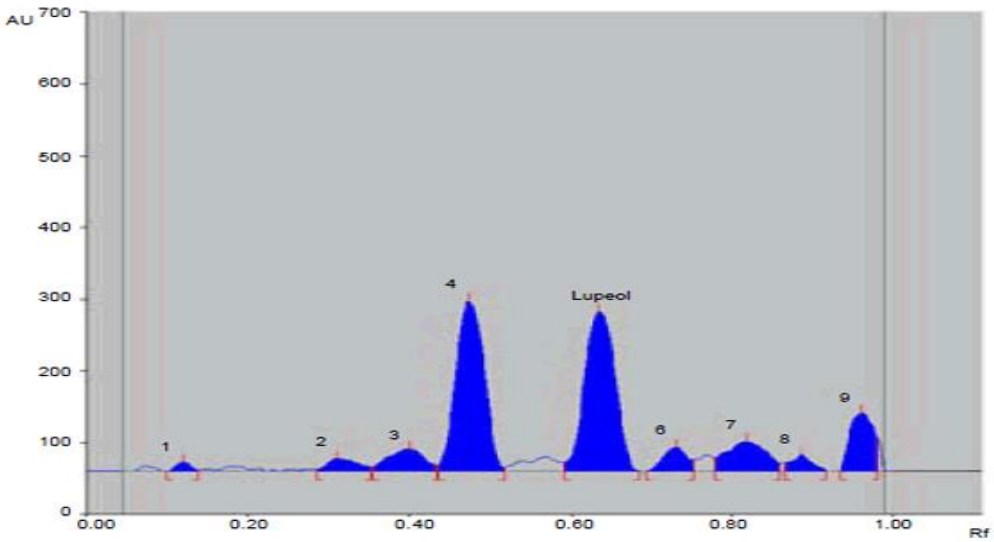

Figure 5 HPTLC chromatogram of sample containing Lupeol

GC-MS

Botanical substances, including sugars, amino acids, steroids, polyphenols, and fatty acid esters. The primary phytoconstituents identified in A. nilotica (Figure 6) were pyrogallol (64.04%), 4-O methylmannose (17.7%), 9,12-octadecadienoic acid (6.8%), methyl oleate (1.9%), methyl linoleate (1.6%), and N,N-Dimethylglycine (1.3%) [25]. Figure 7 showing GC–MS chromatogram of Quercus infectoria extract.

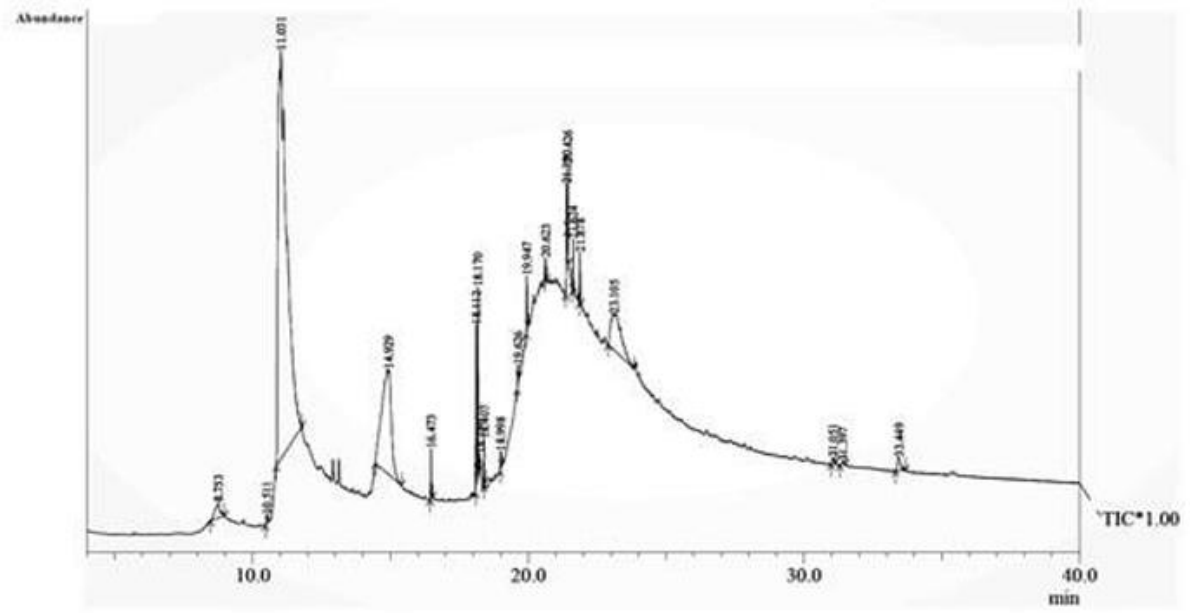

Figure 6 GC–MS chromatogram of methanolic extract of A. nilotica

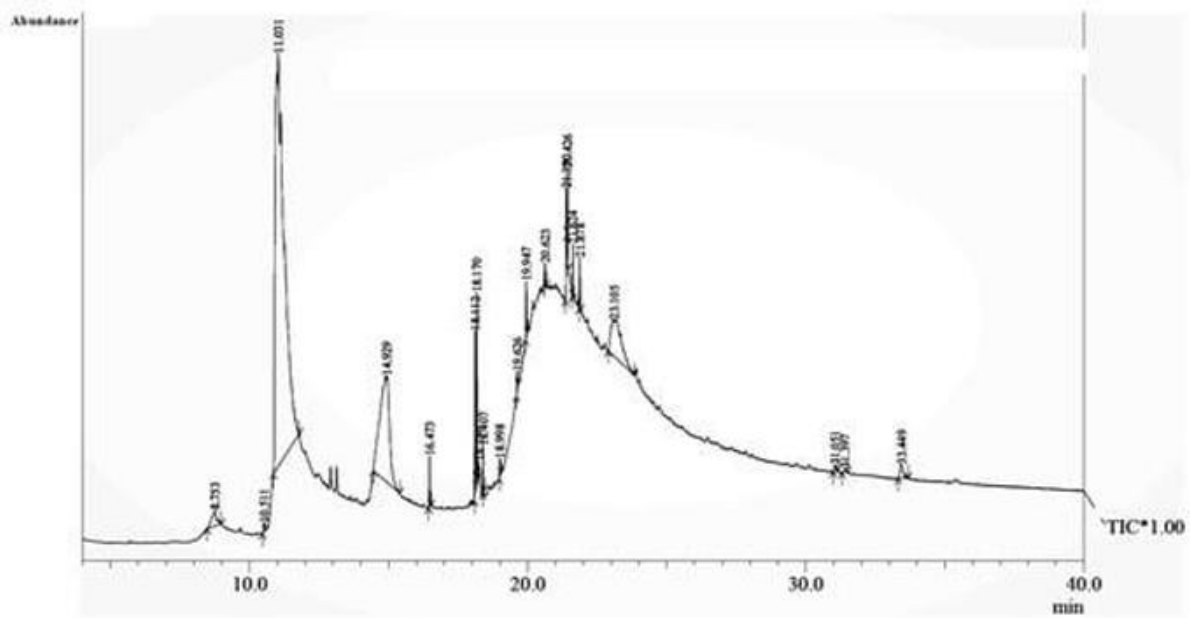

Figure 7 GC–MS chromatogram of Quercus infectoria extract

Antioxidant activity of plant extracts

The antioxidant potential of various samples is summarized in Table 4 based on data obtained through DPPH and FRAP assays [27]. This well-known antioxidant standard itself (89.998±2.883 g/uM an ounce of Trolox), gave the highest activity as measured by DPPH. Compared to the *Quercus infectoria* extract (QIE) from plant extracts, ANE extract of *Acacia nilotica* was more effective as an anti-radical species in the DPPH scavenging assay: Therefore, its DPPH radical neutralization efficacy (76.377±2.988 uM/g) was greater than that for any other plant sample tested (Figure 8). Conversely ascorbic acid had the highest reducing potential (2277±0.026 g uM Fe(II))/g according to FRAP measurements equalizing Fe(II). In the FRAP measurement, ANE once again showed its greater power by comparison with QIE (2002.3938± 10.877

uM Fe(II)/g vs 1739.4984± 11.938 uM Fe(II)/g, respectively). These data show that both ANE and QIE have noticeable antioxidant effects, ANA effect is much better in either test.

Table 4 DPPH and FRAP assay results

Sample	DPPH (microM per gram dry sample)	FRAP (microM Fe(II)/g dry mass)
Control	---	---
Trolox	89.998 ± 2.883	---
Ascorbic acid	---	2277 ± 0.026
ANE	76.377 ± 2.988	2002.3938 ± 10.877
QIE	67.938 ± 3.948	1739.4984 ± 11.938

ANE (*Acacia nilotica* extract) and QIE (*Quercus infectoria* extract)

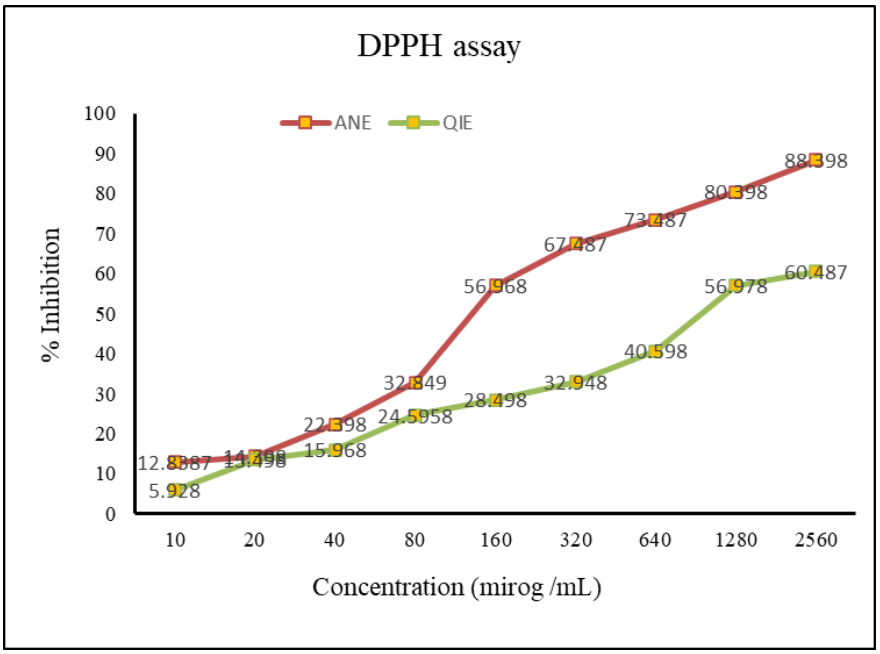

Figure 8 DPPH assay of both plants' extracts

Discussion

The study highlights the therapeutic promise of *Acacia nilotica* and *Quercus infectoria*, grounded in traditional medicine and validated through modern analytical techniques. The physicochemical analyses revealed that *Acacia nilotica* exhibits higher aqueous extractive values (19.35%) and slightly more acidic pH (5.54) compared to *Quercus infectoria*, implying a richer concentration of water-soluble bioactive compounds such as tannins, flavonoids, and glycosides [14]. This is further supported by phytochemical screenings that confirmed the presence of phenolic compounds, saponins, steroids, and proteins across both aqueous and ethanolic extracts.

Spectroscopic and chromatographic profiling using IR, HPTLC provided detailed insights into compound identity and concentration. For instance, *Acacia nilotica* showed substantial levels of ferulic acid (5451.04 µg/mL), chlorogenic acid (4572.26 µg/mL), and quercetin (3733.37 µg/mL), all recognized for their strong antioxidant and neuroprotective properties [25, 8]. Additionally, the HPTLC chromatograms confirmed the presence of betulinic acid and β-sitosterol, which are well-documented for their anti-inflammatory and lipid-lowering effects [22].

Antioxidant activities assessed via DPPH and FRAP assays underscored the potency of both extracts, with *Acacia nilotica* outperforming *Quercus infectoria* in radical scavenging and reducing capacity. The DPPH values of 76.38 ± 2.99 µM/g and FRAP values of 2002.39 ± 10.88 µM Fe(II)/g for *Acacia nilotica* suggest robust oxidative stress mitigation potential, surpassing that of *Quercus infectoria* (67.94 ± 3.95 µM/g and 1739.50 ± 11.94 µM Fe(II)/g respectively) [26, 27].

The integration of qualitative phytochemical profiling with quantitative spectral analysis supports the traditional claims regarding neuroprotective applications of these plants. Quercetin and gallic acid—present in notable concentrations—have been linked to modulation of oxidative pathways and inflammation, contributing to neuronal health and reducing the progression of neurodegenerative conditions [3, 28-34].

In sum, the comparative evaluation affirms *Acacia nilotica* as a slightly superior candidate in terms of

extractive yield and antioxidant efficacy. However, both plants exhibit broad-spectrum phytochemical richness, indicating potential utility in herbal formulations aimed at combating oxidative stress and inflammation-related disorders. Further bioassay-guided fractionation and in vivo models are encouraged to substantiate these findings and unravel molecular mechanisms involved.

CONCLUSION

Using an integrated approach that includes physicochemical characteristics, chemical profiling and in vitro antioxidant studies, it is possible to establish a robust pharmacognostic and neuropharmacological outline of Acacia nilotica (ANE) or Quercus infectoria (QIE). The protective effects of both extracts are fairly productive through the blood-brain barrier. They have a significant antioxidant force because they contain bioactive chemicals including such high antioxidant loads as quercetin and gallic acid. Further work, involving isolation of active compounds from these extracts research into their mechanism at the molecular level and clinical trials with patients is desirable for establishing their pharmacological potential and translational safety.

Data availability

It can be made available on request.

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We are thankful to the institute for providing all facilities required to conduct this work.

Declaration

Authors declared no conflict of interest. ChatGpt was used for writing to improve English language.

Author credit statement

PS – review and proofreading, RP – written original draft.

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