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Evaluation of Phytochemical Composition, Antioxidant Activity, and Bioactive Constituents of *Acacia arabica* bark Extract: A Comprehensive Analysis

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ABSTRACT

Acacia arabica is historically known to have medicinal properties in bark. In this work, its phytochemical components, antioxidant efficacy and bioactive substances was assessed for investigating the possibility of using it as natural antioxidant. Soxhlet extraction was used to obtain a hydroalcoholic extract of *Acacia arabica* bark. Principal ingredients were identified by phytochemical screening and TPC and TFC of a crude extract was determined. DPPH, ABTS, FRAP tests have been used to determine the antioxidant potential of the extract. The extract was analysed with LC-MS to identify bioactive compounds. Phenols, tannins, flavonoids and alkaloids were reported to be present in phytochemical investigation. Antioxidant activity of the extract was very high with IC_{50} values of 6.44 µg/mL (DPPH), 4.38 µg/mL (ABTS) and 0.86 µg/mL (FRAP). They had a quantified total phenolic content (TPC) of 940 and a total flavonoid content (TFC) of 94 mg quercetin equivalent per gram. Bioactive chemicals as spermine, isocitric acid lactone, and 9-deenoic acid, known for their antioxidant activity, were detected by LC-MS. Considering the past use of *Acacia arabica* as a natural antioxidant source, the significant antioxidant properties of its bark extract towards its phenolic and flavonoid content are validated. These findings enabled subsequent studies on the applicability of this in functional foods or nutraceutical formulations.

Keywords: *Acacia arabica*, Antioxidant activity, Flavonoid content, LC-MS, Phenolic content.

INTRODUCTION

Reactive oxygen species (ROS) and free radicals are exceedingly reactive chemicals which are formed during the metabolism of the body or at exposure to external stressors due to ultraviolet ray, pollution and environmental toxins. ROS are needed for immune defense and cell signaling, but too many of them can upset the balance of cells, which can cause oxidative stress that hurts DNA, proteins, and fats. Oxidative stress is a crucial element in the development of various chronic diseases, such as cancer, diabetes, and cardiovascular ailments.[1]

The body's antioxidant defense system

combats ROS through both enzymatic components, such as superoxide dismutase and catalase, and non-enzymatic components, including glutathione, polyunsaturated fatty acids, and vitamins C and E. Under stress situations, this system may prove inadequate, prompting the need for exogenous antioxidants, especially from natural sources, owing to their therapeutic efficacy and safety. Plant-derived compounds, such as phenolics, flavonoids, tannins, and alkaloids, have significant antioxidant properties by eliminating reactive oxygen species, chelating metal ions, and enhancing the body's intrinsic defenses.[2,3]

Acacia arabica, also referred to as Indian gum arabic, is widely recognized in traditional medicine for its anti-inflammatory, antibacterial, and wound-healing attributes[4]. It is abundant in phenolic chemicals and flavonoids, which enhance its pharmacological efficacy.[5] Despite the prolonged use of *Acacia arabica*, its antioxidant activity has not been rigorously evaluated, and the bioactive chemicals responsible for these effects remain unidentified. This work aims to investigate the phytochemical composition, antioxidant activity, and bioactive constituents of *Acacia arabica* bark extract to address this research gap.

MATERIALS AND METHODS

Chemicals

All the reagents used in this research were supplied by CDH Pvt. Ltd in Delhi, Merk Ltd. in Mumbai, Qualigens in Mumbai, and HiMedia Ltd. in Mumbai, and all were of LR or AR grade.

Plant Collection and Authentication

Plant was selected after reviewing the literature, gathering phytoconstituent-based activity data from conventional publications, the internet, and the traditional medical system. The plant's bark was collected from the roadside of a village in Sambhal, District-Moradabad, Uttar Pradesh, India, in December 2023. Plant for authentication was successfully done, and the authentication letter was received. Authentication was done by Dr. Alok Shrivastava from Plant science department, M.J.P. Rohilkhand University, Bareilly, U.P., India with authentication number–MJPRU/PS/2023/0578.

Extraction technique

The powdered bark was subjected to sequential extraction using a soxhlet apparatus. Initially, defatting was conducted using petroleum ether (60–80°C), succeeded by extraction with 70% ethanol. Whatman No. 1 filter paper was employed for filtration of the extracts. This filtrate was concentrated to a minimal volume using a rotary evaporator at 40°C under reduced pressure (70-100 mBar). Subsequent to each extraction phase, the marc was air-dried at room temperature before to advancing to the next solvent. The yield of hydroalcoholic extract was calculated. The hydroalcoholic extract of *Acacia arabica* bark was further analyzed for phytochemical constituents and evaluated for antioxidant and to optimize its production[6].

Phytochemical screening

Standard qualitative methods were employed to detect the presence of significant phytocompounds, including flavonoids, terpenoids, tannins, alkaloids, steroids, saponins, and phenolics in the Acacia arabica bark extract.[7,8]

Estimation of total phenolic content

The evaluation of total phenols in extract was conducted utilizing the Folin-Ciocalteu method. A standard gallic acid solution was prepared by dissolving 25 milligrams of gallic acid in distilled water. The extract was dissolved in various quantities, and flasks were filled with each concentration. The flasks were subsequently calibrated to 25 milliliters, and a UV spectrophotometer (UV-Shimadzu 1900i) was employed to record the absorbance at 750 nm after 90 min of incubation[9]

Estimation of total flavonoids content

Using the aluminium chloride colorimetric method, the complete amount of flavonoid was assessed. By dissolving 25 mg of quercetin in methanol, a standard solution with a concentration of 1 mg/mL was prepared. An additional 100 mg/mL solution was produced by diluting the original. It was then mixed with water, potassium acetate, methanol, and aluminium chloride. Various amounts of the flavonoid quercetin were used to plot a standard curve.[10]

Thin layer chromatography (TLC)

TLC plates (a 0.2 mm thick sheet of pre-coated aluminium silica gel) were taken using the following eluents: mixes of methanol and chloroform

(7:3 and 9:1), ethyl acetate and chloroform (6:4), and chloroform with ethyl acetate and formic acid (5:4:1). A pin-drawn line indicates where the spots were inserted, working with a capillary tube, one centimeter from the bottom. After the substance on the plate dried, it was quickly covered, and they placed it into the chromatographic tank. The plate was taken out, labeled, and dried after the solvent had reached the very top. Applying iodine vapor and spraying spotting reagent at UV wavelengths of 254 and 365 nm allowed for the determination of the number of spots.[11]

Liquid Chromatography-Mass Spectroscopy

The sample extract was subjected to LC-MS analysis in a Waters Xevo TQD triple quadrupole mass spectrometric platform coupled with a Waters Acquity H-class UPLC/PDA system (CSIR-CDRI, Lucknow). The two functional tests, ES+ and ES-, were conducted over a mass range of 150 to 2000 nm for approximately 40 minutes. The column temperature was set at 35°C. The pre-injection wash length was 0 seconds, whereas the post-injection wash period was 6 seconds, with an injection volume of 2 µL. The UPLC eLambda operated at 800 nm served as the detector. The device detected and reported the phytoconstituents after comparing them with those in the linked NIST computer library.[11]

Antioxidant ASSAY

2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging

On a 96-well plate, 5 µL of a different test stock was mixed with 0.1 ml of a 0.1 mM DPPH solution. Three copies of the reaction were made, one with five microliters of a chemical at varying concentrations and the other with 0.2 milliliters of DMSO/methanol. The reagent-free wells (DPPH) were designated as blank, and treatment-free wells were designated as control. The dish was incubated in the dark for 30 minutes. At the end of the incubation, the decolorization was measured in a microplate reader (iMark, BioRad) at 517 nm. Serving as the control was a reaction mixture with 20 µL of deionized water[12]. The display showed the percentage inhibition of the scavenging activity relative to the control. GraphPad Prism 6 software was used to determine the IC_{50}. A graph was prepared between the X-axis (sample concentration) and the Y-axis (% inhibition with respect to control. Scavenging activity (percentage) was estimated by the below mentioned equation 1.

$$\frac{(Absorbance\ \ of\ control - Absorbance\ of\ sample)}{Absorbance\ of\ control} \times 100$$

2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) scavenging

Ammonium Persulfate (APS) at 2.45 mM and 7 mM solutions of ABTS were combined to generate ABTS radicals. The ABTS free radical reagent was subsequently diluted by a factor of 100. A 96-well plate with 200 µL of ABTS free radical reagent was incubated at ambient temperature for 10 min in the absence of light. Samples and 10 µL of a distinct stock of the reference material (ascorbic acid) were included. The untreated wells were assigned as the control group. Assess the absorbance of the decolorization at 750 nm utilizing an iMark microplate reader (BioRad) following incubation. The results for the negative control were provided[13]. The IC_{50} was determined using GraphPad Prism version 9.5.1 software. A graph was constructed with the X-axis representing sample concentration and the Y-axis indicating % inhibition relative to the control 14–16. Scavenging activity was calculated by equation 1.

Ferric reducing-antioxidant power scavenging assay

A mixture of 0.04 mL of 0.2 M sodium phosphate buffer, pH 6.6 and 1% potassium ferricyanide [K3Fe (CN)6] was mixed. After giving the reaction mixture a thorough vortex, it was incubated for 20 min at 50°C. The control wells were those that received no treatment. 0.5 milliliters of trichloroacetic acid (10%) was added to the mixture at the conclusion of the incubation. After that, 50 µL of 0.1% ferric chloride and 50 µL of deionized water were added. Using a microplate reader (iMark, BioRad), the colored solution was read at 700 nm in comparison to the blank[17]. The program Graph Pad Prism 6 was used to determine the IC_{50}. Scavenging activity was calculated by equation 1.

RESULTS

Percentage yield of extract

The yield of hydroalcoholic extract of *Acacia arabica* bark was found to be 36%.

Phytochemical screening of the extract

Phenols, tannins, flavonoids, and alkaloids were identified in the extracts through preliminary phytochemical screening, as shown in Table 1.

Table 1: Results of phytochemical screening of *Acacia arabica* bark

Sr. No	Phytoconstituents	A.arabica extract	Name of the test
1	Phenolic and Tannins	+	Ferric chloride test
		+	Potassium dichromate test
		-	Lead acetate test
		-	Acetic acid test
		+	Dilute HNO_3 test
2	Flavonoids	-	Shinoda test
		-	Lead acetate test
		-	NaOH test
3	Alkaloids	+	Dragendroff's test
		+	Mayer's test

Total phenolic and flavonoid estimation

The phenolic amount in the extract of *Acacia arabica* bark was calculated to be 940 mg/ Gallic acid equivalent per gram and the amount of flavonoid was found to be 94 mg/Quercetin equivalent per gram. All the values are stated as Mean ± Standard deviation (SD). Values are mean of three replicates.

Qualitative phytochemistry: Thin layer chromatography

TLC was performed based on the solvent and were presented in Table 2.

Table 2: TLC analysis of Acacia arabica extract

Sr. No	Solvent used	Ratio	No. of spot	Colour of spot	R_f value
1	Chloroform: Methanol	7:3	1	Light brown	0.91
2	Chloroform: Ethyl acetate	6:4	1	Light brown	0.44
3	Chloroform: Ethyle acetate: Formic acid	5:4:1	2	Light brown	0.64 and 0.94
4	Chloroform: Methanol	9:1	2	Light brown	0.96 and 0.90

LC-MS of *Acacia arabica* extract

The probable identification of compound present in bark extract were presented in Table 3 and Fig. 1 for ES (+) and for ES (-) represented in Table 4 and Fig. 2. The highest relative concentration of ions at m/z, 202.22, which were thought to represent spermine. The other substances found in bark extracts were Isocitric acid lactone, Ethylpiperidine-4-carboxylate, 4-t-Butylbenzeneamine, 9-Decenoic acid, 1-Hexanamine, N-hexyl-N-nitroso-, N-Methyldodecanamide, Diethyl decylphosphonate, N-Methyldodecanamide, Barban, 9-Heptadecanol, Nonadecane, 3-methyl-, 1,3,5-Trinitrobenzene and Heptadecane, 3-methyl-.

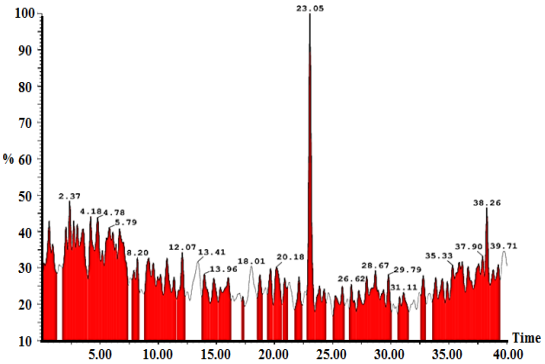

Fig. 1. LC-MS (ES+) spectrum of *Acacia arabica* bark

Table 3: LC-MS (ES+) analysis of bark extracts of *Acacia arabica*

Sample	MS (m/z)	Tentative identification
Bark	402.19	Isocitric acid lactone
	202.22	Spermine
	157.11	Ethylpiperidine-4-carboxylate
	149.12	4-t-Butylbenzeneamine
	223.19	9-Decenoic acid
	214.2	1-Hexanamine, N-hexyl-N-nitroso-
	213a.21	N-Methyldodecanamide
	278.2	Diethyl decylphosphonate
	213.21	N-Methyldodecanamide
	257	Barban

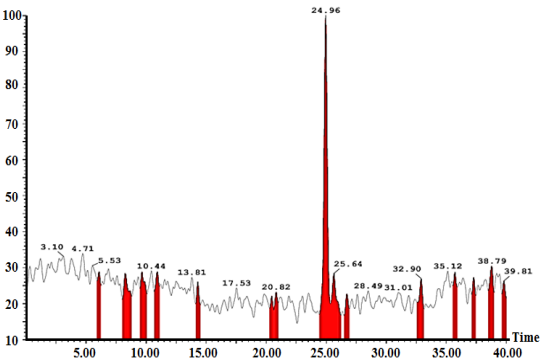

Fig. 2. LC-MS (ES-) spectrum of *Acacia arabica* bark

Table 4: LC-MS (ES-) analysis of bark extracts of *Acacia arabica*

Sample	MS (m/z)	Tentative identification
Bark	256.28	9-Heptadecanol
	282.33	Nonadecane, 3-methyl-
	213	1,3,5-Trinitrobenzene
	254.3	Heptadecane, 3-methyl-

DPPH radical scavenging activity

The IC_{50} value was determined for each substance. The significantly lower IC_{50} value of 6.442 ± 0.17 µg/mL for the *Acacia arabica* extract indicates its robust antioxidant activity in comparison to ascorbic acid's IC_{50} value of 22.05 ± 0.026 µg/mL. These results suggest that Acacia arabica

may serve as a more potent natural antioxidant than ascorbic acid, demonstrating a superior capacity to scavenge free radicals. The comparative percentage scavenging activity of both ascorbic acid and *Acacia arabica* bark extract was illustrated in Figure 3.

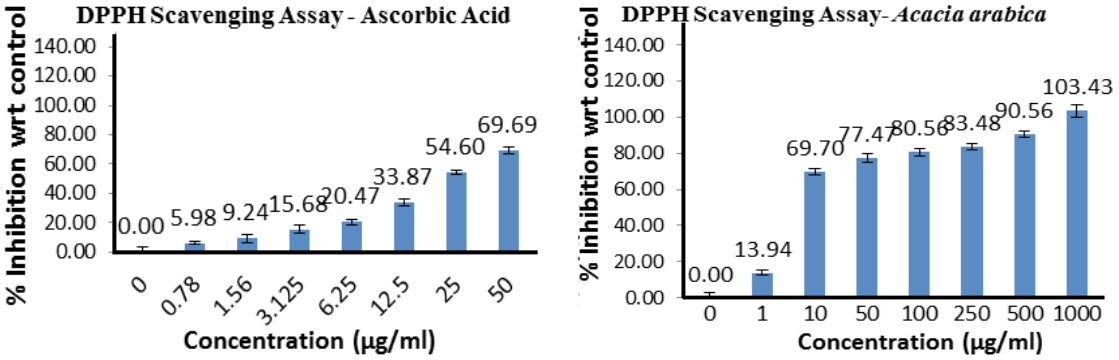

Fig. 3. Comparison graph of DPPH assay of ascorbic acid with *Acacia arabica* bark extract

ABTS radical scavenging activity

The IC_{50} value of extract, representing the concentration required to block 50% of ABTS radicals, was determined to be 4.38 ± 0.089 µg/mL and for ascorbic acid it was found to be 8.414 ± 0.029. The bark extract exhibits significant antioxidant activity, evidenced by its relatively low IC_{50} value, indicating its efficacy in neutralizing free radicals. The comparative percentage scavenging activity of both ascorbic acid and *Acacia arabica* bark extract was presented in Figure 4.

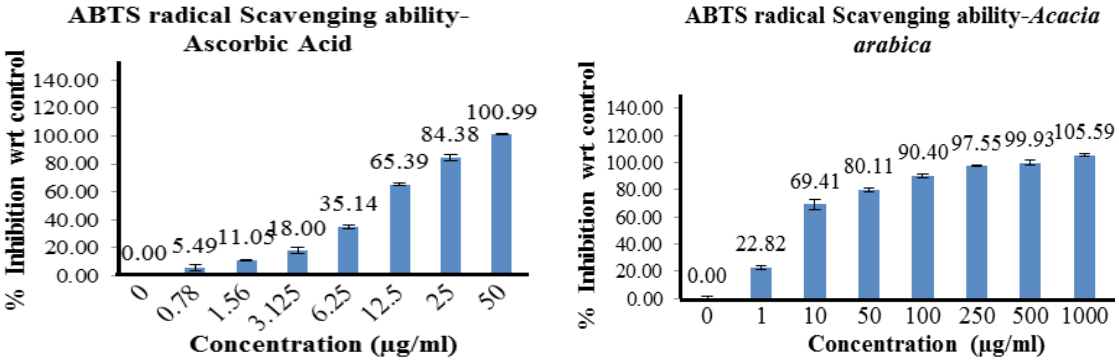

Fig. 4. Comparison graph of ABTS assay of Ascorbic acid with *Acacia arabica* bark extract

FRAP radical scavenging activity

The IC_{50} for ferric ion reduction in extract was established at 0.8602 ± 1.35 µg/mL while ascorbic acid was observed to be 2.39 ± 0.011 µg/mL. This extract demonstrates remarkable ferric ion-reducing action, evidenced by its low IC_{50} value, which underscores its significant antioxidant potential. The comparative percentage scavenging activity of both ascorbic acid and Acacia arabica bark extract was presented in Figure 5.

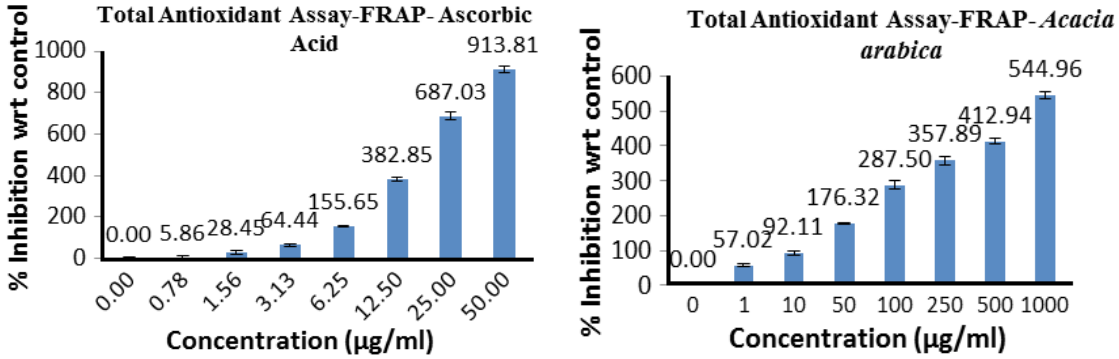

Fig. 5. Comparison graph of ABTS assay of Ascorbic acid (as standard) with *Acacia arabica* bark extract

DISCUSSION

Soxhlet extraction is a process that involves boiling a solid substance to extract soluble components, with polar solvents being better for obtaining the desired compounds. *Acacia arabica* bark extract, an essential component of natural health products, has high antioxidant qualities due to its phenolic components, which may have antibacterial and anti-inflammatory. Phytochemical screening is conducted to identify and describe the bioactive substances contained in the extract, such as tannins, flavonoids, saponin, phenols, and alkaloids which possess anti-inflammatory, antibacterial, antioxidant and anti-diabetic activity[18]

The total phenolic content (TPC) of plant extracts is a significant parameter, because phenolic chemicals are important for maintaining health and preventing illness. The TPC value indicates the presence of phenolic chemicals, which are essential for protecting against inflammation, lowering oxidative stress, and neutralizing free radicals. The high TPC value in the bark extract supports its potential for creating dietary supplements or medications targeting inflammation and disorders linked to oxidative stress[19]. Using a colorimetric technique, the quantity of flavonoids in plant extracts is measured by total flavonoid content, or TFC. As shown, the corresponding quantity of rutin or quercetin of material, it is essential for evaluating the antioxidant and medicinal qualities of the extract.

TLC, or thin layer chromatography, is an essential analytical method for separating and identifying bioactive compounds in the extract. It can create an extract's fingerprint profile, which is essential for control and standardization of herbal medication quality. Key components were found by the use of LC-MS such as phenolics, flavonoids, and alkaloids, validating the extract's potential therapeutic applications and upholding traditional medical usage.[20]

The DPPH assay evaluates plant extracts' antioxidant potential by assessing their ability to neutralize free radicals. The bark extract of *Acacia arabica* has a low IC_{50} value and significant antioxidant activity, perhaps as a result of the flavonoids, phenolic chemicals, and other bioactive components it contains. The ABTS test measures the extract's ability to combat free radicals ABTS•+, demonstrating its potential for medicinal and prophylactic applications. Ferric-to-ferrous ion conversion of the extract is measured using the FRAP test, indicating its strong antioxidant properties. The extract's potential as a preventive measure against oxidative damage is demonstrated by its capacity to convert Fe^{3+} to Fe^{2+}.[12]

CONCLUSION

The findings of this study attributed to robust antioxidant properties of *Acacia arabica* bark extract and the existence of bioactive phytochemicals such as phenols, flavonoids, and tannins. The extract's primary bioactive components from LC-MS were found to be -spermine, isotric acid lactone, ethyl piperidine-4-carboxylate, and 9-decenoic acid etc. These compounds demonstrated significant scavenging free radicals through DPPH, ABTS, and FRAP assays. The study's identified mechanisms of apoptotic inhibitory, inflammatory reducing, and antioxidant effects may be significantly influenced by these substances.

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Conflict of interest

There are no reported conflicts of interest among the authors.

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