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HPLC-BASED IDENTIFICATION AND QUANTIFICATION OF QUERCETIN IN ABELMOSCHUS ESCULENTUS FRUIT EXTRACT AND EVALUATION OF ITS ANTIMICROBIAL ACTIVITY

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ABSTRACT

Background: Abelmoschus esculentus (okra) contains bioactive phytochemicals, particularly flavonoids and phenolic compounds, which are responsible for its therapeutic activity.

Objectives: To identify and quantify quercetin (principle flavonoid) in the aqueous fruit extract of A. esculentus via high-performance liquid chromatography (HPLC) method and to assess its antimicrobial activity.

Materials and Methods: Fresh okra fruits were shade dried, pulverized, and extracted with distilled water. The extract was initially subjected to qualitative phytochemical screening to confirm flavonoid presence. Quercetin was quantitatively determined using reverse-phase HPLC on a C18 column with an isocratic mobile phase (acetonitrile and 0.05% orthophosphoric acid 50:50, v/v). Chromatographic separation was performed with a mobile phase flow rate of 0.7 mL/min and UV detection at 280 nm. Antimicrobial efficacy was assessed against specific organisms using the agar well diffusion assay.

Results: The developed HPLC method exhibited excellent linearity within the concentration range of 10–50 µg/mL (R^2 = 0.999). A characteristic quercetin peak was detected at ≈ 3.88 min retention time, and the extract contained 15.40% (w/w) quercetin. The extract exhibited antibacterial activity, with the greatest inhibition against S. aureus (22 mm), while antifungal activity was observed against A. niger (11 mm) and F. oxysporum (9 mm).

Conclusion: The HPLC method provided a reliable approach for the identification and quantification of quercetin in A. esculentus fruit extract. The measurable quercetin content together with the demonstrated antimicrobial activity highlights the potential of the extract for pharmaceutical applications.

KEYWORDS: Abelmoschus esculentus, Quercetin, HPLC, antibacterial, antifungal

INTRODUCTION

Medicinal plants remain an important source of therapeutic agents and continue to contribute significantly to modern drug discovery. Their extensive range of secondary metabolites has encouraged the exploration of plant-derived compounds for preventing and treating various diseases. Among these metabolites, polyphenols and flavonoids have emerged as compounds of considerable scientific interest because of their well-documented antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. Nevertheless, the successful therapeutic application of herbal medicines depends on proper phytochemical characterization and standardization to ensure their quality, safety, and reproducibility.[1]

Abelmoschus esculentus (okra), belonging to the family Malvaceae, is cultivated worldwide as an edible vegetable and is also valued in traditional medicine owing to its broad spectrum therapeutic properties. Various parts of the plant have been used in the management of inflammatory disorders, gastric ulcers, diabetes mellitus, and microbial infections. Earlier phytochemical investigations have revealed that the fruits contain several biologically active constituents, including flavonoids, phenolic acids, tannins, and polysaccharides, which are believed to contribute to their pharmacological activities.[2]

Quercetin is one of the major flavonoids reported in A. esculentus and has been extensively investigated because of its broad spectrum of biological activities. Additionally to its antioxidant potential, quercetin exhibits antimicrobial effects through diverse mechanisms, such as disruption of microbial cell membrane integrity, inhibition of nucleic acid synthesis, and interference with essential enzymatic processes. Since the biological efficacy of plant extracts is closely associated with the nature of their bioactive constituents, accurate identification and quantification of quercetin are essential for establishment of quality control standards and correlating phytochemical composition with pharmacological activity.[3]

HPLC is one of the widely accepted analytical method for the qualitative and quantitative determination of phytochemicals in herbal extracts. Owing to its high sensitivity, precision, and reproducibility, HPLC has become the preferred technique for the standardization. The technique enables accurate estimation of marker compounds, thereby facilitating batch-to-batch consistency and strengthening the scientific validation of herbal products.[4]

Although the medicinal properties of A. esculentus have been studied, evidence regarding the quantitative determination of quercetin in its aqueous fruit extract using HPLC method remains limited. Furthermore, studies correlating quercetin content with the extract's antimicrobial activity are relatively scarce. Therefore, the present study was commenced to identify and quantify quercetin in the aqueous fruit extract of A. esculentus using reverse-phase HPLC and to evaluate its antifungal and antibacterial activities against selected pathogenic microorganisms. The findings of this study are expected to contribute to the phytochemical standardization of A. esculentus and further substantiate its potential as a natural source of antimicrobial compounds for pharmaceutical use.

MATERIALS AND METHODS

Fresh fruits of Abelmoschus esculentus (okra) were procured from a local retail market in Belagavi, Karnataka, India. The plant was taxonomically identified and authenticated by Dr. Harsha V. Hegde, Scientist-E, at the Indian Council of Medical Research (ICMR) – National Institute of Traditional Medicine (NITM), Belagavi. A specimen of herbarium was deposited at the institute for future reference (Accession No. RMRC-1752).

Quercetin reference standard (purity ≥98.0%) was procured from Yucca Enterprises, Mumbai, Maharashtra, India. HPLC-grade solvents including acetonitrile, methanol, and orthophosphoric acid were procured locally. All chemicals were of analytical reagent grade.

Preparation of Abelmoschus Esculentus Fruit Extract

The collected okra pods were carefully washed using distilled water to eliminate adhering dirt and impurities. The cleaned pods were dried in shade at room temperature for 72 h, followed by drying at 30–40 °C until a constant weight was achieved. The dried material was pulverized using an electric blender, passed through sieve #22, and preserved in a container for further use.

For aqueous extraction, 1 g of the powdered material was boiled for 20 min in 200 mL of distilled water. Whatman No. 1 filter paper was used to filter the mixture, and the filtrate was evaporated to concentrate the extract using a water bath. The concentrated extract was stored at 4 °C for subsequent analysis.3

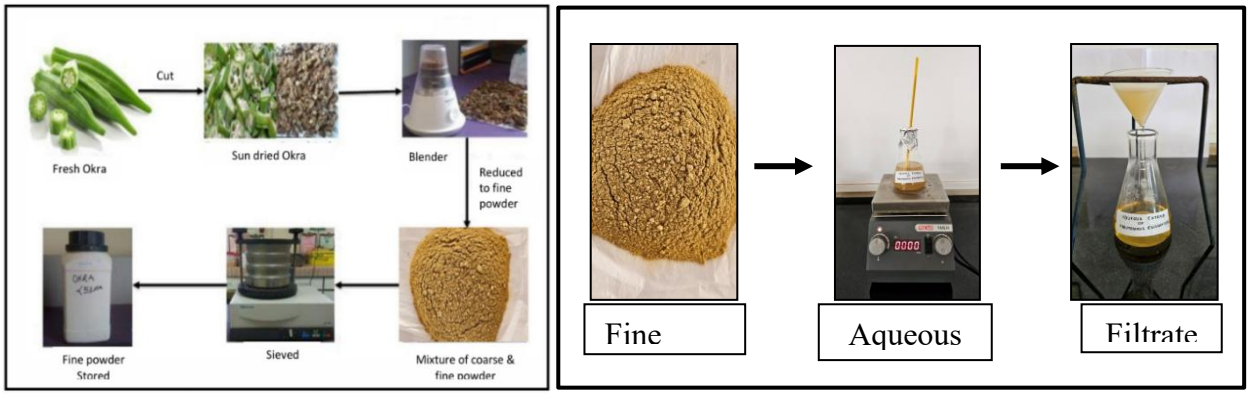

Figure 1: Preparation of Abelmoschus esculentus Fruit Extract

Qualitative Identification of Flavonoids Present in the Extract

The aqueous extract of Abelmoschus esculentus fruits was subjected to qualitative phytochemical analysis for the identification of flavonoids using standard phytochemical procedures described in the literature. Flavonoids were identified by performing the Shinoda test, alkaline reagent test, lead acetate test, and sulfuric acid test.5-9

Tests for Flavonoids

- Shinoda Test: Pieces of magnesium ribbon and concentrated Hcl were mixed with aqueous fruit extract and boiled for a few minutes. Appearance of pink to red colour showed the presence of flavonoid.
- Alkaline Reagent Test: 2 ml of 2.0% NaOH mixture was mixed with aqueous plant crude extract; concentrated yellow colour was produced, which turns colourless upon the addition of 2 drops of diluted acid to mixture. This result showed the presence of flavonoids.
- Lead Acetate Test: To the test solution add a mixture of 10 % lead acetate (2-3 drops). It gives white precipitate.
- Test for Acid: To the small quantity of test solution, 2-3 drops of concentrated sulphuric acid were added. Yellowish orange colour indicates the presence of flavonoids.

Quantitative Estimation of Quercetin in Abelmoschus Esculentus by High-Performance Liquid Chromatography

Instrumentation and Chromatographic Conditions

Quantitative determination of quercetin in the aqueous fruit extract of Abelmoschus esculentus was carried out using a reverse-phase high-performance liquid chromatography (RP-HPLC) system. Chromatographic parameters, including stationary phase, mobile phase composition, flow rate, and detection wavelength, were systematically optimized to obtain satisfactory peak resolution, symmetry, and reproducibility. The optimized HPLC system comprised an Agilent 1100 Series Isocratic Pump (HP-1100, G1310A) equipped with a Younglin UV 730D detector. Chromatographic separation was performed on a C18 reversed-phase column (250 × 4.6 mm, 5 µm particle size). The mobile phase consisted of acetonitrile and 0.05% orthophosphoric acid mixed in the ratio of 50:50 (v/v) and was delivered under

isocratic conditions at a flow rate of 0.7 mL/min. Detection was carried out at 280 nm, and a sample injection volume of 20 µL was maintained for all chromatographic analyses.10–12

Preparation of Standard Solution

A primary stock solution of quercetin (1000 µg/mL) was prepared by accurately dissolving 10 mg of quercetin reference standard in 10 mL of methanol. Working standard solutions containing 10, 20, 30, 40, and 50 µg/mL of quercetin were subsequently prepared by serial dilution of the stock solution with the mobile phase.

Preparation of Sample Solution

For HPLC analysis, 10 mg of the dried A. esculentus fruit extract was dissolved in 10 mL of methanol to obtain a final concentration of 1000 µg/mL. Before chromatographic analysis, the solution was passed through a 0.45 µm membrane filter to remove suspended particles and ensure compatibility with the HPLC system.

Construction of the Calibration Curve

Each working standard solution (10–50 µg/mL) was injected into the HPLC system in duplicate under the optimized chromatographic conditions. The corresponding peak areas were recorded, and the mean values were used to construct the calibration curve by plotting quercetin concentration against mean peak area. Method linearity was evaluated using linear regression analysis. The sample extract was analyzed under identical experimental conditions, and quercetin was identified by comparing its retention time with that of the authenticated reference standard. The amount of quercetin present in the extract was determined using the external standard calibration method derived from the regression equation.

Antimicrobial Study

The antimicrobial potential of the aqueous fruit extract of Abelmoschus esculentus was investigated using the agar well diffusion technique against selected bacterial and fungal pathogens. The bacterial panel comprised Staphylococcus aureus (ATCC 25923), Bacillus subtilis (ATCC 6633), Escherichia coli (ATCC 25922), and Klebsiella pneumoniae (ATCC 13883), whereas the fungal panel included Aspergillus niger (ATCC 16404) and Fusarium oxysporum (ATCC 48112). All microbial cultures were maintained under standard laboratory conditions and freshly subcultured before use to ensure the viability of the inoculum. Antimicrobial efficacy was assessed by measuring the diameter of the zone of inhibition (ZI) surrounding each well, and the results were expressed in millimetres (mm).[13]

Antibacterial Activity

Fresh bacterial suspensions were prepared from actively growing cultures. Sterile Petri dishes containing 15 mL of nutrient agar medium (HiMedia Laboratories, India) were allowed to solidify under aseptic conditions. Subsequently, 100 µL of each bacterial suspension was evenly distributed over the agar surface using a sterile L-shaped glass spreader to obtain a uniform bacterial lawn.

After the inoculated plates had dried, wells of 6 mm diameter were aseptically prepared using a sterile cork borer. Test solutions were prepared at a concentration of 1 mg/mL in distilled water, and 100 µL of each solution was carefully transferred into the respective wells. Streptomycin (1 mg/mL) was employed as the positive control, while dimethyl sulfoxide (DMSO) served as the negative control. The plates were incubated at 37 ± 2°C for 24 h, after which antibacterial activity was determined by measuring the diameter of the inhibition zones surrounding each well.[14-16]

Antifungal Activity

Fresh fungal inocula were prepared from actively growing fungal cultures. Sterile Petri dishes containing 15 mL of Sabouraud Dextrose Agar (SDA) medium (HiMedia Laboratories, India) were prepared and allowed to solidify. A volume of 100 µL of each fungal suspension was uniformly spread across the agar surface using a sterile L-shaped spreader to obtain an even distribution of the inoculum.

Following inoculation, sterile wells measuring 6 mm in diameter were punched into the agar using a cork borer. The extract solution (1 mg/mL) was prepared in distilled water, and 100 µL was dispensed into each well. Miconazole (1 mg/mL) was used as the reference antifungal agent, whereas DMSO served as the negative control. The inoculated plates were incubated at 37 ± 2°C for 24 h, and antifungal activity was evaluated by recording the diameter of the clear zones of inhibition around each well in millimetres (mm).[13,17]

RESULTS AND DISCUSSION

Qualitative Identification of Flavonoids in the Extract

Preliminary phytochemical analysis of the aqueous fruit extract of Abelmoschus esculentus confirmed flavonoids presence through all four qualitative assays employed in the study. In the Shinoda test, the extract developed a characteristic pink to reddish colour, indicating the presence of flavonoid compounds. The alkaline reagent test produced an intense yellow colour that disappeared after acidification, further confirming the occurrence of flavonoids. Likewise, the formation of a yellowish-white precipitate in the lead acetate test and the appearance of a yellowish-orange colour following the addition of concentrated sulfuric acid provided additional confirmation of these phytoconstituents.

The positive responses obtained in all qualitative tests are in accordance with previous studies reporting A. esculentus as a natural abundant source of flavonoids and phenolic content. Flavonoids exhibit a broad spectrum of biological activities, such as antioxidant, anti-inflammatory, antimicrobial, and wound-healing effects. Therefore, the confirmation of flavonoids in the aqueous extract supports its pharmacological significance and provides a scientific rationale for the subsequent quantitative determination of quercetin using HPLC.[7]

Quantitative Estimation of Quercetin by High-Performance Liquid Chromatography

HPLC Method Development

HPLC conditions were systematically optimized to achieve efficient separation of quercetin with acceptable peak symmetry, resolution, and reproducibility. Among the chromatographic systems evaluated, the C18 reversed-phase column provided the most satisfactory performance. An isocratic mobile phase of 0.05% orthophosphoric acid and acetonitrile (50:50, v/v), delivered 0.7 mL/min flow rate, produced a sharp and well-defined quercetin peak with a consistent retention time, demonstrating the suitability of the selected chromatographic conditions for routine analysis.

Linearity of Quercetin

The developed RP-HPLC method exhibited excellent linearity over the concentration range of 10–50 µg/mL. A progressive increase in peak area was observed with increasing concentrations of quercetin, indicating a strong linear relationship between analyte concentration and detector response. The calibration data corresponding to each standard concentration are summarized in Table 1, which indicated that the method was reliable for the quantitative estimation of quercetin.

Table 1: Calibration data of quercetin standard

Concentration (µg/mL)	Mean Peak area
10	88.711
20	173.5036
30	260.3951
40	350.7162
50	438.3293

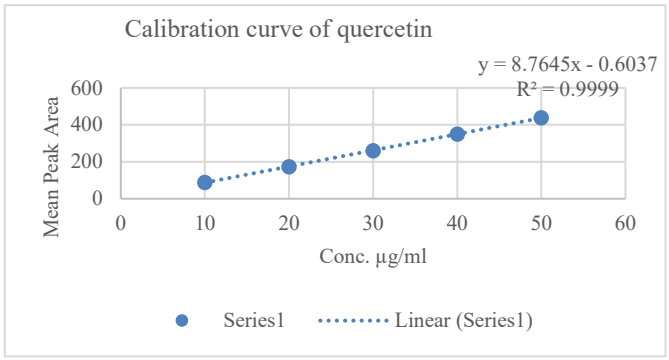

Figure 2: Calibration curve of Quercetin

The calibration curve plot showed excellent linearity within the investigated concentration range of 10–50 µg/mL, yielding a correlation coefficient ($R^2 = 0.999$). A high degree of linearity was observed between quercetin concentration and the corresponding chromatographic peak area (Figure 2), indicating a consistent detector response across the calibration range. These findings confirm the reliability, accuracy, and suitability of the developed RP-HPLC method or the quantification of quercetin in Abelmoschus esculentus fruit extract.

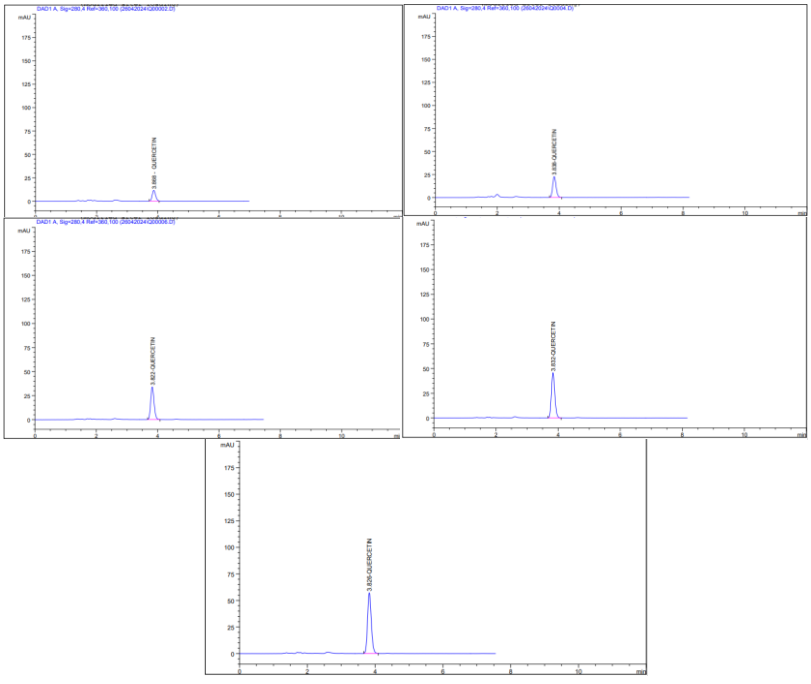

Figure 3: Representative HPLC chromatograms of quercetin standard solutions (10–50 µg/mL)

The chromatogram obtained from the aqueous fruit extract of Abelmoschus esculentus showed a well-defined peak at a retention time of 3.876 min (Figure 3). This retention time closely matched that of the quercetin reference standard (3.83 min), confirming the identity of the detected compound. The corresponding peak area was 1348.795 mAU·s, providing further evidence for the presence of quercetin in the extract. The close correspondence between the retention times of the standard and the sample demonstrates the specificity and reliability of the developed RP-HPLC method for quercetin identification.

The quantity of quercetin present in the extract was determined using the external standard calibration approach. Based on the calibration equation ($y = 8.7645x - 0.6037$; $R^2 = 0.999$), the concentration of quercetin in the analyzed sample solution was calculated to be 153.97 µg/mL. Considering that the sample solution was prepared by dissolving 10 mg of A. esculentus fruit extract in 10 mL of methanol, the quercetin content of the extract was estimated to be 153.97 µg/mg, corresponding to 15.40% (w/w).

Overall, the optimized RP-HPLC method exhibited satisfactory chromatographic performance, characterized by excellent peak resolution, reproducibility, specificity, and linearity across the selected calibration range. The successful detection and quantification of quercetin establish its suitability as a chemical marker for the quality assessment and standardization of A. esculentus fruit extract. These findings also strengthen the phytochemical characterization of the extract and provide a reliable analytical platform for future pharmacological and quality control investigations.

Antimicrobial Study

Antibacterial Activity

The antibacterial efficacy of the aqueous fruit extract of Abelmoschus esculentus was investigated against four clinically important bacterial strains using the agar well diffusion assay. The test panel included two Gram-positive bacteria, Staphylococcus aureus (ATCC 25923) and Bacillus subtilis (ATCC 6633), together with two Gram-negative bacteria, Escherichia coli (ATCC 25922) and Klebsiella pneumoniae (ATCC 13883). Streptomycin (1 mg/mL) and dimethyl sulfoxide (DMSO) were used as the positive and negative controls, respectively.

The extract inhibited the growth of all bacterial strains tested, as indicated by the formation of measurable zones of inhibition (Table 2). In contrast, no inhibitory zone was observed around the DMSO wells, confirming that the solvent did not contribute to the antibacterial activity observed.

Among the tested organisms, Staphylococcus aureus exhibited the greatest susceptibility to the extract, producing a zone of inhibition of 22 mm, which approached the activity of the reference antibiotic streptomycin (26 mm). Moderate inhibitory effects were recorded against Escherichia coli (18 mm) and Klebsiella pneumoniae (16 mm), whereas the smallest inhibition zone was observed for Bacillus subtilis (14 mm). Under identical experimental conditions, streptomycin produced inhibition zones of 22 mm, 25 mm, and 23 mm against Bacillus subtilis, Escherichia coli, and Klebsiella pneumoniae, respectively.

The observed inhibitory activity against both Gram-positive and Gram-negative bacteria suggests that A. esculentus fruit extract possesses broad-spectrum antibacterial properties. This antimicrobial effect may be attributed to the presence of bioactive phytochemicals, particularly flavonoids such as quercetin, which have been reported to impair microbial growth by disrupting cell membrane integrity, altering membrane permeability, inhibiting essential enzymes, and interfering with nucleic acid synthesis. The antibacterial activity demonstrated in the present study supports previous reports on the therapeutic potential of A. esculentus and highlights its promise as a natural source of antimicrobial compounds for pharmaceutical applications.[14]

Table 2: Antibacterial activity

Bacterial strain	Control (DMSO) (mm)	Streptomycin (1 mg/mL) (mm)	Okra extract (1 mg/mL) (mm)
Staphylococcus aureus (ATCC 25923)	0	26	22
Bacillus subtilis (ATCC 6633)	0	22	14
Escherichia coli (ATCC 25922)	0	25	18
Klebsiella pneumoniae (ATCC 13883)	0	23	16

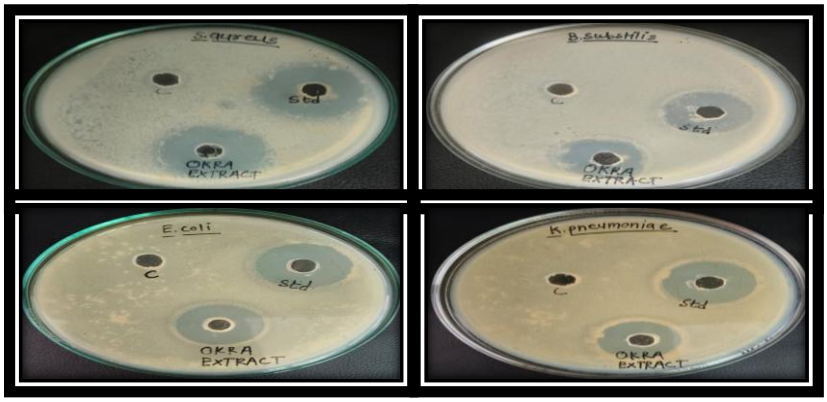

Figure 4: Antibacterial activity

Antifungal Activity

The antifungal potential of the aqueous fruit extract of Abelmoschus esculentus was assessed against Aspergillus niger (ATCC 16404) and Fusarium oxysporum (ATCC 48112) using the agar well diffusion method. Miconazole (1 mg/mL) served as the reference antifungal agent, whereas dimethyl sulfoxide (DMSO) was employed as the negative control.

The extract inhibited the growth of both fungal species, as evidenced by the formation of distinct inhibition zones around the wells (Table 3). No inhibitory zone was observed in the DMSO-treated wells, confirming that the solvent did not contribute to the antifungal activity.

Among the tested fungi, Aspergillus niger was more susceptible to the extract, exhibiting a zone of inhibition of 11 mm, whereas the corresponding inhibition zone produced by miconazole was 15 mm. In the case of Fusarium oxysporum, the extract produced an inhibition zone of 9 mm, while the standard antifungal agent produced a zone of 16 mm.

Although the antifungal activity of the extract was lower than that of miconazole, the results demonstrate that A. esculentus possesses measurable inhibitory activity against both fungal pathogens. The greater sensitivity of A. niger compared with F. oxysporum suggests a species-dependent response to the phytoconstituents present in the extract. This antifungal effect may be associated with the presence of flavonoids, phenolic compounds, tannins, and other secondary metabolites, which have been reported to disrupt fungal cell wall integrity, alter membrane permeability, inhibit essential metabolic pathways, and ultimately suppress fungal growth. Collectively, these findings support the potential of A. esculentus fruit as a natural source of antifungal bioactive compounds and provide a basis for further studies aimed at isolating the active constituents and elucidating their mechanisms of action.[16,17]

Table 3: Antifungal activity

Fungal strain	Control (DMSO) (mm)	Miconazole (1 mg/mL) (mm)	Okra extract (1 mg/mL) (mm)
Aspergillus niger (ATCC 16404)	0	15	11
Fusarium oxysporum (ATCC 48112)	0	16	9

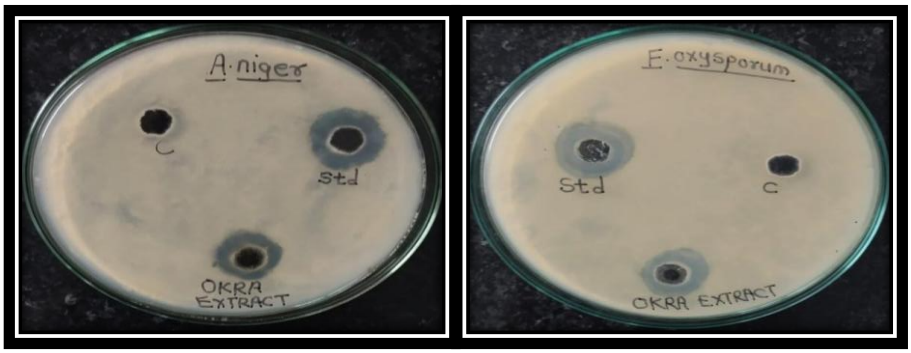

Figure 5: Antifungal activity

CONCLUSION

The present study successfully developed and applied a reliable reverse-phase HPLC method for the qualitative identification and quantitative determination of quercetin in the aqueous fruit extract of Abelmoschus esculentus. Preliminary phytochemical screening confirmed the presence of flavonoids, and the optimized RP-HPLC method demonstrated satisfactory specificity, linearity, and reproducibility, enabling accurate estimation of quercetin. The substantial quercetin content identified in the extract supports its use as a suitable phytochemical marker for the standardization and quality assessment of A. esculentus.

In addition to its phytochemical characterization, the extract exhibited promising antimicrobial activity against both Gram-positive and Gram-negative bacterial strains, along with measurable antifungal activity against the tested fungal species. These biological effects are likely associated with the synergistic action of quercetin and other naturally occurring phytoconstituents present in the extract.

Overall, the findings provide scientific evidence supporting the pharmaceutical potential of A. esculentus fruit as a valuable source of bioactive compounds with antimicrobial properties. The developed analytical method may serve as a useful tool for routine quality control of herbal preparations containing A. esculentus. Further investigations involving isolation of individual bioactive constituents, mechanistic studies, and in vivo pharmacological evaluation are warranted to validate its therapeutic efficacy and facilitate the development of standardized herbal formulations.

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