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Bioactive Potential Of Myrica Esculenta Bark Extracts: A Multi-Assay Investigation

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Abstract
Myrica esculenta Buch.-Ham. Ex D. Don, a Himalayan medicinal shrub, is also known for its diverse phytopharmacological properties. The study aimed to determine the antibacterial, antioxidant, anticancer activities, and phytochemical composition of sequential bark portions (acetone, ethanol, and water) of M. esculenta. Qualitative and quantitative analyses revealed phenolics and flavonoids present in all extracts, with the water extract showing the highest concentrations. Antioxidant assays (DPPH, ABTS, FRAP) indicated the radical scavenging and reducing power activity, particularly in the aqueous extract. Antibacterial screening revealed solvent-dependent efficacy, with acetone fractions being most effective against Pseudomonas aeruginosa and aqueous fractions against Staphylococcus aureus. Cytotoxicity assays using HeLa and MCF-7 cell lines demonstrated strong anticancer activity, particularly in ethanolic fractions MEEF2 and MEEF3. MEEF2 showed the lowest IC_{50} values (249.2 µg/ml for HeLa as well as 244.3 µg/ml for MCF-7), highlighting extract's therapeutic potential. The findings support the conventional use of M. esculenta and suggest its promise as a genesis of natural bioactive compounds for pharmaceutical applications.
Keyword: *Myrica esculenta, phytochemicals, antioxidant activity, antibacterial activity, anticancer activity*

INTRODUCTION

The medicinal shrub grows throughout the Himalayas. Plant species with several pharmacological properties have been in the spotlight. M esculenta Buch. -Ham. Ex D. Don, referred as kaphal, katphala, Himalayan bayberry, or box myrtle, is a plant of the family Myricaceae and genus Myrica. The genus includes several species, including Myrica faya, Myrica arborea, Myrica gale, Myrica inodora, Myrica nagi, etc. Taxonomic and phylogenetic investigation classified Myrica based on molecular markers (Yanthan et al., 2011). Conventionally, different plant parts of Myrica esculenta being used to cure respiratory, digestive, and inflammatory ailments. The phytopharmacological investigations have increased the scope of its utility so much that modern times have not only upheld the traditional claims but also have suggested opportunities for the synthesis of new potent bioactives. Different parts of the plant were reported to exhibit several therapeutic activities. M. esculenta stem bark extract was shown to have anti-allergic activity in Swiss albino mice (Patel et al., 2010a). M. esculenta was found to have radical scavenging (Rawat et al., 2011). Essential oil extracted from bark of the stem of Myrica esculenta has been stated to poseses antimicrobial properties (Agnihotri et al., 2012). Both phytochemical and antifungal activities have been investigated in the fruit and stem bark of the plant (Goyal et al., 2013; Srivastava et al., 2016). One comprehensive research has reported the possibility of wild edible fruits as a nutraceutical, such as M. esculenta, consisting of high phenolic and flavonoid content (Bhatt et al., 2017). Ripened fruit of Myrica esculenta has high polyphenolic and anthocyanin content (Belwal et al., 2019). Fruit of Myrica esculenta has carbohydrates, proteins, fats, high fiber content, and minerals, including manganese, iron, copper, zinc, etc. (Ahmad et al., 2022b). The phytochemical, morphological, and anatomical study revealed distinct traits of Myrica esculenta leaves (Kabra et al., 2019a). Its methanolic extract from the leaves and fruit of Myrica esculenta showed strong abilities to fight against oxidation and harmful microbes (Kabra et al., 2019c; Das et al., 2024). Methanolic extract of Myrica esculenta was found to have neuroprotective and anti-inflammatory consequences in a Parkinson-induced rat model (Kabra et al., 2020). The wild Myrica esculenta fruit from Northeast India was investigated for its biochemical and antioxidant properties to reintroduce it to the daily diet (Rymbai et al., 2023).

Numerous compounds with therapeutic potential have been taken out from the bark and fruit of Myrica esculenta. Fruit of Myrica esculenta was mentioned as a rich source of polyphenols, anthocyanins

(cyanidin-3-O-glucoside, delphinidin-3-O-glucoside), phenolics (gallic acid, vanillic acid, caffeic acid, ferulic acid), flavonoids (kaempferol, rutin, quercetin, myricetin), ellagitannins and dihydrochalcone as isolated and quantified by LC–ESI–MS/MS (Sendri and Bhandari, 2021). The isolated compounds have antioxidative properties. Six bioactive compounds, namely, myricanone, myricanol-5-O-β-D-glucopyranoside, myricetin, myricolol, β-sitosterol, and β-sitosterol-D-glucoside, having antioxidative potential, have been subsequently separated from Myrica esculenta stem bark as aqueous ethanolic extract (Kundal et al., 2024). Gallic acid and myricetin are major phenolic and flavonoid compounds, respectively, being extracted from the Myrica esculenta stem bark by an optimized method of HPTLC (Patel et al., 2010b; Patel et al., 2010c). Catechin is a flavonoid, has been isolated from the fruit of Myrica esculenta using column chromatography and was further characterized by LC-MS, FTIR, and NMR. Catechin was recorded to bear antioxidant properties as reported by an in-vitro and silico docking study (Pathak and Chandra, 2025). Myricetin has been found to have antioxidant activity, protect against oxidative stress, lipid peroxidation, and DNA damage (Semwal et al., 2016), anti-bacterial property against S. aureus, E. coli, and H. pylori, and anti-viral property against herpes simplex virus, and HIV, and influenza virus (Cushnie & Lamb, 2005), anti-inflammatory property by suppressing "TNF-α, IL-6, and COX-2 along with inhibiting NF-κB signalling path way (Takano-Ishikawa et al., 2006; Wang et al., 2010), anticancer activity in breast (MCF-7 and MDA-MB-231 cell lines) (Jiao et al., 2016; Knickle et al., 2018), colon cancer (HCT116) (Zhu et al., 2020), antiatherosclerosis" (Meng et al., 2019). Myresculoside, a monoterpenoid glycoside isolated from methanolic extract of Myrica esculenta leaves, has exhibited ACE inhibition (Nguyen et al., 2010). Myricitrin, a glycosylated flavonoid, has antioxidant, antiatherosclerosis effects in ApoE-/- the mice model (Sun et al., 2013). Myricitrin compound possesses hypoglycaemic effect, improves glucose uptake, and ameliorates diabetic nephropathy in rat models (Dua et al., 2021). The compound was reported to have antioxidative, anti-inflammatory, and antinociceptive characteristics (Zhang et al., 2020). Myricitrin exhibited better hepatoprotection than silymarin (a medication used to cure chronic liver diseases) in the CCl4-intoxicated mice model (Domitrović et al., 2015). Another pentacyclic triterpenoid, 3-Acetylmyricadiol, isolated from Myrica esculenta bark ethyl acetate extract, was recorded to have anti-inflammation in nature activities in LPS-activated Raw 264.7 macrophages (Ahmad et al., 2022a). Myricanol-9-acetate compound is also found to have anticancer activity in MCF-7, MiaPaCa-2, and HCT 116, especially MCF-7 cell line (Ahmad et al., 2021).

Together, research addresses Myrica esculenta as a potentially important phytopharmaceutical resource to be investigated further for bioactive compounds and therapeutic efficacy.

MATERIALS AND METHODS

Material:

The National Centre Cell Sciences in Pune provided Hela and MCF-7 cancer cell lines in breast. The cultured bacterias namely S. aureus, B. subtilis, P. aeruginosa, and E. coli, with accession numbers MCC 2408, MCC 2010, MCC 2265, and MCC 3099, respectively, were acquired from the National Centre for Microbial Resources, Pune.

Collection of plant samples and their extraction

Bark from stem of M. esculenta was gathered from the wild and authentication was done by Dr. Anamika of the Department of Botany, Vardhman College, Bijnor. First bark of the plant was washed, shade-dried at room temperature, and air-dried. Soxhlet extraction of powdered bark was carried out as per Azwanida (2015). Different kinds of compounds were isolated according to their solubility by performing extraction sequentially with 3 solvents, i.e., water, acetone, and ethanol. The proportion of powdered bark material to the solvent recorded was 1:25 w/v. The process of extraction took place at a temperature of 60–80°C, which mitigated some of the impediments normally associated with the process without destroying most of the compounds. The extracts were further condensed and filtered with less pressure in a rotary vacuum evaporator before weighing for yield and stored at 4 °C.

Qualitative estimation of Phytochemicals

Phytochemical screenings were performed following the standard protocol for the qualitative estimation of alkaloids, flavonoids, glycosides, carbohydrates, phenolic compounds, and saponins, among others.

Estimation of Total Phenolic content (TPC) and Total Flavonoid content (TFC)

Compounds like "phenolic and flavonoid content in crude have been estimated following the protocol of Siddhuraju and Becker (2003) and Zhishen et al. (1999), respectively. During estimation of phenolic content, 50µl of crude extract has been incorporated with 950µl of distilled water. 500µl of Folin-Ciocalteu phenol reagent (1:1 with water) and 2500µl of 20% Na_2CO_3 solution were mixed to the diluted extract and mixed well following a 45-minute incubation in the dark. The intensity of the color was noted at 765nm wavelength. A standard curve for gallic acid had been used to estimate the phenolic content in terms of gallic acid equivalents (GAE) /gram dry matter.

For the estimation of flavonoid content, 250µl of crude extract was mixed with 1.25ml of distilled water. 75µl of 5% $NaNO_2$ solution was incorporated to the diluted extract after being incubated at room temperature for five minutes. 150µl of 10% $AlCl_2$ was mixed and filtered. The filtrate was thoroughly combined with 0.275ml of distilled water and 0.5ml" of 1MNaOH. The intensity of pink color was noted at a wavelength 415 nm with a blank reagent. The quercetin linear curve was drawn to estimate flavonoid content in terms of quercetin equivalents per gram dry matter. Both phenolic as well as flavonoid conc. were estimated using the equation "below.

$$\text{Total Phenolic content (TPC) or Total flavonoid content (TFC)} = \frac{C * V}{m}$$

Where C = gallic acid equivalent(mg/ml)/quercetin equivalents(mg/ml)

V = volume of plant extract(ml)

m = weight of pure plant extract in" grams.

Antioxidative assay of extracts

For antioxidative assays, all extracts were diluted to 1mg/ml concentration. DPPH free radical assay, ABTS and FRAP assay were estimated following protocol of "Blois (1958), Re et al. (1999) and Benzie & Strain (1996), respectively. For DPPH assay, 100µl of extracts was incorporated well with 1.5ml of DPPH solution (0.1mM) followed by incubation in a dark room at room temperature for 30 minutes. Absorbances were noted at 517nm against a blank reagent. A standard curve for ascorbic acid was also prepared between different ascorbic acid concentrations" and their % DPPH inhibition to estimate the Vitamin C Equivalent Antioxidant Capacity (CEAC) of all extracts.

For ABTS, 100µL of ABTS reagent (1:1 of 7mM ABTS and 2.45mM potassium persulfate, sixteen hours of dark incubation) was mixed well with 1mL of diluted extract, followed by a 1-minute incubation in the dark. Absorbances were noted at wavelength 734 nm. ABTS reagent was used as a reagent control. CEAC was estimated using an ascorbic acid standard curve. To estimate % inhibition or scavenging of ABTS or DPPH free radicals following equation "was used.

$$\text{DPPH or ABTS inhibition (\%)} = \left(\frac{\text{Acontrol} - \text{Atest}}{\text{Acontrol}}\right) * 100$$

Where Acontrol is the absorbance of the reagent control, and Atest is the absorbance of the extract.

To" estimate FRAP value of crude extracts, 70µl distilled water and 900µl FRAP reagent were combined together well with 30µl of extracts. For ten minutes, the reaction mixture was incubated at room temperature and the absorbances were noted at wavelength 593nm. Ferrous sulfate ($FeSO_4$) has been used as a standard. The mixture of buffer acetate, solution of TPTZ, and ferric chloride was used as control in a ratio of 10:1:1, without adding test extracts. The results of FRAP were expressed in µM $FeSO_4.7H_2O$/g test extract dry weight.

Column purification of extracts

For estimation of antibacterial and anticancer activity crude plant extracts were purified by flash-column chromatography. 30g of pre-activated silica gel with a mesh size 60-120, wet packed into a column (inner diameter 18mm and length 300mm) with chloroform. This gradient elution system uses chloroform and methanol, starting from 100% chloroform to 50% chloroform and methanol solvent, at a flow rate of 1ml/min to effectively separate the extract components based on polarity. Column fractions were dried under ambient conditions and stored at 6^0C.

Antibacterial activity and Minimum inhibitory concentration (MIC) of crude and purified

Anti-bacterial activity of crude extracts and fractions has been tested on Müeller-Hinton agar following CLSI guidelines. The agar was freshly prepared, autoclaved, poured into Petri dishes, and inoculated with 0.1mL of bacterial cultures (S. aureus, B. subtilis, P. aeruginosa, E. coli) at 0.6 OD. On the agar, sterile 6mm discs soaked in the samples were placed, and they were incubated for 24-72 hours at 37°C. Observation was recorded in zones of inhibition, and active samples were subjected to MIC determination using the broth dilution method. In 96-well plates, 0.1mL of bacterial cultures (OD 0.1) were treated with diluted extracts. Controls included wells with culture only and solvent blanks (acetone, ethanol, water). After 24-hour incubation at 37°C, 600nm absorption was measured to estimate MIC, as imipenem used as a standard reference.

Screening of anticancer activity of crude and fractions and their IC50 estimations

The MTT cytotoxicity assay, described by Mosmann (1983), was followed to screen the anticancer "activity of extracts and their fractions at conc. of 0.1mg/mL and 1mg/mL, respectively. Cancer cell lines (HeLa and MCF-7) have been placed. To enable cell adhesion, 96-well plates were filled with approximately 5,000 cells per well and incubated for 24 hours at 37°C in an environment containing 5%CO_2. After incubation, cells were dealt" with 0.1mg/mL of crude extracts and 1mg/mL of fractions for next 24 hours, along with appropriate controls (cell control, DMSO control, and media control). Following treatment, each well received 25µL of MTT solution (5mg/mL), which was then incubated for four hours to assess the cells' vitality. After gently shaking the resulting formazan crystals for 10 to 15 minutes in 100µL of DMSO, the absorbance was measured at 570nm with the help of a microplate reader. The cytotoxicity (%) was computed by employing the following equation:

$$\%\ \text{cytotoxicity} = \left(\frac{\text{Acontrol} - \text{Atest}}{\text{Acontrol}}\right) * 100$$

Where Acontrol is the absorbance of untreated HeLa and MCF-7 cells, and Atest is the absorbance of treated cells.

Samples exhibiting more than 50% cytotoxicity were considered for IC_{50} estimation. For IC_{50} determination, the active fractions were diluted within the range of 50–500µg/mL and tested using same MTT assay method.

RESULT AND DISCUSSION

The extraction yields for Myrica esculenta using acetone (37.4%), ethanol (46.8%), and water (48.8%) (Table 1) highlight solvent-dependent efficiency, with polar solvents (water > ethanol > acetone) producing higher yields. This aligns with literature on solvent polarity influencing phytochemical extraction (Ramesh et al., 2024; Shah et al., 2019). Water yielded the highest extraction (48.8%), likely due to the ability to dissolve polar compounds like polysaccharides and glycosides (Bhatt et al., 2023; Shah et al., 2019). Ethanol (46.8%) performed better than acetone (37.4%), consistent with its intermediate polarity, which balances extraction of both polar and moderately non-polar compounds (Shah et al., 2019).

Table 1: Percentage yield of Myrica esculenta

Sample Abbreviation	Plant	Solvent	Sample weight (g)	Final dry weight(g)	Extraction yield (%)
MEAc	Myrica esculenta	Acetone	5	1.87	37.4
MEEt	Myrica esculenta	Ethanol	5	2.34	46.8
MEW	Myrica esculenta	Water	5	2.44	48.8

Where MEAc: Acetone extract, Et: ethanol extract and W: "water extract

Phytochemical Screening

The Myrica esculenta bark extracts qualitative phytochemical analysis suggested that the presence of several bioactive 2^o metabolites. Alkaloids were found only in the acetone extract (MEAc), as indicated by a positive result with Mayer's test, while absent in ethanol (MEEt) and water (MEW) extracts. Flavonoids were moderately to strongly present in all extracts, with the highest presence being ethanol

and water. Phenolic compounds were detected in all three extracts, suggesting their wide solubility profile. Glycosides showed their presence in all of the plant extracts. Saponins and steroids could not be detected in any of the extracts (Table 2). These findings indicate that M. esculenta contains a rich variety of phytoconstituents, particularly flavonoids and phenols, which are known to contribute to its antioxidant and antimicrobial potential. These outcomes are following prior reports by Ahmad et al. (2022) and Dhiman et al. (2019), who also noted a diverse phytochemical profile for M. esculenta depending on the solvent system used.

Table 2: Results of qualitative phytochemical screening of different plant extracts

Sample	Alkaloid			Flavonoid	Phenol	Glycosides	Tannins	Carbohydrate			Saponins	steroids
	Mayer's test	Dragendorff's test	Wagner test					Molisch	Fehling's	Benedicts		
MEAc	+	-	-	+	+	+	+	-	-	-	-	-
MEEt	-	-	-	++	++	+	+	-	-	-	-	-
MEW	-	-	-	+++	+	+	+	-	-	-	-	-

Where MEAc: Acetone extract, Et: ethanol extract and W: "water extract

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The TPC and TFC of M. esculenta bark extracts differed considerably between solvents. The highest TPC has been observed in the water extract (MEW) at" 29.92mg/g, followed by ethanol extract (MEEt) along with 24.26mg/g, and the lowest in the acetone extract (MEAc) at 9.74mg/g. Similarly, the TFC was highest in the MEW extract (3.69mg/g), followed by the MEEt (2.99mg/g), and lowest in MEAc (0.54mg/g) (Figure 1). These results indicate that polar solvents as water and ethanol are more effective for extraction of phenolic and flavonoid components from M. esculenta bark, compared to less polar solvents like acetone. The strong correlation between extract polarity and phytochemical content supports prior results by Ahmad et al. (2022) and Kumar et al. (2021), who emphasized efficacy of ethanol and aqueous solvents in recovering antioxidant-rich compounds. The far higher level of TPC and TFC in MEW and MEEt also explains their comparatively better performance in antibacterial assays.

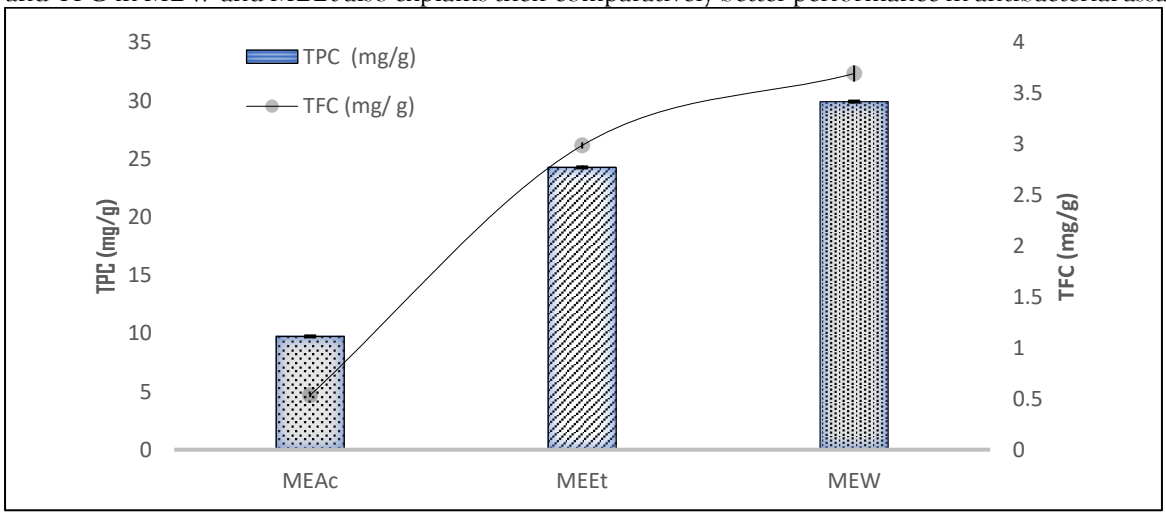

Figure "1: Total phenolic (TPC) and Flavonoid (TFC) content of different plant extracts. Data are represented as mean ± standard deviation.

Antioxidant activity of Myrica esculenta extracts

The" antioxidant potential of Myrica esculenta bark extracts showed marked variation based on the solvent used for extraction. The water extract (MEW) exhibited highest antioxidant activity across all 3

assays. DPPH scavenging activity was highest in MEW (84.28%, CEAC = 0.271 mg/ml), followed by MEEt (69.71%, CEAC = 0.188 mg/ml) and MEAc (39.78%, CEAC = 0.017 mg/ml). ABTS radical inhibition also followed a similar trend with MEW at 90.59% (CEAC = 0.0038 mg/ml), MEEt at 86.38% (CEAC = 0.0035 mg/ml), and MEAc at 58.61% (CEAC = 0.0016 mg/ml). The FRAP value, a measure of reducing power, was significantly higher in MEW (4914.29 µM Fe(II)/g), followed by the MEEt (3657.14 µM Fe(II)/g) and MEAc (514.29 µM Fe(II)/g). These outcomes suggest that the MEW is most potent antioxidant fraction, likely due to the fact that it contains more flavonoids and phenols overall. The observed result supports earlier finding by Ahmad et al. (2022) and Dhiman et al. (2019), who explained strong antioxidant potential in aqueous and ethanolic extracts of M. esculenta due to the enrichment of flavonoids, tannins, and additional polyphenolic compounds.

Table 3: Antioxidant activity of different extracts of Myrica esculenta evaluated using DPPH, ABTS, and FRAP assays.

Sample	**% DPPH scavenging activity**	**CEAC(mg/ml)**	**% ABTS inhibition**	**CEAC (mg/ml)**	**FRAP value(µM Fe(II)/g dry wt.)**
MEAc	39.781±0.491	0.017±0.003	58.614±0.414	0.0016	514.286
MEEt	69.708±0.570	0.188±0.003	86.376±0.132	0.0035	3657.143±98.974
MEW	84.284±0.143	0.271±0.001	90.590±0.127	0.0038	4914.286±98.974

Where MEAc: Acetone extract, Et: ethanol extract and W: water extract of Myrica esculenta. Values "are expressed as mean ± standard deviation. CEAC: Vitamin C equivalent antioxidant capacity. FRAP: Ferric Reducing Antioxidant Power expressed in µM Fe(II)/g dry" weight.

Anti-bacterial activity and MIC (Minimum inhibitory concentration) of extracts

Four bacterial strains were selected to test for antibacterial properties of extracts from Myrica esculenta. The plant extracts were purified using column chromatography. The initial two fractions of each extract exhibited anti-bacterial activity against distinct bacteria. The water fraction-1 showed the greatest activity against the Gram-positive bacteria B. subtilis and S. aureus, with corresponding zones of inhibition of 1.5 and 1.2 cm, respectively. Ethanol fractions (MEEF1 and MEEF2) were moderately inhibitory to Escherichia coli (1.2 and 1.4 cm), whereas the acetone fractions (MEAcF1 and MEAcF2) exerted maximum inhibitory effect against Pseudomonas aeruginosa (1.6 cm), a Gram-negative bacterium typically confronted with antibiotic resistance. This is an agreement with Ahmad et al. (2022b) and Kabra et al. (2019c), whose work described M. esculenta as having an array of antimicrobial actions, mostly due to compounds that include flavonoids like myricetin and phenolic glycosides. The pronounced activity of acetone extracts against P. aeruginosa may be due to the presence of non-polar bioactives such as terpenoids and sterols, as highlighted in the work of Manners & Handique (2021). The solvent-dependent variation in antibacterial activity underscores the importance of extract polarity in targeting specific bacterial groups and supports the traditional use of M. esculenta in treating microbial infections. The MIC values of various solvent fractions of Myrica esculenta bark extract revealed differential antibacterial properties in opposition to Gram-positive and Gram-negative bacteria. Among the examined fractions, MEWF2 displayed significant progress against Staphylococcus aureus (368.6µg/ml) and Pseudomonas aeruginosa (440.9µg/ml), while MEEF1 showed moderate inhibition of S. aureus (374.1µg/ml) and Escherichia coli (514µg/ml). These results indicate a broader spectrum of antibacterial potential in water and ethyl alcohol-soluble fractions. Comparatively, MEAcF2 has the least activity higher MIC values. The observed outcomes are reliable with earlier research by "Dhiman et al. (2019) and Kumar et al. (2021), which reported MICs in a similar range for ethyl acetate and methanol extracts of M. esculenta. This activity is likely characterized by the presence of bioactive flavonoids and phenolic compounds like myricetin and myricanol, as" supported by Ahmad et al. (2022b). These findings suggest that M. esculenta bark fractions, particularly MEWF2 and MEEF1, hold promise as potential antibacterial agents.

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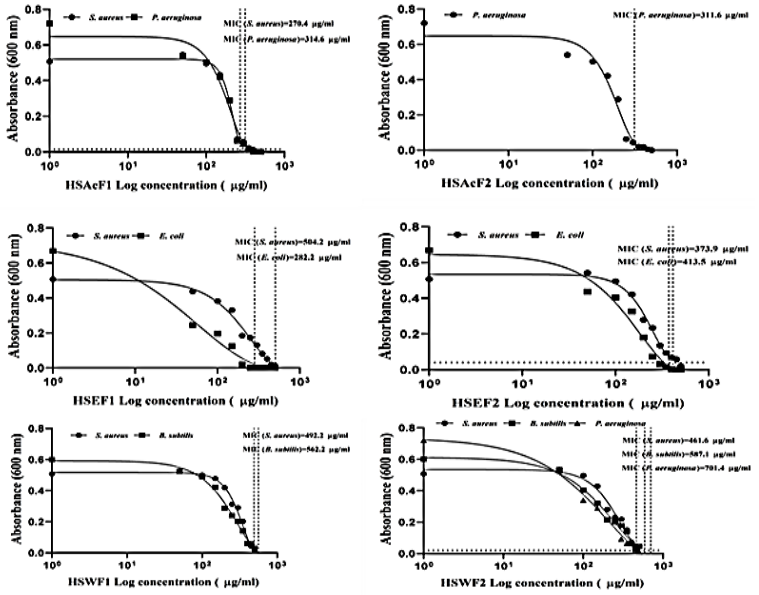


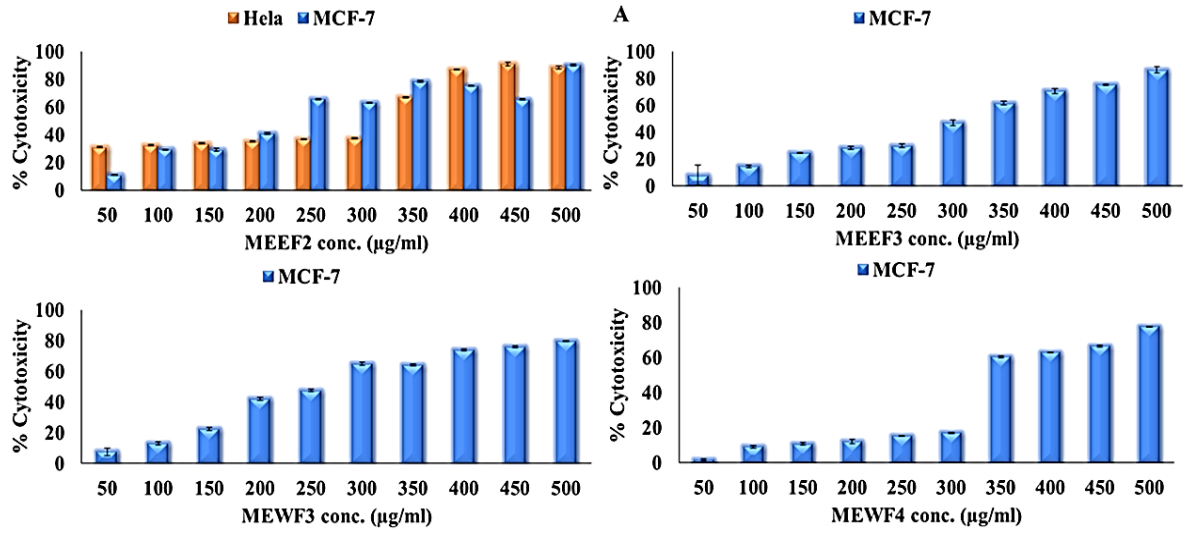
Fractions	MIC (µg/ml)			
	S. aureus	*B. subtilis*	*P. aeruginosa*	*E. coli*
HSWF1	492.2	562.2	-	-
HSWF2	461.6	587.1	701.4	-
HSEF1	504.2	-	-	282.2
HSEF2	373.9	-	-	413.5
HSAcF1	270.4	-	314.6	-
HSAcF2	-	-	311.6	-

Figure 2: Determination of "Minimum Inhibitory Concentrations (MICs) of Extract Fractions Against Bacterial Strains. Dose response curves of MEAcF1, MEAcF2, MEEF1, MEEF2, MEWF1 and MEWF2 against Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, and Escherichia coli. The MIC values table, representing the lowest concentration at which bacterial growth was inhibited, is provided in the table.

Anticancer activity of crude and fractions

All the fractions at their highest concentrations have been screened for their cytotoxic activity against HeLa and MCF-7 cell lines". Among all samples, ethanolic fractions showed markedly higher cytotoxicity, reinforcing the solvent's efficiency in extracting bioactive compounds with anticancer potential. The ethanolic fractions MEEF2 and MEEF3 exhibited significant cytotoxicity, with MEEF2 showing 85.14% inhibition against HeLa and 90.86% against MCF-7, while MEEF3 showed 92.85% inhibition against MCF-7. The aqueous fractions (MEWF3 and MEWF4) also displayed moderate activity against MCF-7 (77.61% and 80.30%, respectively), though no HeLa activity was recorded for them. The fractions with anticancer potential were proceeded with half-maximal inhibitory concentration (IC50) estimation.

A



B

Fractions	IC50 (µg/ml)		Fractions	IC50 (µg/ml)	
	Hela	MCF-7		Hela	MCF-7
MEEF2	249.2	244.301	MEWF3	-	279.7
MEEF3	-	304.9	MEWF4	-	366.6

Figure 3: Cytotoxic Activity of M. esculenta Fractions against HeLa "and MCF-7 cell lines. **A**: Dose-dependent cytotoxicity of fractions (MEEF2, MEEF3, and MEWF3) on HeLa and MCF-7 cell lines. The percentage of cytotoxicity was measured at increasing concentrations (50–500 µg/ml). **B:** IC50 values (µg/ml") calculated for each extract.

MEEF2 shows dose-dependent cytotoxicity on both Hela and MCF-7 cells, with higher concentrations leading to increased cell death. MEEF2 is the most potent among the tested fractions, with the lowest IC50 values of 249.2 and 244.301µg/ml for HeLa and MCF-7 cell lines, respectively. The study on polyphenolic composition and antiproliferative properties of M. esculenta extracts found that different extraction solvents influence the cytotoxic potential, with ethyl acetate and methanol extracts often showing strong antiproliferative influences against cancer cell lines which includes the HeLa cell line (Saini et al., 2013). The cytotoxic effects are believed to be mediated by induction of apoptosis, cell cycle arrest, and oxidative stress in cancer cells, mechanisms commonly associated with polyphenols and flavonoids present in M. esculenta (Bhatt et al., 2023). The existence of substances like myricetin, quercetin, catechins, and triterpenoids (e.g., oleanolic acid, lupeol) likely contributes to the observed cytotoxicity (Kabra et al., 2019b).

CONCLUSION

This study underscores the phytopharmacological potential of Myrica esculenta bark, which exhibited rich phytochemical content, particularly in polar extracts. The aqueous and ethanolic extracts demonstrated superior antioxidant activities, correlating with their high phenolic and flavonoid contents. Antibacterial assays found significant inhibition of both Gram-positive and Gram-negative bacteria, alongside solvent-specific activity, highlighting the importance of the extraction method. Furthermore, ethanolic fractions exhibited potent cytotoxic impacts in opposition human cancer cell lines, with MEEF2 showing promising IC_{50} values against both HeLa and MCF-7 cells. Those results validate the traditional medicinal use of M. esculenta and position it as a potential origin for the development of plant-based antimicrobial and anti-cancer therapeutics. Further in vivo studies and compound isolation are recommended to elucidate mechanisms of action and therapeutic viability.

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