RESEARCH ARTICLE

# Evaluation of Antiviral, Antibacterial and Antioxidant Activity of *Morinda tinctoria* Roxb., *Abroma augusta* L. Educes and *Myristica fragrans* Houtt. Ethereal Oil in Microcapsules

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## ABSTRACT

Viruses and bacteria can interfere with human functioning; scientists are always looking for new ways to harness the healing power of plants. There is potential for a new type of medicine to be developed from plant materials rich in biologically active chemicals. The purpose of this research was to create microcapsules and scrutinize the biological outcomes of a variation of plant educes and ethereal oils. Using chromatographic techniques, we identified the primary components of the educe and ethereal oil, measured the antioxidant activity spectrophotometrically, tracked the development of 09 different infectious agents, and calculated the antiviral result on coronavirus-contaminated avian cells. The extract of *Abroma augusta* L. demonstrated the highest levels of antioxidant and antiviral activity (27.28 – 0.32 and 642.54 – 9.12 g TE/g dw *via* DPPH and ABTS techniques, correspondingly). The FRAP assay found that *Morinda tinctoria* Roxb. extract had the highest antioxidant activity (685.72 – 4.65 mg FS/g dw) and showed antibacterial activity against gram-positive pathogens. Excipients and their quantities were crucial to the emulsion's consistency. The diameter of the enlarged microcapsules containing educes and ethereal oil was greater than twice in intestinal media and less than half that in gastric media, with a starting diameter of 1.86 mm.

**Keywords:** Educes of plants, Ethereal oils, Microcapsules, Extrusion have antioxidant, Antiviral, Antibacterial properties, *Morinda tinctoria* Roxb., *Abroma augusta* L., *Myristica fragrans* Houtt.

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## INTRODUCTION

Medicine, nutrition, flavoring, drinks, dying, pest control, fragrances, pharmaceuticals, and cosmetics are just some of the various applications for plant educes and ethereal oils. Activity as an antioxidant, digestive stimulating action, reduces inflammation characteristics, antibacterial properties, hypolipidemic effects. Effects that counteract or prevent mutations, and potential to prevent or inhibit the growth of cancer cells are only some of the many health benefits attributed to various plants.[1] One of the most noticeable types of subordinate metabolites in plants is phenolic compounds, and their presence may be seen at every stage of metabolism. These chemicals are mostly responsible for the biological activity of plants. Polyphenols contain a broad range of chemicals, including flavonoids, phenolic acid, intricate flavonoids, and colored anthocyanin.[2-4] *Morinda tinctoria* Roxb., often known as mannanunai or Indian mulberry, is the plant in question. It can be found in the untamed and uncultivated regions of southern India.[5] The herbs have been used to treat a wide range of ailments, such as diabetes, liver problems, ulcers in the stomach, viral infections, and diarrhea. The plant in leaves is reported to as cytoprotective,[6] antimicrobial and anti-inflammatory,[7] anticonvulsant,[8] macro-vertebra colonization,[9] antimicrobial,[10] *in-vitro* antioxidant activity[11] and removal of ammonia from polluted waters.[12] The fruits are wound healing,[13] antihyperglycemic and antidiabetic,[14] antiinflammatory.[15] *Abroma augusta* L., often known as Devils' cotton, is a plant use in conventional medicament to treat a wide range of disorders. Plants of the genus *A. augusta* L. can be found virtually anywhere in India.[16-17] Several types of alkaloids can be found throughout the plant itself, from saponins and tannins to flavonoids and amino acids.[18] The antihyperglycemic effects of *A. augusta* can be attributed mostly to abromine, the active ingredient of the

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plant formerly recognized as betaine.[19] *A. augusta* L. extract combined with other plants can yield a combination with potent antibacterial and antioxidant effects. Similar to *A. augusta* L., the phenolic chemicals in this plant are responsible for its biological effects. Monoterpenes, phenylpropanoids, sesquiterpenes, diterpenes, triterpenes, and their oxygenated derivatives.[20] Many studies have shown that the monoterpenes and phenolic chemicals found in *Myristica fragrans* Houtt. ethereal oil has various beneficial effects, including those of an aphrodisiac, reduces swelling, ulcer-fighting compounds, protection against cancer, an antioxidant nature.[21,22] Ethereal oils can be incorporated into a product not just due to their biological benefits but also due to the pleasant aroma they can impart.[23] Microencapsulation can be used to preserve the active medicinal ingredients present in ethereal oils during the manufacturing process. Microencapsulation is a technique used to shield chemical substances from the elements. The core compounds may be shielded by the excipients, which form a shell. Antibiotic resistance has increased during the past few years. Bacteria still have the genetic capacity to spread and develop resistance to medicinal chemicals, which is a major contributing factor. Furthermore, several new multiresistant strains have emerged, which represents a significant risk for both immunocompromised patients and those receiving routine medical care.[24] Plant-based antibacterial medicines hold great potential as a therapy option for a variety of bacterial illnesses. Plants are generally less expensive and more easily accessible than other options, and they have fewer unwanted side effects. Educes from plants that have antiviral properties can be an effective adjunct to standard antiviral treatment. Given that viral particles reside within both the presenter and get incorporated inside the cell that will serve as the host, it is highly challenging to apply antiviral therapy with medications. Antiviral medicines may also kill the host cell.[25] In extrusion, sodium alginate is a common excipient.[26-30] This polysaccharide has excellent chemical stability that may precipitate out of water to form sturdy gel barriers.[31,32] Particles can be formed using the straightforward and low-cost extrusion technique with just items such as a crosslinker solution, syringe pumps, and medical syringes.[33,34] Depending on the desired qualities, extruded microcapsules could be employed either in a liquid or a dry state. The typical particle size range for this technique is between 0.25 and 2.5 mm.[35,36] The quantity of excipients, solution concentration of the crosslinker, and shell material each possess a role in determining the last magnitude.[37]

*M. tinctoria* Roxb., *A. augusta* L., and *M. fragrans* Houtt were selected for this study. An emulsion and extruded microcapsules were created using their educes and ethereal oil, and their character and quality were analyzed.

## MATERIALS AND METHODS

### Plant Material and Reagents

*A. augusta* L. leaves and *M. fragrans* seeds were collected at Kundari Rakabganj, Lucknow, India, while Ranan Nagar, Madurai, Tamil Nadu, produced *M. tinctoria* Roxb. fruits. Dr. Navin K. Ambasht, HOD and Prof. of Botany at C. C. College in Kanpur, India, certified the voucher specimens of both species in the herbarium. This study used distilled water as a control. Reference materials were Sigma Aldrich genistein, daidzein, and glycyrrhizin acid. $AlCl_3$, $(CH_2)6N_4$, $(CH_3)2SO$, $CH_3COOH$, and dehydrated SDA; $K_2S_2O_8$; ethanol (96%); ABTS and 2,2-biphenyl to create a solid gel from sodium alginate used to make microcapsules; calcium chloride salt was used as a cross

### Educe and Ethereal Oil Preparation

*Preparation of M. tinctoria roxb. educe*

The extract was made from dried and powdered *M. tinctoria* Roxb. seeds. The powder was dissolved in 10 mL sterile water for extraction. Four hours were spent macerating the plant materials in water. Next, ultrasound-assisted extraction was performed in a 38 kHz bath. Refining temp. 40 ± 2°C, and the extracting duration was 30 minutes. Specimens were extracted and spun up for 10 minutes at 3382 gm (5500 rpm), and the waste product was decanted. Purified the extract before use in the research.

*Preparation of A. augusta L. educe*

*A. augusta* L. was finely powdered before to usage. Trapezoid-shaped holes in the 0.5 mm sieve were used for the grinding, which was done at 4025 g (6000 rpm). We used a 38 kHz ultrasonic bath to aid in the extraction process. The flower heads were dried and ground down to 0.3 ± 0.001 g before being soaked in 10 mL of halfway ethanol. Refining temp. 40 ± 2°C, and the ultrasonic extracting lasted 10 minutes. After ultrasonic treatment, refluxing the samples in a round-bottom flask at a temperature of 100°C for one hour was done. Next, we cooled the mixture to 25 ± 2°C. The sample supernatant was decanted after 10 minutes of centrifugation at 3382 g (5500 rpm). A paper filter purified the extract before use in the research.

### Ethereal Oil Preparation

*M. fragrans* oil trees were made using a process of modified hydrodistillation. In a mixture of powdered *M. fragrans* distilled water was added seed and magnesium aluminometasilicate (5:1:300). Two hours were spent performing hydrodistillation using Clevenger equipment and a heated mantle. They extracted a colorless, ethereal oil and stored it in a dark bottle. The oil was kept in the fridge at 4°C.

*Quantification of the overall phenolic content*

An extract concentration of 0.5 mL were combined with a phenol reagent dilution of 1:9 (2.5 mL) and a sodium carbonate concentration of 7% (2.0 mL). After 1-hour, the spectrophotometer reading showed a 765 nm absorbance. Gallic acid (0.1–0.1 mg/gm; y =11.108; R2 = 0.9981) was used to generate the calibration curve.

*Quantification of the overall flavonoid content*

Add 0.1 mL of retrieve to 96% v/v solvent ethyl alcohol, 0.015 mL (33% ethanoic acid), 0.15% (10% $AlCl_3$), and 2.0% (5%) methenamine solutions. A 475 nm spectrophotometer

measurement was collected after 30 minutes for analysis. The following formula estimated total flavonoid content (mg RE/gm dw): TFC = C Ve F/M, where C is the standard concentration (mg/L), Ve is the solvent volume (L), F is the sample dilution coefficient, and M is the sample mass (gm). A rutin calibration curve (0–0.5 mg/gm) was created (y = 5.0867, R2 = 0.9985). Rutin units per gram of dry weight were used.

*ABTS radical scavenging activity assay*

The ABTS radicals scavenging activity assay is a popular method for measuring the antioxidant capacity of substances. ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) forms a stable radical cation, ABTS +, which is blue-green. Antioxidants can quench this radical cation, leading to a decrease in absorbance, which can be measured spectrophotometrically. ABTS Stock Solution: dissolve 7 mM ABTS in distilled water and prepare a 2.45 mM potassium persulfate solution. Mix the ABTS solution with potassium persulfate solution in a 1:1 ratio. Allow the mixture to stand in the dark at room temperature for 12 to 16 hours before use. This will generate ABTS radicals' cations (ABTS + ). Dilute the ABTS + solution with ethanol (or PBS, depending on solubility) to an absorbance of 0.70 ( ± 0.02) at 734 nm. Two mL of the test sample and two mL of the ABTS working standard were mixed in the cuvette to assess free radical scavenging. Samples were maintained at room temperature and dark for 30 minutes. Trolox produced a calibration curve with y = 0.0001728x and R2 = 0.9832 mg/g. Data was provided as TE/g dw, or Trolox equivalents per gram dry weight.

*DPPH radical scavenging activity assay*

DPPH in methanol to obtain a final concentration of 0.1 mM. as added to two mL of each sample. After mixing and incubating the reaction mixture at room temperature for 30 minutes, absorbance was 517 nm against a blank. The calibration curve was created by utilizing Trolox (0–0.016 mg/g; y =0.00623x; R2 = 0.9923). Data was provided as TE/g dw, or Trolox equivalents per gramme dry weight.

*Iron diminishing antioxidant potency*

We set up the Iron Diminishing test with 0.3M $CH_3COON_a$ buffer, 10 mM 2,4,6-tripyridil-S- triazine in 40 mM HCL, & 20 mM $F_eCl_3$. Add 10 µL sample and 200 µL iron diminishing reagent and mix well. A spectrophotometer found maximum absorption at 593 nm. Calibration curves were made utilizing $FeSO_4$ (1-mg/g) (y = 2.6272, R2 = 0.9985). Ferrous sulfate per gram of dry weight was observed.

*Antibacterial activity of educe*

Each extract was put on a clean petri plate (1–0.0075 mL for *M. tinctoria* Roxb., 1–0.1 mL for clover). Mueller-Hinton agar medium was added in 5 mL. Reference microbe suspensions were introduced after the agar set. After 20 to 24 hours at 35°C, the samples were kept at room temperature for 24 hours. Antibiotic efficacy was assessed. The sample did not stop bacterial growth if cultures thrived.

*Antibacterial activity of ethereal oil*

In order to cultivate bacteria, sterile petri dishes were filled with 0.5 mL of bacterial solution. About 5 mL of Mueller-Hinton agar were then added to each plate. Once the agar had been set, an 8 mm disk and 30 µm of *M. fragrans* Houtt were added to the top. Oil of clove was put in it. The samples were held at room temperature for 24 hours after being stored for 20 to 24 hours at 35°C.

*Antiviral activity*

A Vero cell line was generously donated by the Department of Microbiology at SVSU, India. The cells were grown at 37°C in a 5% $CO_2$ incubator with DME media with 10% fetal bovine serum. To avoid microbiological contamination, 50 g/mL of gentamycin and 100 units/mL of nystatin were utilized. Infectious bronchitis virus (IBV) strain Beaudette, modified for usage in Vero cells, was employed. The viral stocks were made and then kept in aliquots at -80°C. In 96-well plates with Vero cells, we determined the TCID50 of untreated and treated IBV. IBV was diluted serially by ten-fold increments. Experiments were performed twice, each time using octuplicates of each sample. We looked at CPE after 72 hours. Using the Kärber method to calculate titers and standard deviations, the ability to inhibit viruses was assessed.

*Emulsion preparation*

The process began with the preparation of 4% sodium alginate by adding sodium salt of alginic acid to distilled water. Throughout the experiment, it was used as the shell to produce emulsions. The following ingredients were added to a sodium alginate solution: excipients (maltodextrin, inulin, and/or gum Arabic), which were agitated for 15 minutes using a magnetic stirrer. The resulting emulsion included ethereal oils of *M. tinctoria* Roxb., *A. augusta* L., and *M. fragrans* Hout. For 15 minutes at 5000 rpm, the solution was homogenized using a homogenizer. The centrifuge test confirmed that the emulsion was stable. Three separate 5-minute runs were conducted at 23°C with 3000 rpm. To measure emulsion stability, a centrifugation index (CI) was determined.

$$CI\ (\%) = V_e / V_i \times 100$$

where Ve is the volume of the concentrated emulsion and Vi is the volume of the unconcentrated emulsion before centrifugation.

*Extrusion-based microcapsule preparation*

To make microcapsules, extrusion was used. Microcapsules were made from droplets using a medical syringe and NE-1000 Programmable Single Syringe Pump. Squirting emulsion droplets into the crosslinker with the needle. The needle was held 10 to 15 cm above the fluid while the pump ran at 3 mL/min. Crosslinking was done using 5% calcium chloride. A magnetic stirrer made microcapsules. After 15 minutes in the crosslinker solution, the microcapsules were rinsed with distilled water and filtered using filter paper. After production,

capsules were dried for one hour at room temperature (20 ± 2°C). Dry and moist microcapsules were tested in sealed tubes.[38]

### Characteristics of Microcapsules

*Dimensions of dehydrated and hydrated microcapsules*

A Digital Calipiper micrometer was used to determine the size of the microcapsules. About 30 capsules, both dried and newly produced, were measured for diameter, and an average was derived.

*Firmness of microcapsules*

The texture analyser TA. TX. Plus was used to determine the level of stiffness of newly created microcapsules. Five microcapsule units were collected for analysis, and the amount of force needed to crush two mim was determined using a P/100 probe.

*Characteristics of microcapsule swelling*

Gastric and intestinal media were used to rehydrate the dried microcapsules.[43] At 0, 0.5, 1, 2, 4, and 24 hours, microcapsules were weighed again. Microcapsules that had become bloated were taken out, filtered through metal mesh, and dried using paper towels. The formula used for calculating the swelling index (SI):

$$SI (\%) = W_s – W_i / W_i \times 100$$

where Ws is the weight of the microcapsules when they are swollen and Wi is the weight of the microcapsules after they have dried.[39]

*Statistical data analysis*

Statistical analysis was performed in SSPS 20.0. In order to ensure precision, the trials were conducted three times. The findings are presented as a mean ± SD. The Friedman and Wilcoxon tests were utilized to examine similarities and differences between the three variables. At the degree of importance ($p < 0.05$), the outcomes were confirmed. The antiviral trials were analyzed using Fisher's criteria and the student's t-test to compare various approaches and educes. In this study, results were contemplated compelling at $p$ <0.05 level.

## RESULTS AND DISCUSSION

### Phenols and Antioxidant Activity of Educe and Ethereal Oil

Educes and ethereal oils include several components, including carotenoids, vitamins C and E, and phenolic compounds, making antioxidant activity difficult to assess. Because complex interactions can prevent or enhance effects.[40] Thus, educes were examined for entire phenolic component count, flavonoid concentration, and antioxidant activities using ABTS, DPPH, and iron diminishing**.** This study employed *M. tinctoria* Roxb. and *A. augusta* L. educes.

### Total Phenolic and Flavonoid Content

There were 44.15 ± 0.07 mg GA/g dry weight of phenolics in *M. tinctoria* Roxb. extract. The educe from the leaves of *A. augusta* L. yielded the highest findings, 75.01 ± 0.16 mg GA/g dw (Table 1). When comparing total phenol and total flavonoid content, ethanolic *A. augusta* L. extract were found to have considerably greater entire phenol than aqueous *M. tinctoria* Roxb. extract ($p < 0.05$). Lower molecular weight polyphenols, particularly glycosides, are more easily extracted with ethanol, and the solvent polarity can account for the variations in phenolic chemical solubility caused by different solvents.[41,42]

The ethereal oil of *M. fragrans* Houtt. has little phenol content (8.52 ± 0.04 mg GA/g dw) due to its manufacturing process. Our sample was mostly monoterpenes (68.76%). Ethereal oil contained 6.95 ± 0.07 mg RU/g dw of total flavonoids.

### Antioxidant Activity of Educes and Ethereal Oil

To measure antioxidant activity in educes and ethereal oils from natural sources, a number of different assays have been established in the scientific literature. Educes of *M. tinctoria* Roxb. and *A. augusta* L., as well as *M. fragrans* Houtt. ethereal oil, were tested for their antioxidant properties in this work using the DPPH, ABTS, and FRAP techniques. Antioxidants that can scavenge free radicals are ethereal for this line of defense against free radical damage to cells.[43] Many chronic disorders, including lipid peroxidation, can be slowed by taking antioxidants.[44] *M. tinctoria* Roxb. and *A. augusta* L. had comparable antioxidant activity as measured by the DPPH technique (Table 2). *M. tinctoria* Roxb. extract demonstrated higher ferric reducing capacity (685.72 ± 4.65 mg FS/g dw), while *A. augusta* L., educes showing greater antioxidant activities using the ABTS technique (642.54 ± 9.12 µg TE/g dw). *M. tinctoria* Roxb. and *A. augusta* L. have both been found to be effective antioxidants in scientific studies. While the estrogenic effects of abromine in *A. augusta* L. are well-documented, the extract's antioxidant capabilities may instead stem from other phenolic components like quercetin, hyperoside, or clovamides.[45]

*M. fragrans* Houtt ethereal oil, along with educes of *M. tinctoria* Roxb. and *A. augusta* L., has been credited with antioxidant action. However, the ethereal oil concentration in this research was only 1%. Both -pinene and -pinene are active antioxidants and antimicrobials. However -pinene's antimicrobial action is stronger.[22]

### Antimicrobial and Antiviral Activity

*M. tinctoria* Roxb. and *A. augusta* L. educes were tested for antibacterial activity using dilute educes. Against gram +ve bacteria such *S. aureus, S. epidermidis, E. faecalis*, and *B. cereus*, *M. tinctoria* Roxb. extract showed antibacterial action. Furthermore, it inhibited the development of the yeast *Candida albicans*.

A 0.2% extract concentration suppressed these pathogens, while a 2% *M. tinctoria* Roxb. extract solution inhibited *Bacillus aureus*. Gram -ve bacilli like *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *P. vulgaris* were immune to the antibacterial effects of *M. tinctoria* Roxb. extract. Table 3 displays the findings.

When tested against a reference culture of microbes, 'I' showed an inhibitory impact (antimicrobial effect), but 'N' showed no such effect really low level of growth.

*A. augusta* L. extract inhibits gram-positive bacteria more than they do gram-negative bacteria, however, this extract's antibacterial activity was not expressed. Growth of *Staphylococcus epidermidis* was inhibited by 0.75 mL of extract in 5 mL agar media (14.04% concentration of extract), while the same concentration weakly inhibited growth of *S. aureus* and *B. cereus*. Other pathogen development was not stifled (Table 4). Abromine, the active ingredient in *A. augusta* L. responsible for its antibacterial properties, was present in our investigation but at a concentration too low to provide noticeable results. Because of their volatility, ethereal oils' effects are hard to quantify. In this study, the antibacterial activity of a single concentration of ethereal oil was tested against nine different diseases. It was discovered that *M. fragrans* Houtt. Growth of *S. aureus*, *B. cereus*, *E. coli*, and *K. pneumoniae* were all inhibited by the ethereal oil, while the extension of the other two gram+ve and two gram-ve pathogens was not.

When tested against a reference culture of microbes, 'I' showed an inhibitory impact (antimicrobial effect), but 'N' showed no such effect really low level of growth.

This study found that compared to what other researchers had found, a concentration of nutmeg ethereal oil that was twice as high was able to inhibit the growth of *C. albicans, S. aureus, B. cereus, B. luteus, E. coli, and P. aeruginosa*.[22] *M. fragrans* Houtt ethereal oil had a zone of inhibition 1.22 times larger than that of gentamicin in the other study, which compared the two compounds for their ability to halt the extension of *S. aureus*.[46] Growth of *B. subtilis* were reported to be inhibited by an extract of *A. augusta* L. The non-growth zone measured 20 mm in diameter, while the ethereal oil nongrowth zone were 12 mm. When compared to ethereal oil, the educes was 40% more effective against *B. subtilis*.[47] *A. augusta* L. extract was also evaluated for its antibacterial action, and it was found to be ineffective against *S. aureus*, *E. coli*, *S. typhimurium*, and *B. cereus*.[48] Therefore, it follows that clover extract, rather than ethereal oil, should be utilized to boost products' antibacterial activity. An ethanolic extract of *M. tinctoria* Roxb. fruits were shown to inhibit the development of *B. cereus, B. subtilis, K. pneumoniae*, and *S. aureus* in published studies.[49] However, the educes had no action on the extension of *E. faecalis*. To effectively inhibit *E. faecalis* growth, we observed that a concentration of 0.01 mL of *M. tinctoria* Roxb. educe in a volume of 5 mL of agar medium was sufficient.

**Table 1:** The outcomes of the samples indicate the overall amount of phenol and flavonoid substance

| *S.No.* | *Samples* | *Total phenols (mg GA/g dw)* | *Total flavonoids (mg RU/g dw)* |
|---|---|---|---|
| 1. | *M. tinctoria* extract | 44.15 ± 0.07 | 16.89 ± 0.02 |
| 2. | *A. augusta* L. extract | 75.01 ± 0.16 | 19.50 ± 0.04 |
| 3. | *M. fragrans* Houtt. ethereal oil (1%) | 8.52 ± 0.04 | 6.95 ± 0.07 |

**Table 2:** The antioxidant properties of ethereal oils and plant educes

| *S.No.* | *Samples* | *DPPH (μg TE/g dw)* | *ABTS (μg TE/g dw)* | *FRAP (mg FS/g dw)* |
|---|---|---|---|---|
| 1. | *M. tinctoria* educe | 27.21 ± 0.25 | 524.65 ± 6.33 | 685.72 ± 4.65 |
| 2. | *A. augusta* L. educe | 27.28 ± 0.32 | 642.54 ± 9.12 | 526.86 ± 3.21 |
| 3. | *M. fragrans* Houtt. ethe real oil (1%) | 8.69 ± 0.01 | 92.14 ± 1.26 | 176.04 ± 0.13 |

## Antiviral Activity of Used Plant Educes and Ethereal Oil

The CC50 for vero cells was found to be 4.8 mg/mL for *A. augusta* L. and 10.0 mg/mL for *M. tinctoria* Roxb. In contrast to *A. augusta* L., for which the diluent ethanol at concentrations of 8.0% (*A. augusta* L., 4.8 mg/mL) and 6.67% (*A. augusta L.*, 4.0 mg/mL) had a virucidal effect upon IBV (Table 5), *M. tinctoria* Roxb. diluent had no such effect. Almond EO's carrier oil improved its virucidal efficacy ($p < 0.05$).

**Table 3:** Influence of *M. tinctoria* Roxb. educe on the development of reference microorganism cultures as an antimicrobial agent

| *Reference culture of microorganisms* | *Amount of **M.** tinctoria educe (mL)* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *1.1* | *1.2* | *1.3* | *1.4* | *1.5* | *1.6* | *1.7* | *1.8* | *1.9* | *1.10* |
| | *1* | *0.75* | *0.5* | *0.25* | *0.1* | *0.075* | *0.05* | *0.025* | *0.01* | *0.0075* |
| *Bacillus cereus* | I | I | I | I | I | N | N | N | N | N |
| *Candida albicans* | I | I | I | I | I | I | I | I | ± | N |
| *Enterococcus faecalis* | I | I | I | I | I | I | I | I | I | N |
| *E. coli* | N | N | N | N | N | N | N | N | N | N |
| *K. pneumoniae* | N | N | N | N | N | N | N | N | N | N |
| *P. aeruginosa* | N | N | N | N | N | N | N | N | N | N |
| *P. vulgaris* | N | N | N | N | N | N | N | N | N | N |
| *S. aureus* | I | I | I | I | I | I | I | I | I | N |
| *S. epidermidis* | I | I | I | I | I | I | I | I | I | N |

Dilutions of 1:15 and 1:30 were selected for comparison of the virucidal activity of educes based on cytotoxicity and control data. Moderate to significant virucidal activity was seen with both plant preparations (1:15 and 1:30), and viral titers were reduced by 90%. *A. augusta* L. demonstrated moderate virucidal activity (viral reduction 2.75 log10) at a dilution of 1:15 (2 mg/mL), while at a dilution of 1:30 (1-mg/mL), virus reductions ranged from 1.125 log10 to 1.75 log10. Both concentrations of other plant educe demonstrated virucidal action. Almond concentration had no effect ($p > 0.05$), but the virucidal action of *A. augusta* L. and *M. tinctoria* Roxb. was dose-dependent ($p > 0.05$). *M. tinctoria* Roxb. and *A. augusta* L. educes were evaluated for their antiviral properties (Table 6). It was demonstrated that the educes could shield the Vero cells from infection with IBV. Both post-infection extract treatment and pretreatment of cells with IBV failed to completely prevent CPE. A promising formulation with significant antioxidant, antibacterial, and antiviral activities can be created by combining these educes.

**Table 4:** *M. fragrans* Houtt and *A. augusta* L. educes' antimicrobial effects. The effect of ethereal oils on the development of cultures of reference microorganisms

| *Reference Cultures of Microorganisms* | *Amount of Clover Extract (mL)* | | | | | *Amount of M. fragrans Houtt. Ethereal oil (µg)* |
|---|---|---|---|---|---|---|
| | *2.1* | *2.2* | *2.3* | *2.4* | *2.5* | *4* |
| | *1.0* | *0.75* | *0.5* | *0.25* | *0.1* | *30.0* |
| *B. cereus* | I | ± | N | N | N | N |
| *C. albicans* | ± | N | N | N | N | N |
| *E. faecalis* | N | N | N | N | N | N |
| *E. coli* | N | N | N | N | N | I |
| *K. pneumoniae* | N | N | N | N | N | I |
| *P. aeruginosa* | N | N | N | N | N | N |
| *Proteus vulgaris* | N | N | N | N | N | N |
| *S. aureus* | I | ± | N | N | N | I |
| *S. epidermidis* | I | I | N | N | N | N |

## Emulsion Physical Stability

The stability of six different emulsion samples was measured (the emulsion's ingredients are shown in Table 7). After all the materials were combined, sample E3 had an uneven consistency and a poor centrifugation index.

Single excipient (maltodextrin/inulin/gum Arabic) were shown be ineffective at maintaining emulsion stability (CI 50%) when tested against others. The emulsion's stability was improved by about 46% when two excipients were added to the mixture in comparison to E1. After determining which of three excipients provided the most stability for the emulsion, they were all added. The most stable emulsion was E6, with a CI of 100%. The Mastersizer 3000 was using to assess the particle size, dispersion, and ethereal oil drop diameter of an emulsion (Figure 1). The average drop size of the emulsion containing ethereal oil of *M. fragrans* Houtt., was 0.571 m. D10 = 0.328 ± 0.051 m, D50 = 0.46 ± 0.03 m, and D90 = 0.89 ±

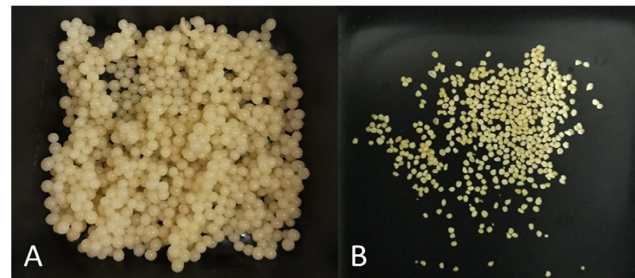


**Figure 1:** Extruded microcapsules (A) immediately after extrusion and (B) after drying the microcapsules.

**Table 5:** Plant educes virucidal effects on IBV were compared

| *Materia, dilution & concentration* | | | *TCID50* | *Virus reduction (TCID 50)* | | |
|---|---|---|---|---|---|---|
| *Name* | *Dilution* | *Concentration* | | *Log10* | *%* | *Reduction factors* |
| Almond oils (control) (µg/mL) | 1:15 | 13.3 | 3.88 ± 0.18 | 1.34 | 94.82 | conducive |
| | 1:30 | 06.6 | 3.75 ± 0.16 | 1.34 | 96.94 | conducive |
| *M. tinctoria* Roxb. (mg/mL) | 1:15 | 13.3 | 3.50 ± 0.18 | 1.72 | 97.25 | conducive |
| | 1:30 | 6.63 | 4.00 ± 0.19 | 1.24 | 95.36 | conducive |
| *A. augusta* L. (mg/mL) | 1:7.5 | 04 | NA | NA | NA | NA |
| | 1:15 | 02 | 2.50 ± 0.26 | 2.72 | 99.83 | modest |
| | 1.30 | 01 | 3.50 ± 0.00 | 1.74 | 97.43 | conducive |
| *50%* Ethanol (control) *(%)* | 1:15 | 03.33 | 5.22 ± 0.16 | - | - | - |
| | 1:30 | 01.67 | 5.24 ± 0.16 | - | - | - |
| *M. fragrans* Houtt ethereal oil (µg/mL) | 1:15 | 13.3 | 4.13 ± 0.18 | 1.10 | 93.56 | conducive |
| | 1:30 | 06.6 | 4.00 ± 0.18 | 1.24 | 95.40 | conducive |
| Water (%) | 1:30 | 06.66 | 5.22 ± 0.16 | - | - | - |
| | 1:30 | 03.33 | 5.24 ± 0.16 | - | - | - |
| IBV (%) | Undiluted | 100 | 5.25 ± 0.16 | - | - | - |

* This impact is dose dependent ($p < 0.05$) ** Fresh M. blossoms the ethereal oil concentration were 1% Houtt., and it was diluted with almond oil. Due to the virucidal action of ethanol, this is not relevant (NA).

**Table 6:** Antiviral activity of plant educes

| *Plant extract* | *Antiviral effect evaluated by CIA 100* | | | |
|---|---|---|---|---|
| | *Virus pre-treatment with extract* | | | *Cell pre-treatment* |
| | *Prior to infection* | *During infection* | *After infection* | *Prior to infection* |
| *M. tinctoria* | + | + | - | - |
| *A. augusta* | + | + | - | - |

+: had an effect; -: no effect.

**Table 7:** Emulsions' composition

| *Ingredient* | *Sample & ingredient quantities* | | | | | |
|---|---|---|---|---|---|---|
| | *E1* | *E2* | *E3* | *E4* | *E5* | *E6* |
| 4% $NaC_6H_7O_6$ solution (gm) | 48 | 48 | 48 | 48 | 48 | 48 |
| *M. fragrans* Houtt ethereal oil (mL) | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 |
| *M. tinctoria* Roxb educe (mL) | 16.7 | 16.7 | 16.7 | 16.7 | 16.7 | 16.7 |
| *A. augusta* L. educe (mL) | 4.3 | 4.3 | 4.3 | 4.3 | 4.3 | 4.3 |
| Maltodextrin (gm) | 12.1 | - | - | 8.7 | 10.4 | 6.5 |
| Insulin (gm) | - | 12.1 | - | 3.5 | - | 4.2 |
| Gum arabic | - | - | 12.1 | - | 1.8 | 1.6 |
| Distilled water | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 |

0.094 m are the measured percentiles based on the evaluation. D10, D50, and D90 represent the percentage of particles that are equivalent to or smaller than those sizes.[50] Particle size in the emulsion varied from 16.70 to 55.56 nm while using various ethereal oils. Soy protein isolates and gum Arabic was used to create the basis.[51] We were unable to locate any studies that replicated this methodology using *M. fragrans* Houtt. ethereal oil, educes, gum Arabic, maltodextrin, or inulin. The extrusion method was utilized to create microcapsules from this stable emulsion (E6).

### Microcapsules' Physical Parameters

All of the microcapsules produced in Figure 1 were perfectly round and a light brown color after being formed in a crosslinker solution of 5%. Microcapsule production yielded 68.67 ± 3.08%. The microcapsules' diameter was 1.86 ± 0.35 mm, and they were soft (easily crushed between fingers) and pliable after being freshly made. The crushing force was in the range of 3758.52 to 4135.87 g (as measured by a Texture analyzer). Since the dried microcapsules were harder than the device's maximum force value of 6500 gm, force measurements were not taken. The dried microcapsules had a diameter of 0.76 ± 0.11 mm.

Although extrusion technology is rarely employed, identical research results have been obtained when used to make microcapsules containing ethereal oils and educes. A 2% crosslinker solution was employed to create microcapsules of 2.120 to 2.280 mm in size with a strength of 4333.46 to 5116.70 gm in a study utilizing *M. fragrans* Houtt. ethereal oil.[52] Microcapsules containing 1% rosemary ethereal oil and no additional excipients had a non-dry and dry diameter of 0.950 and 0.756 mm, respectively.[29] Sodium alginate 4% solution was the only excipient utilized. The microcapsules' swelling index ranged from 19.74 to 13.96% in gastric media, while the alginate microcapsules showed no swelling. After 30 minutes, the microcapsules' swelling index had returned to baseline. In contrast, microcapsules expanded in the intestinal medium from 25.22 to 121.59%. The microcapsules had lost their integrity and were mushy after a day. Weight loss was observed in another investigation using alginate microcapsules (soy protein microcapsules infused with thyme ethereal oil) in a gastric medium.[30] After 40 to 60 minutes, the microcapsule count in the stomach medium peaked and then gradually declined.[53] The intestinal medium causes alginate microcapsules to swell, as shown by another investigation; the swelling index may be greater than twenty times.[37] However, in gastric medium (pH = 1–2.5), microcapsules either hardly expanded or shrank.[54] This study's findings that sodium alginate microcapsules expand in the intestinal medium are supported by another research. As a polymer, sodium alginate can be used to create hydrogels. Hydrogels are well-known for their responsiveness to external stimuli such pH, ionic strength, temperature, and electric current. The stomach and intestines have different pH levels, making pH sensitivity an ethereal consideration for GI medication release regulation. Sodium alginate microcapsules or gels have not been shown to expand in the intestinal medium, and a number of research has supported this finding. Alginate does not swell in an acidic media because the pH regulates the degree of dissociation of the guluronic and mannuronic acid groups.[55,56] Drugs that are ineffective in the stomach acid can be encapsulated in alginate microcapsules.

## CONCLUSION

Based on the results of this research, the selected plant educes and ethereal oil are quite rich in their respective active components. Phenols and terpenes made up the bulk of the extract from *M. tinctoria* Roxb., coming in at 348.27 ± 15.56 g/g, while the extract from *A. augusta* L. included 173.59 ± 12.45 g/g of a bromine. Sabinene, α-pinene, β-terpinene, and β-myrcene were the four primary chemical components of nutmeg ethereal oil. Antioxidant properties can be found in every plant extract. The *A. augusta* L. extract showed the greatest effectiveness, both overall and against viruses. *M. tinctoria* Roxb. extract showed the strongest antibacterial activity, especially against gram-positive bacteria. Therefore, there is reason to believe that antibacterial drugs produced from plants will be effective. Ethereal oil and educes in the form of extruded microcapsules could be added to other pharmaceutical forms for medicinal purposes. Research findings will be used to inform future evaluations of microcapsules' ability to release their active ingredients.

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