

## Particle Micronization of Medicinal Plants Extract using Electro spraying

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### ABSTRACT

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Phytochemical compounds are non-nutritive plant bioactive components that contribute to disease prevention through their antioxidant and anti-inflammatory activities. In this study, particles of polyvinylpyrrolidone (PVP), chitosan, and starch-containing phytochemical compounds from medicinal plant extracts were prepared through an electro spray process. This method is used to increase the bioavailability of phytochemical compounds as well as to facilitate the storage process and durability. The effect of process parameters in this study was investigated, the type of polymers (medicinal plant extract/PVP, chitosan, and starch) and the electric voltage used. Particle characterization and phytochemical properties were evaluated using FTIR, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity analyses. The results showed that PVP exhibited the best performance in binding phytochemical compounds from medicinal plants. This was exemplified by the *O. aristatus*/PVP at an applied voltage of 16 kV, which resulted in the highest total phenolic and flavonoid contents of 0.5816 mg GAE/g sample and 0.7638 mg CE/g sample, respectively. Antioxidant activity, evaluated using antioxidant efficiency (AE), also showed the highest value for the *O. aristatus*/PVP at 18 kV, reaching 0.8511 min<sup>-1</sup>. FTIR analysis indicated that PVP was able to physically interact with phytochemical compounds, as evidenced by the overlap between the polymer and extract spectra. In contrast, chitosan and starch exhibited relatively weaker interactions, leading to more limited encapsulation capability and consequently lower retention of phytochemical compounds within the particles.

**Keywords:** Electro spraying, Polyvinylpyrrolidone (PVP), Chitosan, Starch, Medicinal plants

### 1. INTRODUCTION

Bioactive compounds are essential or non-essential compounds ingested as food ingredients (usually vitamins, antioxidants, or probiotic bacteria) that have been shown to have several beneficial effects on human health [1], [2]. These compounds are most commonly found in medicinal plants which are processed by extraction. To facilitate the process of storing water-soluble extracts, encapsulation in polymers was carried out using electrodynamic

spraying (electro spraying). Electro spraying is a drying technique based on the electrohydrodynamic processing of polymer melts, solutions, or dispersions, into nano- and micro-sized particles at high voltage (up to 30 kV) without use of high temperatures [3], [4]. Electro spraying was employed to produce inorganic particles, drug particles, polymeric drug-delivery particles, and particles entrapping the active ingredient [5], [6], [7], [8]. Some of the advantages of electro spray for

encapsulation of foodstuffs include, the high encapsulation efficiency and small particle size that can be achieved, and the possibility to adjust the size and morphology of the obtained encapsulation structure by adjusting the processing conditions [9]. In the context of drug manufacturing, bioactive molecules are mixed into polymer solutions prior to electrospray and then can be emulsified [10]. These techniques have been used by researchers to convert emulsions into capsules to control the release and enhance the bioactivity of various compounds [5], [7], [11], [12].

The main purpose of encapsulation of therapeutic molecules in biodegradable polymers is to protect substances from enzymatic degradation, aggregation, and denaturation, which prolongs their half-life in the body [7], [12]. Biodegradable polymers are widely used in controlled drug delivery systems and are used in various nanoparticle constructions, such as polyvinyl pyrrolidone (PVP), chitosan, and starch. These polymers have been utilized in many applications, such as medicine, cosmetics and pharmaceutical applications. They have been widely used as an encapsulating matrix material because of its physical and chemical properties, such as physiologically compatible, non-toxic, chemically inert, temperature-resistant, non-ionic, and colorless [5].

In this study, electro spraying was employed in micronization of bioactive compounds from extract medicinal plants with polymers PVP, chitosan and starch in order to improve their bioavailability. The effect of polymer type with medicinal plant extracts and electric voltage on the formation of microparticles was also evaluated. The content of phenolic compounds, flavonoids and antioxidant efficiency was carried out using a UV-vis spectrophotometer. Finally, the structure of the polymer raw materials and the resulting particles were examined by FTIR.

## 2. MATERIALS AND METHODS

### 2.1 Materials

Extract medicinal plants (*Orthosiphon aristatus*, *Andrographis paniculata* (Burm.F) Ness and *Gynura segetum*) produced from

subcritical water extraction. Polyvinyl pyrrolidone (PVP, (C<sub>6</sub>H<sub>9</sub>NO); MW: 29000), Chitosan and Starch were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA), and used as an encapsulant without further modification. Sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>, 99.8 %), sodium nitrite (NaNO<sub>2</sub>, 98.5%), aluminum chloride hexahydrate (AlCl<sub>3</sub>.6H<sub>2</sub>O, 98.0%), sodium hydroxide (NaOH, 97.0%), and ethanol (C<sub>2</sub>H<sub>5</sub>OH, 99.7%) were purchased from Wako Pure Chemical Industries Inc. (Tokyo, Japan). Catechin (C<sub>15</sub>H<sub>14</sub>O<sub>6</sub>, C217500) was purchased from Toronto Research Chemicals, Inc. (Toronto, Canada). Folin-Ciocalteu's phenol reagent, and gallic acid (C<sub>7</sub>H<sub>6</sub>O<sub>5</sub>, 97.9 %) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

### 2.2 Preparation of precursor solution

A 3 mL extract and 3 mL of ethanol were mixed and a 6% w/w concentration of PVP was used. Chitosan solution was prepared at a concentration of 0.2% w/v using 2% acetic acid and maintained at pH 5 by adding 1 M NaOH dropwise. The solution was stirred until homogeneous using sonication. The extract prepared for the electro spraying process was added to a chitosan solution with a concentration of 50% v/v. The solution was stirred until homogeneous using sonication. The starch polymer solution at a concentration of 2% w/v using 30% ethanol solution was stirred until homogeneous. The extract prepared for electro spraying was added to the polymer starch solution at a concentration of 50% v/v.

### 2.3 Experimental procedure

Schematic of the electro spraying apparatus used for encapsulating phytochemical compounds from medicinal plants using PVP, chitosan and starch are presented in Figure. 1. The electro spraying apparatus consists of a high-voltage power supply (model HGR30-20 N, Japan) connected to aluminum-covered collector plate foil and stainless steel with an internal diameter of 0.5 mm placed inside an enclosed acrylic chamber. Electro spraying of different solution samples is carried out at a rate of 0.1 ml/hr with a syringe pump (KD Scientific IC3100, USA) through a single-use plastic syringe. Pipe (poly ether ether

ketone (PEEK)) with an internal diameter of 0.5 mm is used to transfer the feed solution from a syringe to a stainless steel needle. The distance between the stainless steel needle and the collector plate is 8 cm, and the voltage is regulated at 18, 16 and 14 kV. All experiments were performed in an enclosed space at temperature ( $22 \pm 1$  °C), relative humidity 20-30% and below atmospheric pressure and carried out for 6 hours. The result of electrospraying sticks on the aluminum-covered collector plate, the collected particles are immediately stored in a vacuum desiccator at room temperature until further analysis.

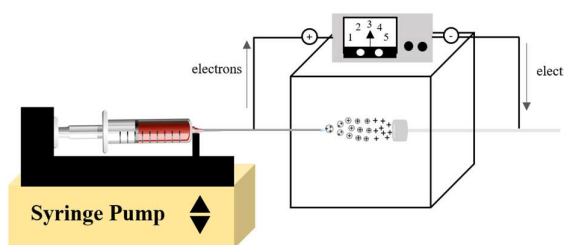


Figure 1. Schematic of the electrospraying apparatus

## 2.4 Methods of analysis

All experiments were conducted in repeated runs to ensure reproducibility. The reported data represent representative results obtained under stable operating conditions. Data analysis was performed using a comparative descriptive approach based on trend evaluation and relative performance among different polymer systems and operating voltages.

### 2.4.1 Particle mass analysis

The mass of electrospraying particles was analyzed using a gravimetric method. The aluminum foil sheets used as particle collection media were weighed before the electrospraying process. After the electrospraying process was completed, the aluminum foil containing the particles was collected and reweighed. The particle mass was determined based on the difference between the mass of the aluminum foil after electrospraying and the mass of the untreated aluminum foil.

### 2.4.2 Determination of total phenolic content

The particles were dissolved using ethanol and distilled water to analyze the total phenol content using Folin Ciocalteu reagent. The solution (1 ml) mixed with 2.5 mL of Folin-Ciocalteu's reagent (1:10 ratio of Folin-Ciocalteu's reagent and deionized water) and shake for 4 minutes. Thereafter, 2.5 mL sodium carbonate solution (7.5 % w/v) was added to the solution until completely mixed. The resulting solution was placed in darkness for 90 minutes prior to measuring its absorbance at 765 nm using a Genesys 10 UV-Vis Scanning spectrophotometry (Thermo Fisher Scientific, Waltham, USA) [13]. Total phenolic content (TPC) was expressed as mg gallic acid equivalent per gram of sample (mg GAE/g).

### 2.4.3 Determination of total flavonoid content

The total flavonoid is expressed as milligrams of catechin equivalents (CE) per gram of dried sample. The solution of particles (1 mL) mixed with a 0.3 mL  $\text{NaNO}_2$  solution (7%, w/v), the resulting solution shook and stored under ambient conditions in darkness for 6 min. Thereafter, a 0.3 ml  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  solution (10 % w/v) was added and homogenized. The solution was stored under ambient conditions in darkness for 5 min. Then 2 mL NaOH solution (1 M) was added to it. The resulting solution was measured absorbance at 507 nm using a Genesys 10 UV-Vis Scanning spectrophotometry (Thermo Fisher Scientific, Waltham, USA) [13], [14], [15]. Total flavonoid content (TFC) was expressed as mg catechin equivalent per gram of sample (mg CE/g).

### 2.4.4 Determination of antioxidant efficiency

Antioxidant efficiency was determined based on the effect arrest on DPPH free radical activity. DPPH (1,1-diphenyl-2-picrylhydrazil) is one of the most widely available organic nitrogen radicals commercially. A 25 ppm of DPPH solution was dissolved in methanol; the solution was stored in a dark place at 4°C in the refrigerator. Measuring absorbance DPPH solution using UV-VIS spectrophotometer at wavelength 516 nm with methanol as a blank. The absorbance measured is the control absorbance. The absorbance of the particle

solution was measured by adding 2 mL to 1 mL of 25 ppm DPPH solution and then shaken until mixed. The particle solution that has been mixed with the DPPH solution is measured every minute until constant absorbance at the same wavelength. Antioxidant efficiency (AE) was calculated using Equation (1).

$$AE = \frac{1}{(EC_{50} \times tEC_{50})} \quad (1)$$

EC50 is the particle concentration that causes 50% reduction initial DPPH absorbance, and tEC50 is the time achieving steady state at EC50 concentration [15].

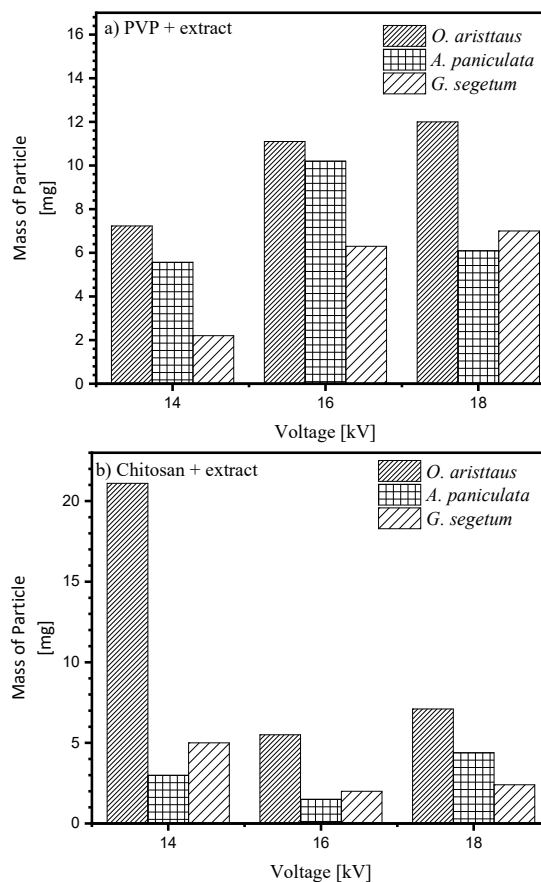
### 3. RESULTS AND DISCUSSION

#### 3.1 Effect of Voltage on particle mass

Currently, biodegradable polymers are widely used in controlled drug delivery and several methods for the formation of particles or nanoparticles, such as the electro spray method [4], [12]. The most commonly used biodegradable synthetic polymers in electro spray are PVP, chitosan and starch. The type of polymer affects the particle size, the physical properties of the particles resulting from the electro spray process, as well as the polymer's ability to interact or bind to phytochemical compounds. In addition, the solvent used to dissolve the polymer also has a significant effect on the particle vibration process and the particulate characteristics of the product. If the solvent evaporation rate is slow, the particle morphology will be more spherical, because there is plenty of time for the solvent to evaporate [4].

The different masses of the electro spraying results were presented in Figure 1, with the same stress and different polymer types occurring because each polymer solution has different conductivity, different polymers occurring product properties, the presence or absence of a Taylor cone under the same operating conditions [16]. However, an increase in the magnitude of the electro spray parameter such as flow rate, viscosity, and percent solid solution tends to be sprayed to

increase the particle diameter [17], [18]. These parameters control the particle size because these parameters are related to the ability and ability to bind bioactive compounds. The conductivity decreases the surface tension of the feed solution and jet causes unstable [19]. Thus, it is in line with the results of the study under the same stress conditions that different polymer solutions, the size and mass of the resulting particles will be different.



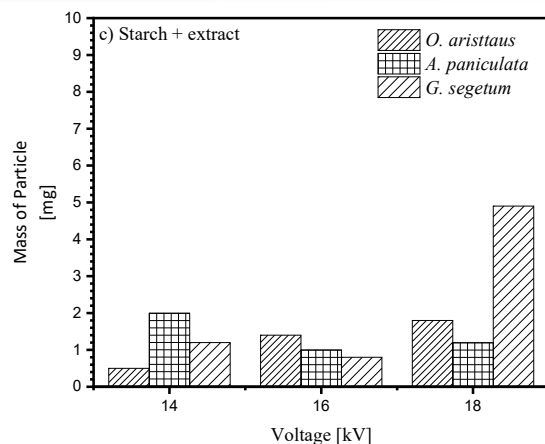


Figure 2. Mass of Particle

High voltage regulates the electric field strength between the precursor at the end of the capillary to the collector plate, so it will produce attractiveness. When a high voltage is applied to the polymer solution in the syringe through the metal needle, the polymer solution will be polarized and the induced charge will spread across the surface polymer solution. Furthermore, this applied voltage can cause conical deformation from polymer solution droplets [20]. The applied voltage will cause the precursor solution to atomize. Figure 2 explain that the higher stress on the same type of polymer, the resulting particles are thicker. This is due to the higher stress because it will attract more charged material from the nozzle so that the particle mass is more [21], [22]. However, too high a voltage also results in an unstable jet and the mass of the resulting particles decreases.

### 3.2 Total content of phenolics, flavonoids and antioxidants in the particles

Phenolic compounds have an important role in human health by controlling various kinds of diseases. In this study, the results of the analysis of the total phenolic and flavonoid content in medicinal plant particles resulting from electrospaying with various types of polymers with a voltage of 14-18 kV are presented in Figure 3-4.

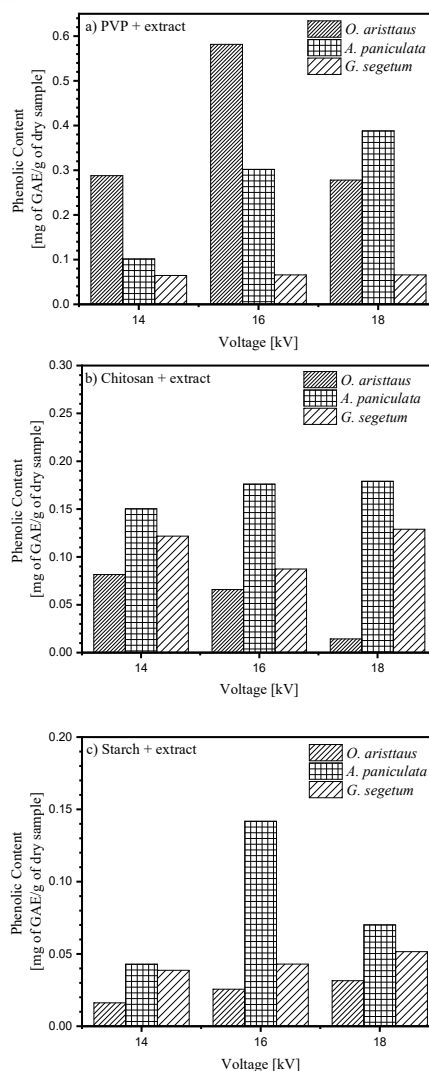
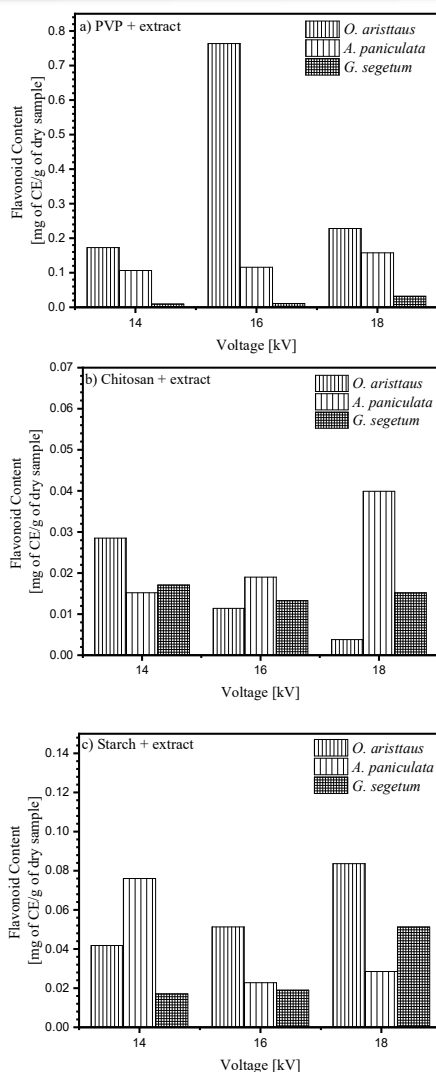


Figure 3. Total phenolic content of the particles using polymer (a) PVP (b) Chitosan and (c) Starch



**Figure 4.** Total flavonoid content of the particles using polymer (a) PVP (b) Chitosan and (c) Starch

Figure 3-4 show the results of the analysis of phenolic and flavonoid compounds from electrosprayed particles along with the extract produced after the process of subcritical water extraction. It was found that the content of phenolic and flavonoid compounds were small in the particles. The volume of the extract used during the electrospray process was small, which was about 0.6 mL compared to the extract which as a whole included the total extraction volume produced, which was  $\pm 450$  ml. In addition, the ability of polymers to bind compounds in the extract is also a major factor

as a determinant of the compounds produced in the particles.

In general, total phenolic and flavonoid content will increase with increasing process stress. This is because the higher stress changes can affect the conductivity and solubility of phenolic and flavonoid compounds in solvents, so that the polymer can bind extracts optimally. This is in line with the results of this study, in Figure 2 and 3 (a), when using 6% PVP polymer for *A. paniculata* and *G. segetum* there was an increase in the content of phenolic compounds and flavonoids when the voltage was increased. However, the highest content of phenolic and flavonoid compounds in *O. aristatus* at voltage of 16 kV was 0.5816 mg GAE/g sample and 0.7638 mg CE/g sample. Meanwhile, at the 18 kV voltage variable, phenolic and flavonoid levels decreased. This is because at a voltage of 18 kV, the shape of the Taylor cone at the end of the syringe begins to change shape into multiple jets which causes instability in the varicose (electrical thread), so that the distribution of particles becomes irregular and does not stick to the aluminum foil plate.

In part (b) of Figure 3 and 4 using chitosan polymer, it can be seen that the highest phenolic and flavonoid content at a voltage of 14 kV for *O. aristatus* were 0.0817 mg GAE/g sample and 0.0285 mg CE/g sample. This is because at a voltage of 14 kV the particles are not formed (wet). Then, for *A. paniculata* and *G. Segetum*, the highest content was at 18 kV. The content of phenolic and flavonoid compounds were 0.1791 mg GAE/g sample and 0.0399 mg CE/g sample for *A. paniculata*, and 0.1289 mg GAE/g sample and 0.1289 mg GAE for *G. segetum*. Figure 3 and 4 section (c), when using starch polymers in *O. aristatus* and *G. Segetum*, the phenolic and flavonoid content increased with increasing stress. However, the upward trend was different in *A. Paniculata*. The highest phenolic content was at a voltage of 16 kV 0.1418 mg GAE/g sample and decreased at a voltage of 18 kV. The increasing phenolic content at a voltage of 16 kV may be due to the increased ability of starch polymers to bind phenolic compounds at that voltage. The ups and downs of the phenol content in the extract can occur because each

solution has a different conductivity and viscosity.

Figure 5 shows the results of the the antioxidant (AE) from electrospayed particles along with the extract produced after the process of subcritical water extraction.

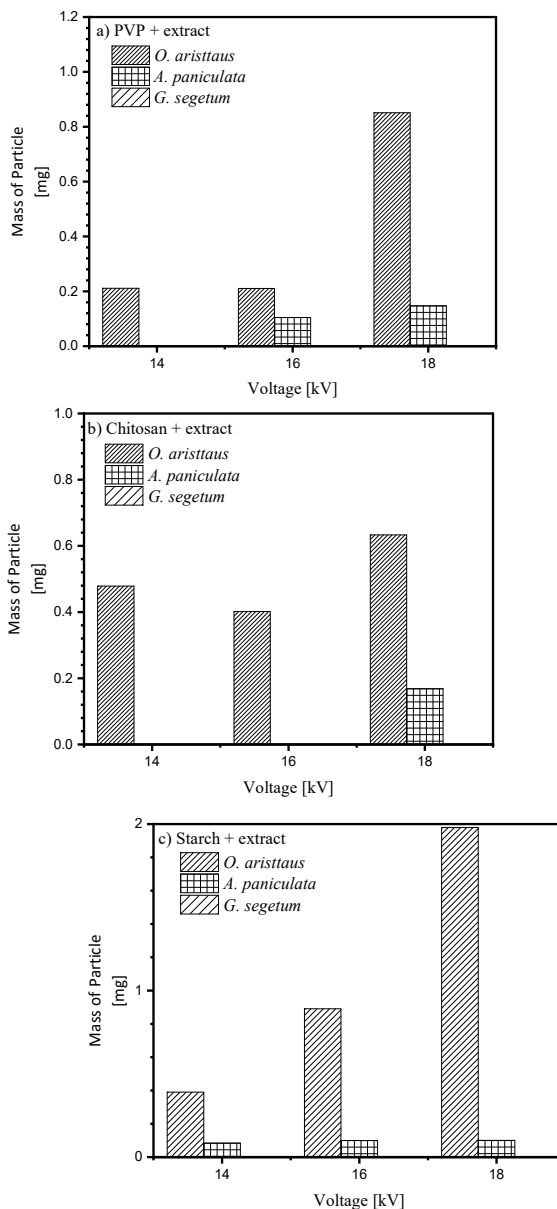


Figure 5. Efficiency of Antioxidant Particles

Based on Figure 5, it can be seen that the efficiency of the anti-oxidant (AE) produced is influenced by the operating voltage. This observed trend can be attributed to the increase in particle mass with increasing

applied voltage. Higher voltages enhance the electrostatic forces acting on the solution, promoting more efficient atomization and material deposition, which consequently increases the mass of electrospayed particles under stable operating conditions [23], [24]. Different results were shown in the extract of *G. segetum* using PVP polymer, chitosan and starch which had no antioxidant efficiency values (there was no change in colour during the analysis process). In this case, DPPH which was initially purple, gradually turned yellow. The colour change of the solution from purple to yellow indicates the efficiency of free radical scavengers. The absence of antioxidant efficiency may be due to the number of particles produced being too small.

### 3.3 FTIR spectra of particles

FT-IR PVP analysis of raw materials, medicinal plant particles/PVP is presented in Figure 6. The difference in FTIR spectrum between PVP (PVP raw material) and electrospun PVP product spectrum (PVP electrospun product with medicinal plant extract) is quite small, indicating that the medicinal plant particles produced by the process have identical functional groups with PVP raw material. This shows that the electro spray process does not divert the PVP function. It should also be noted that the intensity of the FTIR spectrum of medicinal plant/PVP particles is lower than that of the original PVP (raw material). However, the peak intensity between 1647 – 1640  $\text{cm}^{-1}$  in the PVP spectrum – *G. segetum* extract was longer than the PVP spectrum – *A. paniculata* and *O. aristatus*. This might be induced by the high hydrogen interaction between the O-H group of *G. segetum* extract and the C=O absorption band of PVP [5], [7]. All bonds from the two samples, both PVP and medicinal plant extracts/PVP, appeared at the same wave number without any significant difference, which means that the bond between the core material of the medicinal plant extract and the PVP wall material is only a physical bond [25]. Therefore, we suspect that the medicinal plant extracts were successfully encapsulated in PVP by electro spray.

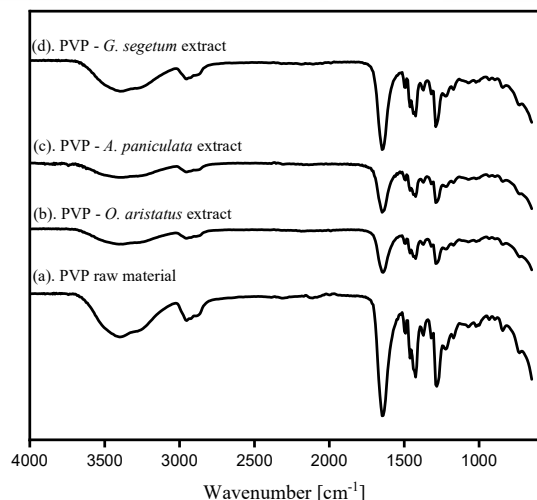


Figure 6. FT-IR spectra of electrospun products using PVP polymer

Figure 6 shows the FT-IR raw material of chitosan with medicinal plant extract/chitosan resulting from electrospay. In chitosan powder, the absorption band at 3000 – 3600  $\text{cm}^{-1}$  is the presence of N-H and O-H stretching vibrations. At 2877  $\text{cm}^{-1}$  and 1312  $\text{cm}^{-1}$  showed symmetrical and asymmetrical vibrations of the  $\text{CH}_2$  strain. The absorption bands of 1647  $\text{cm}^{-1}$  and 1565  $\text{cm}^{-1}$ , respectively, indicated the presence of C=O in the amine group and the  $\text{NH}_2$  group. And at 1029  $\text{cm}^{-1}$  there is a C-O-C range [26]. The results of the FT-IR spectrum of raw material chitosan with the spectrum of medicinal plants/chitosan clearly have differences. The intensity that appears in the extract/chitosan product is found bonded with a weak intensity. This is related to the composition of the constituent materials of the electrospay product. The analyzed particles came from raw material consisting of 50% chitosan solution and 50% V/V% medicinal plant extract, where each medicinal plant extract had a different composition of bioactive compounds even though the raw material used during the electrospay process had the same ratio. This causes the peaks that appear on the particles to be lower than the peaks of the constituent raw materials.

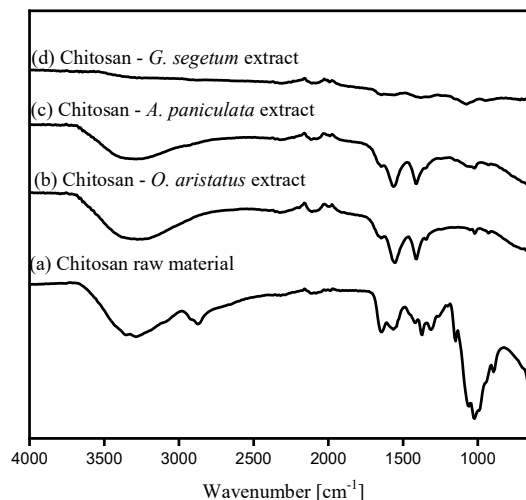


Figure 7. FT-IR spectra of electrospun products using Chitosan polymer

FT-IR analysis for starch raw material in Figure 7, at 3265  $\text{cm}^{-1}$  associated with the hydroxyl group and at 2929  $\text{cm}^{-1}$  including O-H bond stretching vibration. The peak at 1640  $\text{cm}^{-1}$  corresponds to the esterification group of starch, which results from the C=O and C=C bonds; the peak at 998  $\text{cm}^{-1}$  represents the amorphous and crystalline region of the starch [11]. Figure 8 shows the peak spectrum of starch polymer particles with medicinal plant extract. It can be seen that the resulting particles have relatively small peaks or almost invisible spectrum peaks. This is because the solubility of starch in 30% ethanol solvent is very low, which reduces the ability of starch polymers to bind bioactive compounds found in the matrix of medicinal plants [27]. So that the number of unencapsulated phytochemical compounds will increase and reduce the number of particles on the collector plate. Several studies have confirmed that the behavior of electrospay always depends on the composition of the surrounding matrices and their resistance.

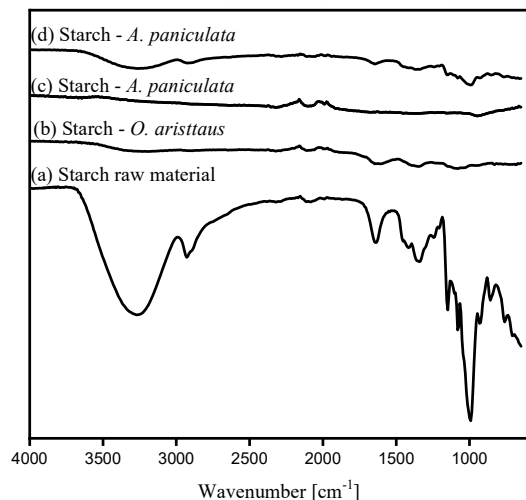


Figure 8. FT-IR spectra of electrospun products using Starch polymer

The FT-IR results also show that PVP is able to bind bioactive compounds. It can be seen that the spectrum of the raw material overlaps with the extract. However, starch and chitosan polymers cannot bind bioactive compounds in plants. It can be seen in the spectrum, which is dominated by the medicinal plant extract spectrum.

#### 4. CONCLUSION

This study confirms that the electrospaying process is feasible for encapsulating phytochemical compounds from medicinal plant extracts, and that polymer type and applied voltage are critical parameters influencing the process outcome. Among the polymers evaluated, polyvinylpyrrolidone (PVP) exhibited the best performance in binding and retaining phytochemical compounds compared to chitosan and starch. The use of PVP, particularly in combination with *O. aristatus* extract, resulted in higher phytochemical content and antioxidant efficiency. In addition, increasing the applied voltage was observed to increase the mass of electrospayed particles under stable operating conditions, indicating its important role in particle formation and material deposition. Overall, these findings demonstrate that PVP-based electrospaying is the most suitable system for phytochemical encapsulation in this study and provides a basis

for improving phytochemical stability and potential bioavailability.

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#### CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

**Nadya Rizkita:** Analyzed and interpreted the data; Wrote the paper.

**Bisma Hendra Setiyawan, Rais Fakhirazin:** Performed the experiments; Analyzed and interpreted the data

**Siti Machmudah, Wahyudiono, Sugeng Winardi:** Planned and designed the experiments; Contributed reagents, materials, experimental apparatus, analysis tools, and data; Interpreted the data.

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