FORMULATION AND EVALUATION OF HERBAL HYDROGEL CONTAINING *Ageratum Conyzoides* LEAVES ETHANOL EXTRACTS

Annisa Kartika Sari[1*], Ummu Afifah Azzahra[1], Tazkia Fauziatus Sa'adah[1], Yuliansa Nurista[1], Elisa Putri Jihan Amir[1], Hikmatul Hasanah[1]

[1]Departemen of Pharmacy, Faculty of Health Sciences, Universitas Muhammadiyah Surabaya, Indonesia

Email: annisakartika@um-surabaya.ac.id

Abstract

Ageratum conyzoides Linn. can be used for wound healing. The main compound found in *Ageratum* leaves is 5'-methoxy nobiletin, a class of polymethoxyflavone that may treat pain and inflammation. The pharmaceutical preparation chosen for the development of *Ageratum* leaf as a wound-related pain relief is a hydrogel, because of its several advantages, namely, it is more convenient, practical, and forms a rinseable film layer that gives a cool feeling to the skin. This study intends to explore the impact of numerous concentrations of ethanol extract of *Ageratum* leaves on the physical evaluation of hydrogels. Hydrogel preparations were made with varying concentrations of *Ageratum* leaf ethanol extract 5%, 6%, and 7.5%. The resulting formula was physically assessed to ensure its quality. The assessment included organoleptic, homogeneity, degree of acidity, spreadability, viscosity, and FT-IR analysis. Statistical data processing of the physical quality of hydrogels was carried out using two-way analysis of variance (ANOVA) to test whether there was a significant difference between formulations. The results showed that all formulas were in accordance with the evaluation requirements including the organoleptic, homogeneity, acidity degree (pH), spreadability, viscosity, and FT-IR analysis. There was no significant change in the spreadability and pH of the formulations over 28 days. Based on the results of organoleptic tests, homogeneity, viscosity, acidity degree, spreadability, and FTIR analysis, it is known that all formulas achieved all physical requirements. This indicates that the hydrogel composed of carbomer 940 and hydroxypropyl methylcellulose can be an ideal base for ethanol extract of *Ageratum* leaves.

Keywords*: Ageratum conyzoides L, Hydrogel, Carbomer 940, Hydroxypropyl methylcellulose 8060-N*

Background

Burns are a type of tissue loss or injury brought on by coming into touch with heat-producing substances like fire, hot water, chemicals, electricity, and radiation. Burn injuries can range from minor soreness and red skin to charring of the skin. Burns are divided into three categories, first-, second-, and third-degree burns, based on the severity of the abnormalities they cause. The heat's temperature and the length of time that the skin is in touch with it both affect how deeply the burn will heal. The effectiveness of many natural compounds as burn treatments is now being researched. *Ageratum* leaf, also referred to as Bandotan leaf, is one of these organic components (Yadav *et al*., 2019). *Ageratum conyzoides* Linn. better known as wild plants (weeds) in the garden and fields. This plant is believed by the Dayak community as a wound healer by chewing or crushed and then placed on the wound (Kuswandi and Hidayat, 2017). The methanol extract of *Ageratum* leaves which were identified as containing alkaloids, tannins, phenolic compounds, and flavonoids turned out to be effective in healing wounds (Kotta *et al*., 2020).

The pharmaceutical preparation chosen for the development of *Ageratum* leaf as a wound-related pain relief is a hydrogel. Mostly because of their favorable healing properties, pharmaceutical hydrogels have shown to be extremely useful for the treatment of wounds. They are created by mixing one or more hydrophilic polymers that have been chosen for use on burns and wounds because of their ability to absorb more water than their weight and form films, which minimize the possibility of discomfort when in touch with tissue (Liang *et al*., 2021). High bioadhesion to the wound surface, moisture, and vapor permeability required for the injured area to heal, infection control, and wound healing are just a few of their benefits when applied to wounds and burns. They also act as anti-bacterial agents by attaching to microbes and removing them from the wound, which promotes wound healing. (Koehler *et al*., 2018). Hydrophilic polymers selected for *Ageratum* leaf hydrogels are Carbomer 940 and hydroxypropyl methylcellulose. Carbomers are used in semisolid pharmaceutical formulations as a rheology modifier. In water, they are utilized as gelling agents at concentrations ranging from 0.5% to 2.0%. It is a thickening polymer that would be transparent, stable, non-toxic, and non-irritating, making it ideal for gel formulations (Huang *et al*., 2019). The maximum viscosity is produced by Carbomer 940, which has a range of 40,000 to 60,000 centipoises in an aqueous dispersion containing 0.5% of this polymer (Shah *et al*., 2020). Pharmaceutical formulations that are administered to the mouth, eyes, nose, and skin typically contain hydroxypropyl methylcellulose. The non-toxic properties, easiness of compression, and capacity to accommodate a high amount of drug loading are advantages of these hydrophilic polymers (Vanti *et al*., 2020). Carbomer can form a gel mass with a low concentration, while hydroxypropyl methylcellulose requires a high enough concentration to form a gel mass. Therefore, the use of a combination of carbomer and hydroxypropyl methylcellulose can overcome the weakness of hydroxypropyl methylcellulose in forming a gel mass with low concentration (Suryani *et al*, 2020)

Following the above description, it is required to establish research to formulate a hydrogel preparation of the extract of *Ageratum* leaf to maximize the use of this plant as a wound healing agent by knowing the optimum concentration of *Ageratum* leaves by optimizing the formula based on the physical properties of the hydrogel, so that hydrogel preparations can be obtained that are hydrophilic and stable.

Materials and Methods

Materials

The material applied for this study includes *Ageratum* leaf (*Ageratum conyzoides* Linn.) obtained from Bangkalan, Madura, East Java, Indonesia.

All the hydrogel materials used pharmaceutical quality, namely carbomer 940 (obtained from Fagron), hydroxypropyl methylcellulose (obtained from PT. Indo Sukses Pratama), propylparabens and methylparabens were obtained from Salicylates and Chemicals Private Limited (SCPL), triethanolamine (TEA) and glycerin were obtained from CV. Aloin Labora, aquadest were obtained from PT. Bratachem, Surabaya, Indonesia.

Tools

The equipment utilized in this study includes a rotary evaporator (Heidolph), scale (Ohaus), scaled glass plate, spreadability test kit, Brookfield Viscometer, pH meter, FTIR-ATR CARY 380 (Agilent), hot plate, glassware, and magnetic stirrer.

Preparation and Determination of Ethanol Extract of Ageratum Leaf

Ageratum conyzoides L. leaf was collected from Bangkalan, Madura, East Java, Indonesia. The authentication of the plant was carried out by the Assessment Services Unit, Universitas Airlangga, Surabaya, Indonesia. *Ageratum* leaves were gathered, thoroughly cleaned with water, and allowed to dry at room temperature before being ground into powder. *Ageratum* leaf powder was macerated with 96% ethanol solvent for 3 x 24-hour periods while being stirred. After that, the filtrate and precipitate were separated using a vacuum and filter paper. The precipitate underwent another maceration, and the end product was mixed with another maceration product solution before being evaporated in a rotary evaporator at 60°C. The condensed extract that had evaporated was put into a jar and covered with aluminum foil for storage.

Preparation of Ageratum leaf Hydrogels

Numerous concentrations of the extract were formulated into hydrogel dosage forms, namely Formulation of *Ageratum* leaf ethanol Extract (FAE) 1, Formulation of *Ageratum* leaf ethanol Extract (FAE) 2, and Formulation of *Ageratum* leaf ethanol Extract (FAE) 3 as presented in **Table 1.**

Table 1. Hydrogel Formulations in this Research

Formulations	**Concentration (in %)**		
	FAE 1	**FAE 2**	**FAE 3**
Ageratum ethanol extract	5	6	7,5
Carbomer 940	0,75	0,75	0,75
hydroxypropyl methylcellulose	0,75	0,75	0,75
Glycerin	5	5	5
TEA	0,5	0,5	0,5
Propyl parabens	0,02	0,02	0,02
Methyl parabens	0,18	0,18	0,18
Aquadest	Ad 100	Ad 100	Ad 100

Annotations:
FAE 1: Hydrogels with *Ageratum* leaf ethanol extract at 5%
FAE 2: Hydrogels with *Ageratum* leaf ethanol extract at 6%
FAE 3: Hydrogels with *Ageratum* leaf ethanol extract at 7,5%

The first step in producing the hydrogel was to create mixture 1 by combining carbomer 940 with triethanolamine (TEA) and extract in 10 ml of water at 70°C. After the addition of the extract, hydroxypropyl methylcellulose develops in 10 ml of water at 70 °C, producing mixture 2. Glycerin was used to dissolve methyl paraben and propyl paraben, to create mixture 3. The three mixtures were combined and mixed as 150 grams of water was added, then swirled until thoroughly mixed.

Assessment of Physical Properties of Ageratum Leaf Hydrogels

The hydrogel's physical properties were assessed. Organoleptic, homogeneity, pH, viscosity, spreadability, and FTIR analysis present the evaluation.

1. Organoleptic
   Direct examinations of the hydrogels are forms, hues, and scents. After preparation and during stability tests, the hydrogels for each formulation were measured in triplicate.
2. Acidity Degree (pH)
   By weighing 10 g of hydrogels, dissolving them in 100 ml of aquadest, and stirring them equally, the pH of the hydrogels was ascertained. The solution's pH was then determined using a pH meter. For each formulation, the pH of the hydrogels was measured in triplicate before and during stability tests.
3. Spreadability
   A sample of 0.5 grams was obtained from each hydrogel and placed on a 20 cm diameter round glass with another glass placed on top for 1 minute. The hydrogels' spread's diameter was measured. Then, it was given an additional 150 grams of load, left to stand for an additional minute, and then its constant diameter was measured. Following preparation and stability tests, the spreadability of the hydrogels was assessed in triplicate for each formulation.
4. Viscosity
   A beaker glass containing no more than 100 ml of hydrogels was placed on a viscometer with spindle number 1 attached. The spindle was then lowered onto the gel until it reached the specified limit. The viscosity number displayed on the tool was used together with a speed setting of 60 rpm. For each formulation, the hydrogels' viscosity was measured in triplicate.
5. Homogeneity
   Hydrogels were applied to a piece of object glass to conduct a homogeneity test. The preparation had to exhibit a uniform construction without any discernible coarse granules. For each formulation, the homogeneity of the hydrogels was assessed in triplicate.
6. FTIR-ATR
   Using FTIR-ATR spectrum analysis, bioactive components in the extract of *Ageratum conyzoides* L. were identified, as well as interactions between the extract and hydrogels. A few drops of extracts and hydrogels were applied to the glass of the instrument for FTIR spectra, through which the laser passes between 400 and 4000 cm-1, and their functional groups were determined using characteristic peaks.

Statistical Analysis

The results of different assays were expressed as the mean (n = 3) and standard deviation (mean ± SD). Two-way analysis of variance (ANOVA) was used to statistically evaluate the data. A difference was deemed significant when $p < 0.05$.

Result and Discussion

Ageratum leaves contain phytochemicals such as quercetin, kaempferol, glycoside, tannin, and other polyphenolic components that can significantly reduce inflammation (Kotta *et al*., 2020). The ethanol extract of *Ageratum conyzoides* is insoluble in oil. *Ageratum conyzoides* is a medical water plant that can be optimally dissolved in distilled water, so it can be applied in the form of hydrogel for wound-related pain relief (Retno Widyawati *et al*., 2021). Pharmaceutical hydrogels have been shown to be extremely useful for the treatment of wounds. They are created by mixing one or more hydrophilic polymers that have been chosen for use on burns and wounds because of their ability to absorb more water than their weight and form films, which minimize the possibility of discomfort when in touch with tissue (Liang *et al*., 2021). This study aims to maximize the use of *Ageratum* leaves as wound healing by knowing the optimum concentration of ethanol extract of *Ageratum* leaves by optimizing the formula so that stable and hydrophilic properties of hydrogel preparations can be obtained.

Based on its biocompatibility, strong adherence to the wound site, good permeability, and capabilities to provide a moist environment for cell migration to effectively stimulate cell migration and facilitate wound healing, Hydrogel is an ideal dressing candidate that overcomes the limitations associated with traditional dressings (Su *et al*., 2021).

The selection of the hydroxypropyl methylcellulose base was due to the organoleptic of a clear gel and compatible with other materials and good hydrogel-forming materials. Meanwhile, Carbomer is freely dissolved in water and, in low concentrations, can provide a suitable gel base with sufficient viscosity. To preserve stability and moisture content in the hydrogel's material properties, glycerin was added as a humectant in the hydrogel formula. The base and humectant have the most impact on the hydrogel's physical properties. In the hydrogel formulation, the base hydrogel will create an essential structure. By absorbing moisture and preventing water from the hydrogel formulation from evaporating, the humectant keeps the hydrogel formulation stable. As a preservative, methylparaben and propylparaben were utilized because hydrogel has a high moisture content that can result in microbial contamination.

Figure 1. Herbal Hydrogel Containing *Ageratum Conyzoides* Leaves Ethanol Extracts

To ensure the quality of the hydrogel formulation, the hydrogel's characteristics should be tested. *Ageratum* leaf hydrogel formulations' organoleptic evaluation was done by visually assessing their appearance, color, and odor. The *Ageratum* leaf hydrogels are homogeneous, semisolid (gel), and green in hue. Owing to the addition of the active ingredient *Ageratum* leaf extract, these physical characteristics became more prominent. The intensity of the color was also enhanced by a higher extract concentration. The hydrogel containing the ethanol extract of *Ageratum* leaf had a contrasting color from the basic hydrogel, because the *Ageratum* leaf ethanol extract was excluded from the basic hydrogel, it appeared transparent. (**Table 2; Table 3**).

Table 2. Results of Organoleptic of *Ageratum* Leaf Hydrogels

Results	FAE 1	FAE 2	FAE 3
Consistency	Soft and thick	Soft and thick	Soft and thick
Color	Green	Green	Green
Odor	Specific	Specific	Specific

Table 3. Results of Organoleptic of *Ageratum* Leaf Hydrogels (Day 0 – Day 28)

Formula	Results	Days				
		Day-0	Day-7	Day-14	Day-21	Day-28
FAE 1	**Consistency**	Soft and thick	Soft and thick	Soft and thick	Soft and thick	Soft and thick
	Color	Green	Green	Green	Green	Green
	Odor	Specific	Specific	Specific	Specific	Specific
FAE 2	**Consistency**	Soft and thick	Soft and thick	Soft and thick	Soft and thick	Soft and thick
	Color	Green	Green	Green	Green	Green
	Odor	Specific	Specific	Specific	Specific	Specific
FAE 3	**Consistency**	Soft and thick	Soft and thick	Soft and thick	Soft and thick	Soft and thick
	Color	Green	Green	Green	Green	Green
	Odor	Specific	Specific	Specific	Specific	Specific

As the normal pH of skin is between 4.5 and 6.5, the pH of topical formulations must be between 5 and 7 (Sinko, 2013). Test results are presented in **Table 4**, **Table 5**, and **Figure 2**. The hydrogel in this research has a pH range from 6.07-6.67, which is suitable for the pH of the skin. When applied to the skin, if the pH of a product is not in range with the pH levels of the skin, it will irritate the skin and make it less comfortable. Statistic analysis for the formula didn't show a different result ($p > 0.05$). There was no significant change in the pH of the formulations over 28 days.

Table 4. Results of Physical Properties Test of *Ageratum* Leaf Hydrogel in Different Formulas

Test/ Formula	Homogeneity	pH (mean±SD)	Spreadability (cm^2) (mean ± SD)	Viscosity (dPa.s) (mean ± SD)
FAE 1	Homogeneous	6.13 ± 0.08	5.03 ± 0.32	205.06 ± 0.05
FAE 2	Homogeneous	6.10 ± 0.05	5.46 ± 0.47	205.33 ± 0.05
FAE 3	Homogeneous	6.07 ± 0.05	5.03 ± 0.15	205.43 ± 0.11

Table 5. Results of pH (Day 0 – Day 28)

Formula	Day 0	Day 7	Day 14	Day 21	Day 28
FAE 1	6.13 ± 0.08	6.11 ± 0.10	6.18 ± 0.04	6.22 ± 0.02	6.16 ± 0.05
FAE 2	6.10 ± 0.05	6.13 ± 0.05	6.14 ± 0.03	6.14 ± 0.10	6.09 ± 0.10
FAE 3	6.07 ± 0.05	5.96 ± 0.07	6.08 ± 0.05	6.09 ± 0.05	6.04 ± 0.02

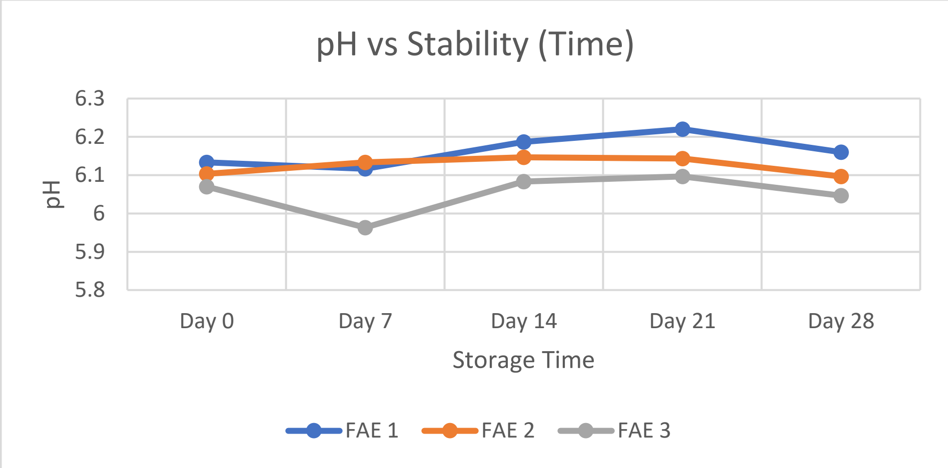


Figure 2. Chart of pH vs Stability (time)

The hydrogel's capacity to spread on the skin was tested for spreading ability. Spreadability test approval criteria are 5-7 cm (Bakhrushina *et al.*, 2022). The application will become easier by improving the topical formulation's spreadability. The result of the statistical test showed no significant difference ($p > 0.05$) for all formulas. This study specifically defines the qualifying spreading ability. While the gel spreading ability is unaffected by variations of *Ageratum* extract concentration. There was no significant change in the spreadability of the formulations over 28 days (**Figure 3; Table 3; Table 6**)

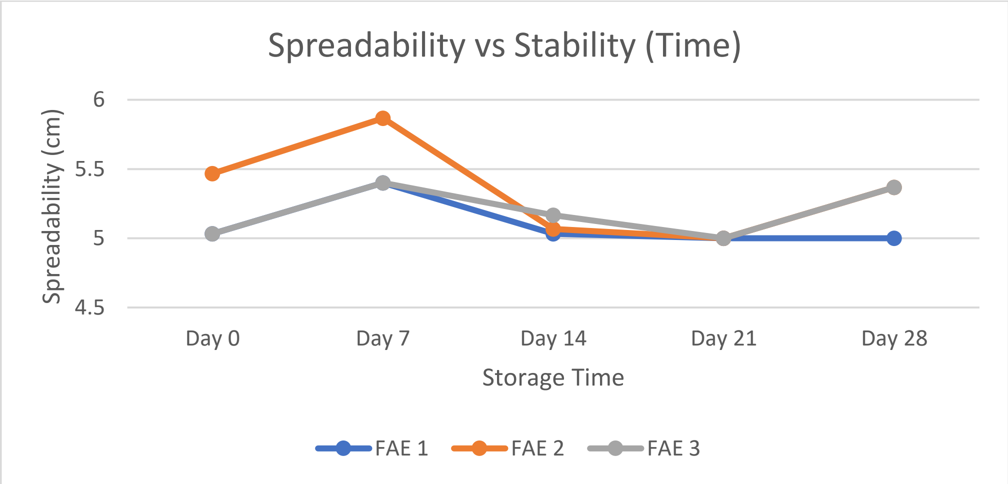


Figure 3. Chart of Spreadability vs Stability (time)

Table 6. Results of Spreadability (Day 0 – Day 28)

Formula	Day 0	Day 7	Day 14	Day 21	Day 28
FAE 1	5.03 ± 0.32	5.40 ± 0.36	5.03 ± 0.23	5.00 ± 0.10	5.00 ± 0.50
FAE 2	5.46 ± 0.47	5.86 ± 0.21	5.06 ± 0.31	5.00 ± 0.17	5.36 ± 0.11
FAE 3	5.03 ± 0.15	5.40 ± 0.26	5.16 ± 0.49	5.00 ± 0.10	5.36 ± 0.37

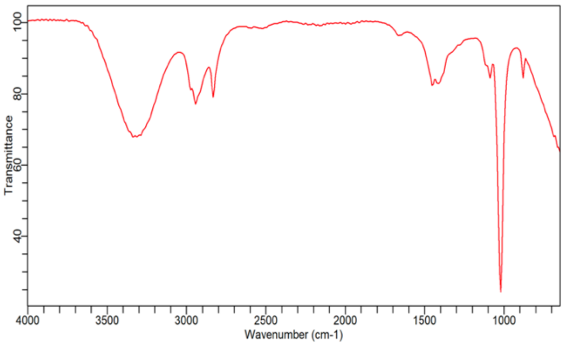
The results of the viscosity test of *Ageratum* leaf hydrogel (*Ageratum conyzoides L*.) showed that the viscosity test results at FAE 1, FAE 2, and FAE 3 showed good viscosity test results (**Table 4**) because they were still in the appropriate viscosity range literature (Chasanah *et al*., 2021). Statistic analysis for the formula didn't show a different result ($p > 0.05$).

Utilizing fourier transform infrared (FT-IR) spectroscopy, bioactive chemicals in the ethanol extract of *Ageratum conyzoides L*. were identified, as well as interactions between the extract and hydrogels. The FT-IR spectrum analysis shows the shifting in the group (**Table 7; Figure 4**). This happens because of the interaction between the extract of *Ageratum* leaf with the hydrogels.

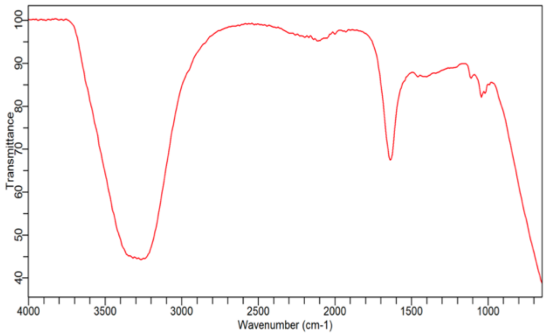
Table 7. The FT-IR spectra of ethanolic extract *Ageratum conyzoides* L and Hydrogels

Peak (wave number cm^{-1})				Function Group
***Ageratum* leaf extract**	**FAE 1**	**FAE 2**	**FAE 3**	
1572.9	1595.3	1617.6	1543.1	C=C stretching cyclic Alkene
1386.5	1379.1	1468.5	1386.5	C-H bending Alkane
1110.7	1110.7	1110.7	1110.7	C-CO-C stretch and bending in ketone
1088.3	1051.1	1043.6	1051.1	C-O stretching vinyl ether
1021.2	1021.2	1021.2	1021.2	C-O stretching primary alcohol

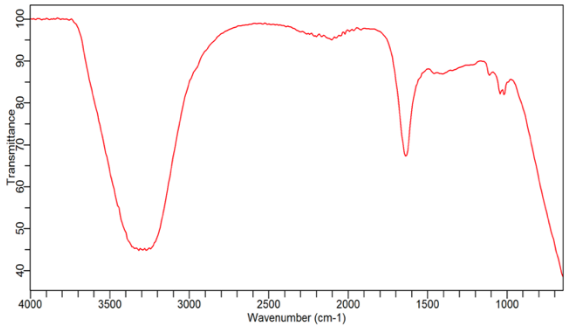
The *Ageratum* leaf ethanol extracts (A) and the hydrogel formula FAE 1 (B), FAE 2 (C), and FAE 3 (D) exhibit the presence of functional groups, as shown by the FTIR spectra in **Figure 4**. It can be inferred from the fact that the spectra of *Ageratum* leaf ethanol extracts and the formula are identical by the presence of functional groups C=C stretching cyclic alkene at wave numbers (1660-1600 cm^{-1}), C-H bending Alkane at wave numbers (1450-1375 cm^{-1}), C-CO-C stretching and bending in ketone (1300-1100 cm^{-1}), C-O stretching vinyl ether (1300-1000 cm^{-1}), and C-O Stretching primary alcohol (1260-1000 cm^{-1}), so it can be concluded that spectra of *Ageratum* leaf ethanol extracts (A), FAE 1 (B), FAE 2 (C), and FAE 3 (D) are identical.



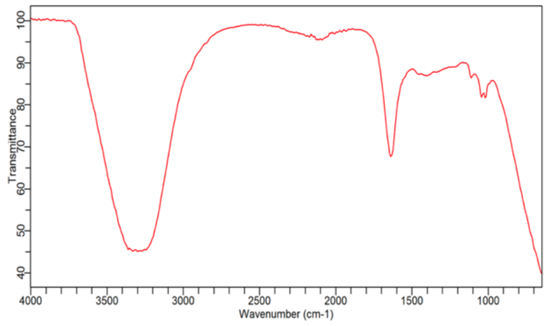
(A)



(B)



(C)



(D)

Figure 4. FT-IR spectrum of the extract of *Ageratum* leaf (A); FAE 1 (B); FAE 2 (C); FAE 3 (D)

Conclusion

A hydrogel made from the ethanol extract of *Ageratum* leaf can be manufactured by combining several hydrophilic polymers (carbopol 940 and hydroxypropyl methylcellulose 8060-N). The physical characteristics of hydrogels did not significantly change if *Ageratum* leaf extract concentration increased. The organoleptic, homogeneity, spreading ability, and acidity degree (pH) during 28 days of storage did not significantly change. To determine the effectiveness of herbal hydrogel containing *Ageratum conyzoides* as wound healing, an in vivo study must be done.

Acknowledgment

This research could be completed thanks to Universitas Muhammadiyah Surabaya for providing equipment and laboratory.

References

Bakhrushina, E. O., Anurova, M. N., Zavalniy, M. S., Demina, N. B., Bardakov, A. I., and Krasnyuk, I. I. (2022). 'Dermatologic Gels Spreadability Measuring Methods Comparative Study'. *International Journal of Applied Pharmaceutics*, **14**(1), p. 164–168. https://doi.org/10.22159/ijap.2022v14i1.41267

Chasanah, U., Mahmintari, N., Hidayah, F., el Maghfiroh, F. A., Rahmasari, D., and Weka Nugraheni, R. (2021). 'Thin-layer hydration method to prepare a green tea extract niosomal gel and its antioxidant performance'. *European Pharmaceutical Journal*, **68**(1), p. 126–136. https://doi.org/10.2478/afpuc-2021-0011

Kotta, J. C., Lestari, A. B. S., Candrasari, D. S., & Hariono, M. (2020). 'Medicinal Effect, in Silico Bioactivity Prediction, and Pharmaceutical Formulation of Ageratum conyzoides L.: A Review'. *Scientifica*, **12**. https://doi.org/10.1155/2020/6420909

Retno Widyawati, D., Sahrial Hamid, I., Sukmanadi, M., Agus Sudjarwo, S., and Hambal, M. (2021). 'The Effectiveness of Bandotan (Ageratum conyzoides L.) Leaf Extract on Mortality of Boophilus microplus In Vitro'. *Journal of Parasite Science*, **5**(1). https://e-journal.unair.ac.id/JoPS

Sinko, P. J. (2013). Coarse dispersions. In *Martin's Physical Pharmacy and Pharmaceutical Sciences: Physical Chemical and Biopharmaceutical Principles in the Pharmaceutical Sciences: Sixth Edition* (p. 410–441).

Su, J., Li, J., Liang, J., Zhang, K., Li, J., Hydrogel, J., and Walles, H. (2021). '*Life Hydrogel Preparation Methods and Biomaterials for Wound Dressing'*. **11**(10). https://doi.org/10.3390/life11101016