International peer reviewed open access journal

Journal of Medical Pharmaceutical and Allied Sciences

Journal homepage: www.jmpas.com CODEN: JMPACO

Research article

Phytochemical profile and bioactive compounds of beluntas leaves extract (*PLUCHEA INDICA L.*) and its hydrogel preparations

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Received - 14-09-2023**, Revised** - 17-11-2023, **Accepted** - 05-12-2023 **(DD-MM-YYYY)**

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Nur Habibah, Heru Santoso Wahito Nugroho, I Gusti Ayu Sri Dhyanaputri, I Gusti Agung Ayu Dharmawati, Ida Bagus Oka Suyasa, 2023. Phytochemical profile and bioactive compounds of beluntas leaves extract (*PLUCHEA INDICA L.*) and its hydrogel preparations. Journal of medical pharmaceutical and allied sciences, V 12 - I 6, Pages - 6184 – 6190. Doi: https://doi.org/10.55522/jmpas.V12I6.5672.

ABSTRACT

"Beluntas" (*Pluchea indica L.)* is a plant that has been used as a traditional medicinal plant for various types of diseases. The most common formulation for *Pluchea indica L*. utilization is in the form of extract. However, *Pluchea indica L*. extract cannot be used directly. It is needed to develop in other formulations, such as a hydrogel. This research aimed to determine the phytochemical profile, the content of bioactive compounds, and the preparation of *Pluchea indica L*. extract incorporated carbopol-HPMC hydrogel. This type of research was a quasi-experimental method. The sample in this study was *Pluchea indica L*. extract. Qualitative tests were carried out for the determination of the phytochemical profile. At the same time, the content of total phenol, flavonoids, total alkaloids, tannins, and antioxidant activity was analyzed using the Folin-Ciocalteu, $AlCl_3$, Folin-Denis, and DPPH methods by visible spectrophotometry. The results showed that *Pluchea indica L*. extract had several phytochemical compounds, namely: alkaloids, tannins, flavonoids, and phenols. The highest level of bioactive compounds was found at a parameter of total phenol with a value of 456.431 mg/g. The sample had strong antioxidant activity, expressed with an IC_{50} value of 0.503 ppm. Preparation of *Pluchea indica L*. extract incorporated carbopol-HPMC hydrogel produced hydrogel that meets the consistency, color, scent, homogeneity, spreadability, pH, and viscosity requirements.

Keywords: "*Beluntas"* leaves extract, Phytochemical profile, Bioactive compounds, Hydrogel preparation

INTRODUCTION

Indonesia is one of the megadiversity countries that has significant biodiversity. The potential of this biodiversity provides enormous opportunities for the development of natural medicinal plants. As many as 1,260 Indonesian plant species are estimated to have medicinal properties [1]. Various plants have been developed as natural medicinal for treating various diseases, including the "beluntas" (Pluchea indica L.). Pluchea indica L. is a bush plant that grows wild and is generally used as a hedge plant. Traditionally, parts of the flowers, leaves, stems, and roots have been used as a deodorant, fever reducer, treating digestive disorders in children, appetite enhancer, diarrhea medicine, and pain medicine [2]. Empirically, Pluchea indica L. contain various bioactive compounds such as flavonoids, alkaloids, phenolic compounds, tannins, and essential oils with substantial pharmacological activity, including antioxidants, analgesics, anti-inflammatories, anti-larvicides, antibacterial, diuretics, antipyretics, anticancer and the ability to cure diabetes mellitus [2–6].

Most of the utilization of bioactive compounds in beluntas plants has been carried out in the form of extract. The extract is usually a thick paste, making it difficult for the reconstitution process

to obtain a homogeneous mixture to be tested. A thick extract can affect the results of the activity test. The extract also cannot be used directly, so it needs to be formulated into other formulations without disturbing the main activity of the ingredients. One way to overcome these limitations is by making beluntas leaves extract into gel or hydrogel formulations. Gels or hydrogels are semi-solid systems of suspended inorganic particles or organic molecules interpenetrated by a liquid. In the pharmaceutical field, hydrogels are widely used as matrices for encapsulating various bioactive compounds in drug preparations [7]. Gel or hydrogel formulations have several advantages compared to extracts because they are relatively more homogeneous and easy to use for topical. They are not sticky and dry quickly to form a thin film. Hence, they are easy to wash, provide a cooling sensation to the skin, and are more stable during storage times [8–11].

Currently, more hydrogels are synthesized using synthetic polymers because they have advantages such as more durability, excellent water absorption, better gel resistance, easy-to-modify gel structure, and more stability to temperature changes [7]. The gelling agent component plays a vital role in providing the physical properties of the resulting gel so that it meets the specified requirements. Several gelling agents can make gel preparations, including Hydroxypropyl Methylcellulose (HPMC), Carboxymethylcellulose (CMC), and carbopol or carbomer [10]. The various types of gelling agents have their respective advantages. One gelling agent widely used for semi-solid formulations is carbopol or carbomer and HPMC. Several research results report that carbopol or carbomer can form gel formulations, is stable in prolonged storage, is non-toxic, and provides the lowest irritation effect compared to other gelling agents such as HPMC and Na-CMC [10, 12–16]. The use of HPMC as a gelling agent can produce precise gel formulas, so HPMC is widely used as an emulsifier, suspending agent, and stabilizing agent in ointments and gel preparations [10].

Combining carbopol and HPMC as gelling agents is widely used as a base in gel formulations. Previous research proves that the combination of carbopol and HPMC at a ratio of 1:9 can produce antiseptic gel formulations that meet the requirements based on homogeneity, pH, spreadability, viscosity, consistency, and stability test [10].

However, using different active ingredients and polymers in developing hydrogel formulas can affect the physical properties and stability of the resulting hydrogels, as well as their functional ability as a carrier matrix for medicinal ingredients. Therefore, this research will study the phytochemical profile at a parameter of phenols, flavonoids, alkaloids, tannins, saponins and determine the bioactive compound of Pluchea indica L. extract and the development of Pluchea indica L. extract as the bioactive ingredient in hydrogel as an alternative candidate for topical drug release matrices.

MATERIALS AND METHODS

Research Design

The type of research used was a quasi-experimental method. In this study, all research objects were tested without being randomly selected. The sample extract was then tested at a parameter of alkaloids, tannins, flavonoids, phenols, and saponins, while the quantitative bioactive compounds are total phenols, tannins, flavonoids, alkaloids, and antioxidant activity. Subsequently, *Pluchea indica L.* extract incorporated carbopol-HPMC hydrogel was developed to obtain alternative candidates for topical drug release matrices. Furthermore, the obtained data was recorded, processed, and presented as narratives and tables. Ethical approval was obtained from the Ethics Commission of Health Polytechnic Denpasar, number: LB.02.03/EA/KEPK/0528/2021.

Procurement of *Pluchea indica L.* leaves

Pluchea indica L. leaves were obtained from Pikat Village, Klungkung, Bali, Indonesia; with authentication number of TL.02.04/D.XI.5/16536.311/2023 from Testing Laboratory, Functional Implementation Unit for "Traditional Health Services" Tawangmangu, Indonesia. From each beluntas stem, beluntas leaves were selected that met the sample requirements, including light-dark green leaves, no holes, no rot and no dryness. Next, the selected leaves were washed thoroughly with running water, then the leaves were air-dried to remove residual water from washing. Next, the leaves were dried using an oven at a temperature of 40 ± 1˚C for approximately 20-40 hours until completely dry. After drying, the leaves were sorted to separate them from the stems and other parts that were involved in drying. Leaves that had passed the dry sorting stage were then crushed using a blender and sieved to obtain simplicia powder of relatively the same size. Next, the simplicia powder was stored in a closed and airtight jar at room temperature.

Preparation *of Pluchea indica L. Extract*

The obtained *Pluchea indica L.* leaves powder was then used in the extraction process. The extraction process was performed by weighing 700 g of dried leaves, which are then dissolved in 96% ethanol at a ratio of 1:5. The re-maceration process was carried out two times to increase the effectiveness of the extraction process. In addition, agitation was performed for 15 minutes each day so that the ethanol could reach all parts of the leaf powder. The obtained extract was filtered with Whatman Paper number 1. The obtained filtrate was concentrated in the rotary evaporator vacuum at 30˚C. The extract yield obtained based on the mass of simplicia powder was 3.142%

Phytochemical Analysis

Alkaloids 1 mL of the sample was added to the N-ammonia-chloroform solution. Furthermore, the mixture was shaken for 1 minute and then filtered. Next, 5 mL of H_2SO_4 was added and

shaken. After settling, separate the aqueous phase and test it by adding Mayer's reagent [17–19]. **Flavonoids** 5 mL of sample was put into a glass beaker, added with 10 mL of ethyl acetate, then boiled and filtered. Furthermore, 0.5 mL of the filtrate was added with 1 mL of dilute ammonia solution, then the changes were observed in the sample [4, 18–20]. **Tannins** A total of 1.6 mL of sample was added to the $FeCl_3$ solution. Subsequently, color changes were observed [4, 17–19]. **Phenol** A total of 2 mL of sample were pipetted, and added a few drops of $FeCl_3$ [4, 17-19]. **Saponins** 10 mL of the sample was added to 5 mL of distilled water and then shaken vigorously until foam formed. Then three drops of olive oil were added, after which it was shaken again and observed for the formation of an emulsion [17, 18].

Quantitative Analysis of Bioactive Compounds

Total phenol 0.1 mL of sample was added to 0.3 ml of 70% ethanol. After that, 0.4 ml of Folin-Ciocalteu reagent was added and then incubated for 6 minutes. After the incubation, 4.2 ml of 5% Na_2CO_3 was added, then vortexed and incubated for 90 minutes. The absorbance was read at a wavelength of 760 nm. The reading results were compared with a standard curve made using gallic acid [18,21]. **Tannin** A total of 0.25 ml of sample was then added to 0.25 ml of Folin-Denis reagent, then vortexed, and added 2 ml of 5% Na_2CO_3. The solution was vortexed and then incubated for 30 minutes. The absorbance was measured using a spectrophotometer at a wavelength of 725 nm. The readings were compared with the standard curve using tannic acid [18,22]. **Flavonoid** A 1 ml of sample was mixed with 4 ml of distilled water and 0.3 ml of $NaNO_2$ (10%) solution was added. After that, it was incubated for 5 minutes and 0.3 ml of $AlCl_3$ solution (10%) and 2 ml of NaOH solution (1%) were added, then immediately tested with a spectrophotometer at a wavelength of 510 nm [18, 20]. **Total alkaloid** Some concentrated sample extract was added 25 mL of 2% HCl and 25 mL of n-hexane and then extracted in a 250 mL separating funnel. The hydrochloric acid extract was added with 35% w/w ammonium hydroxide to pH 9, with 25 mL of chloroform, and extracted in a 250 mL separating funnel [23,24]. Subsequently, 1 mL of test extract and 5 ml pH 4.7 phosphate Buffer were added, and 5 ml BCG (bromocresol green) solution and shaken in a mixture with 4 ml of chloroform. The extracts were collected in a 10-ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against the blank prepared as above but without extract [25].

Antioxidant Activity

A 0.1 g concentrated sample extract was dissolved into 100 mL of methanol. Various concentration (% v/v) of the sample was then prepared, namely: 0.0%, 0.2%, 0.4%, 0.6%; 0.8%; and 1. Next, 2 mL sample was added to 2 mL of 0.1 mM DPPH solution in methanol. The DPPH stock solution volume was 100 mL. The absorbance of the solution was then measured at 517 nm after incubation for 30 min at 27 °C. Antioxidant activity was expressed by the procentation of inhibition, which was calculated based on = [(AC-AS)/AC] x 100%, where AC = control absorbance, and AS = sample absorbance[3, 26].

Preparation of *Pluchea indica L.* Extract Incorporated Carbopol-HPMC Hydrogel

A 4.5 g of HPMC was added to the hot water and continuously stirred until it thickened to obtain A hydrogel (gel made from dissolving 4.5 grams of HPMC). Next, 0.5 g of carbopol was added to the water, then added with 0.25 g of TEA (Triethanolamine) and stirred continuously until B hydrogel (gel made from dissolving 0.5 grams of carbopol + 0.25 grams of TEA) was obtained. The two hydrogels were mixed, and then 0.7 g of phenoxy ethanol and 0.3 g of caprylyl glycol were added. A series concentration (1, 3, 5, and 10% w/w) of *Pluchea indica L.* extract was added to the hydrogel and mixed well via stirring.[14,16] Concentrations 1, 3, 5, and 10 were used for the initial hydrogel formulation. A clearer gel composition is shown in Table 1.

Table 1: Gel composition

Material	Base formula	Formula 1	Formula 2	Formula 3	Formula 4
Beluntas leaf extract (g)	-	1	3	5	10
HPMC (g)	4.5	4.5	4.5	4.5	4.5
Carbopol	0.5	0.5	0.5	0.5	0.5
TEA (g)	0.25	0.25	0.25	0.25	0.25
Glycerin (g)	5	5	5	5	5
Ethanol phenoxy (g)	0.7	0.7	0.7	0.7	0.7
Caprylyl glycol (g)	0.3	0.3	0.3	0.3	0.3
Aquades (g)	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100

Next, the optimal concentration was determined based on the test results of the resulting hydrogel. The prepared *Pluchea indica L.* extract incorporated carbopol-HPMC hydrogel was stored at 4°C for the further use. Drug stability testing is carried out at the next stage, namely after in-vitro effectiveness testing. Gel test parameters include organoleptic, homogeneity test, pH, viscosity, spreadability test.

RESULTSAND DISCUSSION

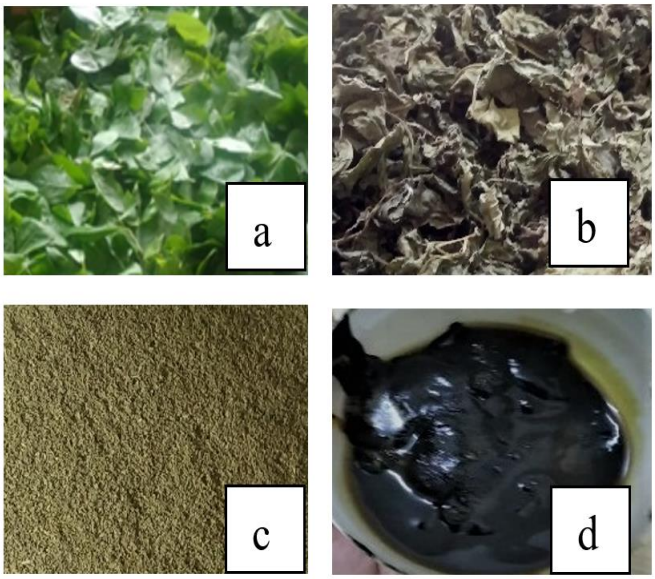
Pluchea indica L. Extract

Pluchea indica L. extract sample was prepared by extracting dried leaves powder with a maceration method using 96% ethanol solvent. A total of 9L macerate was obtained in the extraction process. The macerate was then evaporated to remove the solvent so that 22 g of concentrated extract in the form of a paste was obtained. The yield of the extraction in this study was 3.142% (Figure 1).

Pluche indica L. is a potential plant to be explored as a natural medicinal because it contains various bioactive compounds with considerable pharmacological effects. In order to optimize the activity of various bioactive compounds, an extraction process is carried out to isolate and separate the active compounds. In this study,

the maceration method carried out the extraction process. Maceration is the most common method widely used for extracting natural materials. This extraction method provides a simple procedure. The solvent used in the maceration process was 96% ethanol. This solvent is used because it is selective, neutral, less toxic, can dissolve various secondary metabolites, and quickly evaporates [27–32].

Figure 1: a. *Pluchea indica L.* leaves; b. *Pluchea indica L.* dried leaves; c. *Pluchea indica L.* leaves powder; d. *Pluchea indica L.* extract



During the maceration process, the solvent will diffuse through the cell wall to dissolve the cell's constituents and stimulate the cell's solution to diffuse out so that the secondary metabolites will dissolve in the ethanol and be separated more easily. After the maceration process, the filtrate is filtered and separated from the residue. This evaporation process is essential for further analysis because it could minimize the matrix intervention, especially from the solvent used.

Phytochemical Profile And Bioactive Compounds

The result of the phytochemical qualitative test of the *Pluchea indica L.* extract is presented in Table 2. The qualitative test revealed that the sample contains phenols, flavonoids, alkaloids and tannins. Phenols were identified based on the formation of a greenish blue color in the sample solution. The presence of alkaloids was indicated by the formation of an orange-brown color or white precipitate. The formation of a reddish-brown color indicated the content of flavonoids. Steroids were indicated by the color change of the sample to dark green. The appearance of a blue-black color indicated tannins. The color change and the precipitate formation in the sample indicate the presence of phytochemical compounds.

The quantitative analysis of bioactive compounds at a parameter of total phenol, flavonoids, total alkaloids, tannins, and antioxidant activity was conducted using Folin-Ciocalteu, $AlCl_3$, The Folin-Denis, and DPPH methods by visible spectrophotometry. The quantitative analysis of bioactive compounds is presented in Table 3. Among the five bioactive compounds, the highest level was found at a parameter of total phenol. Phenolic compounds play an important role in antioxidant activity. The results showed that the extract samples had potent antioxidant activity, with an IC_{50} value of 0.503 ppm. This result proves the sample extract is good against DPPH free radical scavenging.

Table 2: Qualitative result of phytochemical compounds of *Pluchea indica L.* extract

Phytochemical compounds	Results
Phenols	+
Flavonoids	+
Alkaloids	+
Tannins	+
Saponins	-

Table 3: Quantitative analysis of bioactive compounds of *Pluchea indica L.* extract

Bioactive compounds	Result
Total Phenol (mg/g)	456.431
Flavonoids (mg/g)	94.414
Alkaloids (mg/g)	69.930
Tannins (mg/g)	67.241
Antioxidant (IC50, ppm)	0.503

The results of qualitative tests revealed that *Pluchea indica L.* extract contains alkaloids, flavonoids, steroids, tannins, and phenols. The results of this qualitative test follow several previously reported. Alkaloids, tannins, and phenols are secondary metabolites commonly found in various types of plants. In the qualitative alkaloid test, the alkaloid compounds in the sample were extracted by adding chloroform under alkaline conditions. Furthermore, the extracted alkaloid compounds were salted with sulfuric acid so that a precipitate formed, indicating the presence of alkaloids in the sample. Quantitatively, the alkaloid content in the sample was 69.930 mg/g. Alkaloids are essential compounds with one or more nitrogen atoms in their cyclic structure. Alkaloids have physiological activity as analgesics [25].

Flavonoids are a large group of phenolic compounds that are found in plants. The flavonoid content in the sample was identified based on the formation of a reddish-brown color in the sample. The change in the color of the sample is since the flavonoids in the form of phenolic compounds can change color when added with a base or ammonia as a result of the conjugation system of the aromatic group. In this study, the levels of flavonoids were analyzed using the $AlCl_3$ method, based on the principle of the reaction of forming a complex compound between $AlCl_3$ and flavonoids, followed by absorbance measurement at a wavelength of 510 nm. The sample flavonoid content was 94.414 mg/g [3, 33, 34].

Phenol is a large group of secondary metabolite compounds that are widely distributed in various types of plants. Phenol compounds have the characteristic of having an aromatic ring containing one or more hydroxyl groups in their structure. Most phenolic compounds bind to other compounds, such as sugar, so they

dissolve quickly in water. Quantitative analysis of total phenol is based on the principle of the oxidation reaction of phenolic hydroxyl groups. The total phenol levels were analyzed using the Folin-Ciocalteu method with gallic acid as a reference standard. The Folin-Ciocalteu reagent will oxidize the phenolic group and reduce the hetero-poly acids (phosphomolybdic-phosphotungstic) into molybdenum-tungsten complexes. The phenolic hydroxyl group will react with the Folin-Ciocalteu reagent during the reaction process to form a blue molybdenum-tungsten complex [21, 33, 35, 36]. Furthermore, the intensity of the blue color formed was measured spectrophotometrically at a wavelength of 760 nm. The intensity of the blue color is proportional to the total phenolic compound in the sample. Among the five bioactive compounds, the highest level was found at a parameter of total phenol with a value of 456.431 mg/g.

The levels of flavonoids and total phenols are closely related to a substance's antioxidant capacity [33, 37]. Antioxidants can neutralize free radicals because they have hydroxy groups and double bonds in their structure [33, 38]. The antioxidant would donate its hydrogen atom to the free radical so that the free radical loses its reactivity. This reaction is indicated by the discoloration of the free radical solution after adding antioxidant substances. The degree of discoloration indicates the scavenging potentials of the substances. The reaction is characterized by a decrease in absorbance and is measured at a wavelength of 517 nm. The antioxidant activity of the substances is expressed by the IC_{50} value, which shows the substance's ability to inhibit 50% of the free radical concentration. The IC_{50} value is inverse proportionally to the antioxidant activity. The smaller the IC_{50} value of the substances, the higher its power to inhibit the free radical concentration. Based on the result, the *Pluchea indica L.* extract has potent antioxidant activity with an IC_{50} value of 0.503 ppm. This result proved that phenolic compounds are critical for antioxidant activity.

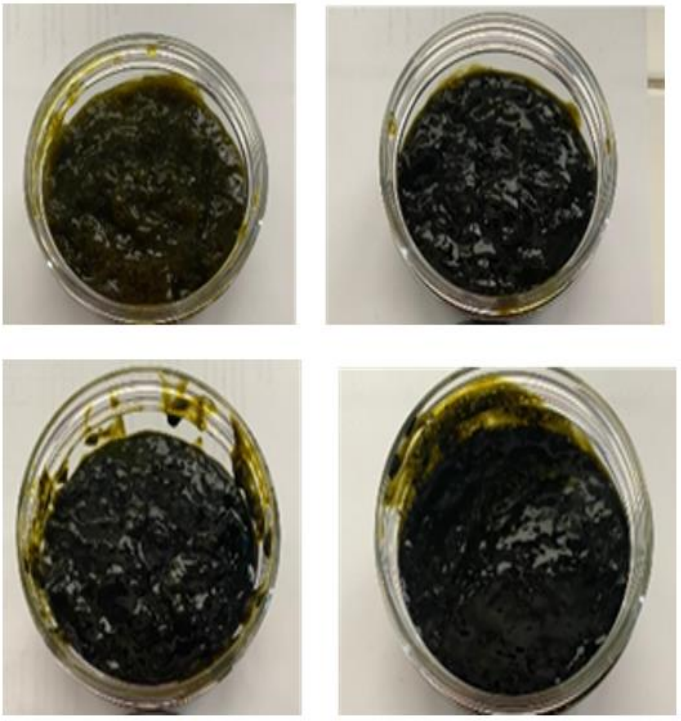
Pluchea indica L. Extract Incorporated Carbopol-HPMC Hydrogel

Pluchea indica L. incorporated carbopol-HPMC hydrogel was synthesized by dissolving and mixing gelling agent, developer, preservative, and extract sample. The appearance of hydrogel containing *Pluchea indica L.* extract was shown in Figure 2.

The prepared hydrogel in this research was shown of good quality based on the organoleptic and physicochemical test at a parameter of consistency, color, scent, homogeneity, spreadability, pH, and viscosity.

Hydrogel synthesis in this study was carried out using a combination of 2 gelling agents: HPMC and carbopol. This gelling agent combination is used to obtain a hydrogel preparation that meets the physical requirements of the gel as an antiseptic gel preparation.

Figure 2: *Pluchea indica L.* extract incorporated carbopol-HPMC hydrogel



This study chose HPMC as a gelling agent because HPMC can produce precise formulations. This gelling agent is combined with carbopol because carbopol was able to form a good gel formula, durable, non-toxic, and less irritation effect compared to other gelling agents such as HPMC and Na-CMC [7, 10, 12, 13]. Other ingredients used in the hydrogel formulation process in this study were TEA, glycerin, phenoxy ethanol, caprylyl glycol, and distilled water. TEA functions as a pH stabilizer, emulsifier, and gel character-forming; glycerin acts as a humectant to maintain the consistency and stability of the gel during storage and retain moisture; caprylyl glycol for and phenoxy ethanol functions as a preservative against microbes contaminants, distilled water is used as a solvent [16, 38]. The addition of extract into the hydrogel preparations affects the appearance and physicochemical properties of the hydrogels. The color intensity of hydrogel was increased proportionally to the amount of the extract. Increasing the concentration of *Pluche indica L.* extract causes a decrease in viscosity and pH but is still within the normal range. However, on the parameters of spreadability, pH, and homogeneity, an increase in the concentration of the extract did not change the physicochemical properties. Hydrogel preparation in this study produced hydrogel that meets the quality standard of consistency, color, scent, homogeneity, spreadability, pH, and viscosity so that the prepared hydrogel has the potential to be further tested and developed into topical drug release matrices.

CONCLUSION

Pluchea indica L. extract has several phytochemical compounds, there were phenols, flavonoids, alkaloids, and tannins. The highest level of bioactive compounds was found at a total phenol

with very strong antioxidant activity. Preparation of *Pluchea indica L.* extract incorporated carbopol-HPMC hydrogel was able to produce hydrogel that meet the requirements for consistency, color, scent, homogeneity, spreadability, pH, and viscosity. So that the prepared hydrogel has the potential to be further tested and developed into topical drug release matrices.

ACKNOWLEDGEMENTS

Thanks and high appreciation to Director of Poltekkes Kemenkes Denpasar who supported this research.

Author Contributions

All authors contributed equally in terms of concept, research implementation, analysis and interpretation of data, as well as in the process of preparing articles. All authors share equal responsibility for all aspects of the work in this study.

Conflict of Interest

There is no conflict of interest related to this research and publication.

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