PHYTOCHEMICAL SCREENING OF JAPANESE PAPAYA LEAF EXTRACTS (*Cnidoscolus Aconitifolius* Mill.) WITH THE MACERATION METHOD USING DIFFERENT TYPES OF SOLVENTS

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Abstract

Introduction: Japanese Papaya Leaf is a wild bush plant that grows up to 6 meters, and this leaf is easy to grow in tropical countries like Indonesia. The flavonoid compounds contained can lower cholesterol levels in the blood. **Method:** In this study, population and samples of Japanese Papaya Leaf (*Cnidoscolus aconitifolius* Mill.) were used that grow in Bekasi Timur Regency, Bekasi. This research is an experimental qualitative descriptive study that aims to determine the results of the macroscopic examination, and phytochemical screening using a test tube on Japanese papaya leaves extracted by maceration using 70% ethanol, n-hexane, and aqua distillate as solvents. **Results:** The results of the research uses an macroscopic examination, which included leaf morphology test showed dark green color with a slightly shiny slippery surface and round and finger shape leaves. The results of the extract yield from various solvents, obtained aqua distillate by 27%, ethanol 70% by 10.2%, and n-hexane by 4.5%. In this case the amount of yield has a different polarity. Phytochemical screening test of Japanese Papaya leaf extract from 70% ethanol and aqua distillate show positive for alkaloids, flavonoids, saponins, triterpenoids/steroids, tannins, while for n-hexane solvent showed positive for flavonoids and steroids. **Conclusion:** The conclusion of this study is that extracts from Japanese Papaya leaves contain secondary metabolites, namely alkaloids, flavonoids, saponins, tannins,triterpenoids and steroids.

Key words : Extract Yield, Jepanese Papaya, Macroscopic, Phytochemical Screening

INTRODUCTION

Japanese Papaya comes from Yucatan, Mexico, and Central America. They were first discovered in an open forest area by I.M. Johnst. Local people commonly use this plant as vegetables and medicine. The benefits of Japanese Papaya leaves cannot be separated from the content of substances in them, such as Protein, Calcium, Iron, Phosphorus, Vitamin A, Niacin, Riboflavin, and Vitamin C. In contrast, the carposide content in Japanese Papaya leaves is efficacious as an anthelmintic. According to Loarca-Pina (2010) in Fatimah, (2016) stated that in addition to the above content, Japanese papaya is also beneficial in biological activities such as antimutagenic, antioxidant, hypoglycemic, and anti-inflammatory, antiprotozoal, and antibacterial. According to Purwati *et al*. (2017) in Hasibuan *et al*., (2021) explained that phytochemical screening is the first step to determining the active ingredients which are secondary metabolites in plants. The flavonoid compounds contained in Japanese papaya leaves are known to be suitable for lowering cholesterol levels in the blood (Rahmawati, 2018). Extraction is the first step taken to get the compound to be extracted. The choice of extraction method is also adjusted to the presence of compounds contained in it. In this case, maceration with the appropriate solvent is used, which meets the specified criteria (Agustina *et al*., 2018). An important factor in the extraction process is the selection of an appropriate solvent. The solvent used must be able to extract most of the secondary metabolites in the simplicia. The effectiveness of the solvent for extracting is very dependent on the solubility of the compound in the solvent, has the principle of like dissolves like, where a polar solvent will dissolve polar compounds and non-polar solvents will dissolve non-polar compounds. The use of the type of solvent can influence the yield of the resulting compound (Kemit *et al*., 2017). The results of research from Obichi *et al*., (2015) on Japanese papaya leaves show the content of tannins, saponins, alkaloids, and flavonoids. The difference between the previous research and the one studied is in the solvent and the sampling site used so researchers are interested in conducting research with phytochemical screening to identify secondary metabolites of Japanese Papaya leaves in the maceration method with three different types of solvents.

METHOD

A. Research Design

The research design is a descriptive qualitative experimental study that was conducted to determine thesecondary metabolites contained in Japanese Papaya (*Cnidoscolus aconitifolius* Mill.) leaves by maceration extraction using three solvents, namely 70% ethanol, n-hexane, and aqua distillate.

B. Research Sites

This research was conducted at the Laboratory of the Mitra Keluarga College of Health, East Bekasi.

C. Research Population

The population is the object of research in the form of animals, humans, objects, and also plants thathave certain characteristics research conducted. In this study, the population of Japanese Papaya (*Cnidoscolus aconitifolius* Mill.) leaves growing in Bekasi Timur Regency, Bekasi.

D. Research Variable

The independent variables in this study were the content of secondary metabolites in the phytochemicalscreening test of Japanese Papaya leaf extract (*Cnidoscolus aconitifolius* Mill.) and the percentage yieldof Japanese Papaya leaf extract (*Cnidoscolus aconitifolius* Mill.).

E. Japanese Papaya Leaf Sampling

Sampling began in February 2022 in the Bekasi Timur Regency area, Bekasi. The plant part used is theleaf part of the Japanese Papaya plant. Japanese Papaya leaves have been collected and then weighed todetermine the weight obtained.

F. Extract Making

The Japanese Papaya leaves used were fresh Japanese Papaya leaves which were dark green as much as 100 grams each then macerated with 70% ethanol, n-hexane, and 500 mL aqua distillate as a solvent. Each Japanese Papaya leaf powder was covered with aluminum foil to protect it from light and left for 3 x 24 hours and homogenized by stirring occasionally. Then the macerate in the form of a liquid extract was filtered using filter paper until a filtrate was produced (accommodated). After that, the residue sample was continued by maceration 1 x 24 hours with aqua distilled solvent, 70% ethanol, and 250 mL of new n-hexane. Furthermore, the maceration sample was filtered and the filtrate obtained was collected into one with the macerate filtrate. The solvent filtrate of 70% ethanol, n-hexane, and aqua distillate will be concentrated or thickened using a rotary evaporator at 400 C to obtain a thick extract (Erlina *et al*., 2018).

RESULTS

A. Japanese Papaya Leaf Morphology Examination

Based on the data in Table 3.1 shows the morphological results of Japanese Papaya leaves, namely witha length of 16-17.5 cm, a width of 14.4-30 cm, dark green, leaf shape round and fingered, leaf tip(apex) tapered, leaf base (base), grooved / incised, leaf margins (margin) are jagged, leaf surface textureis slippery, slightly shiny, leaf veins spread, and the leaf arrangement is single.

Table 3.1 Japanese Papaya Leaf Morphology Examination

Parameter	Observation result	References (Harahap, 2017)
Lenght	16-17,5 cm	10-30 cm
Width	14,4-30 cm	10-30 cm
Color	Dark green	Dark green
Leaf shape	Round and Finger	Round and Finger
Leaf tip (apex)	Tapered	Tapered
Leaf base	Notched/cut	Notched/cut

Leaf edge (margin)	Jagged	Jagged
Surface texture	Slick a little shiny	Slick a little shiny
Leaf bone	Spread	Spread
Leaf arrangement	Single	Single

B. Powder Organoleptic Test

Based on Table 3.2 states that the results of the organoleptic test on Japanese Papaya leaf powder arepowder form/texture, dark green/green leaf color, strong distinctive odor/aroma, and bitter taste.

Table 3.2 Powder Organoleptic Test Results

Organoleptic Test	Observation Result
Shape/texture	Powder
Color	Dark green/green leaf
Smell/scent	Strong characteristic
Flavor	Bitter

C. Extract Organoleptic Test

Based on Table 3.3 the organoleptic test of Japanese Papaya leaf extract stated that the results of the three types of solvents were aqua distillate and 70% ethanol showed the results of the form/texture of the extract being thick, brown-black in color, and characteristic odor/smell, and n-hexane showing the results. green-black shape/texture, green-black color, and distinctive smell/aroma.

Table 3.3 Extract Organoleptic Test Results

Solvent variation	Observation Result		
	Shape/texture	Color	Smell/scent
Aqua Destillate	Thick extract	Dark brown	Strong characteristic
Ethanol 70%	Thick extract	Dark brown	Strong characteristic
N-Hexane	Thick extract	Green-black	Strong characteristic

D. Extract Yield Calculation

Based on Table 3.4, the results of the calculation of the extract yield show that the 3 types of solvents are aqua distillate solvent as much as 27%, ethanol 70% as much as 10.2%, and n-hexane as much as 4.5%.

Table 3.4 Extract Yield Calculation Results

Solvent variation	sample weight (gram)	extract weight (gram)	Yield (%)	Yield Requirements (Depkes RI, 2008)
Aqua destillate	100	27	27	No less than 7,2%
Ethanol 70%	100	10,2	10,2	
N-Hexane	100	4,5	4,5	

E. Pytochemical Screening

Based on Table 3.5, the results show that the phytochemical screening test on aqua distillate was positive for alkaloids, flavonoids, saponins, triterpenoids, tannins, and 70% ethanol solvent was positive for secondary metabolites of alkaloids, flavonoids, saponins, triterpenoids, and tannins, while for the solvent n-hexane was positive. contains flavonoids and steroids.

Table 3.5 Phytochemical Screening Results

Phytochemical Test	Observation Results		
	Ethanol 70%	N-Hexane	Aqua destillate
Alkaloids	Dragendorff's: (+) Mayer: (+) Wagner: (+)	Dragendorff's: (-) Mayer: (-) Wagner: (-)	Dragendorff's: (+) Mayer: (+) Wagner: (+)
Flavonoids	(+)	(+)	(+)

Saponins	(+)	(-)	(+)
Steroids	(-)	(+)	(-)
Triterpenoids	(+)	(-)	(+)
Tannins	(+)	(-)	(+)
Glycoside	(-)	(-)	(-)

DISCUSSION

This study aims to determine the morphology, organoleptic and secondary metabolites present in Japanese papaya leaves with different types of solvents, namely aqua distillate, 70% ethanol, and n-hexane. Tests based on physical parameters include organoleptic examination of powders and extracts and leaf morphology. The organoleptic test aims to observe the shape, color, and smell of Japanese papaya leaves. Based on organoleptic observations of Japanese papaya leaf powder, the results showed that the leaves were dark green, had a powder shape or texture, a distinctive odor or aroma, and a bitter taste. In the research that has been done on organoleptic extracts, the results obtained are that 70% ethanol and aqua distillata have a thick extract form/texture, brown-black in color, with a characteristic odor. As for the solvent n-hexane, the extract organoleptic results were the form/texture of the extract was thick, green- black in color, had a distinctive smell. The purpose of the yield calculation is to determine the percentageof extract obtained so that it will be known the number of simplicia needed to make a certain number of extracts (Lusiyaningrum, 2021). The results obtained from this study indicate that the yield of aqua distillate has the largest yield of 27% and 70% ethanol has a yield of 10.2%, from the results obtained it is included in the extract yield requirements in the Indonesian Herbal Pharmacopoeia (2008) which is more than 7, 2%, while in the solvent n-hexane has the smallest yield of 4.5% so it is not included in the yield requirements. From the yield data, it has something to do with secondary metabolite compounds contained in a sample so that if the number of yield samples increases, the number of secondary metabolites contained in the sample will also increase. The amount of yield depends on each solvent that has a different polarity (Wijaya *et al*., 2022).

The results of a qualitative test through phytochemical screening on samples of Japanese Papaya (*Cnidoscolus aconitifolius* Mill.) leaves growing in the Bekasi Timur Regency area, Bekasi using a variety of solvents, namely 70% ethanol, n-hexane, and also aqua distillate. while on the results of the qualitative test through phytochemical screening conducted by (Nyam *et al.,* 2020) on samples of Japanese papaya leaves (*Cnidoscolus aconitifolius* Mill.) from the Department of Plant Science & Biotechnology, University of Jos, Nigeria using 70% ethanol as solvent. showed positive secondary metabolite compounds, namely alkaloids, tannins, flavonoids, terpenoids. Thus, in these two studies, there were similarities in secondary metabolites, the only difference being terpenoids and glycosides. In this study (Nyam et al., 2020) glycoside compounds were detected (positive), while in this study no glycoside compounds were detected (negative).

The results of this study showed positive secondary metabolites with aqua distillate solvent, namely alkaloids, flavonoids, saponins, triterpenoids, and tannins, while the qualitative test results through phytochemical screening conducted by (Ogbu and Igboanusi, 2019) on samples of Japanese papaya leaves (*Cnidoscolus aconitifolius* Mill.) from Ogbatai, Woji in Obio Akpor, South-south region, Nigeria using aqua solvent, namely alkaloids, tannins, saponins, and steroids. So in the two studies, there are similarities in secondary metabolite compounds, only the difference is in terpenoids and glycosides. In this study (Ogbu & Igboanusi, 2019) phytochemical screening for terpenoids and glycosides was not carried out, while in this study, phytochemical screening results obtained were terpenoids (positive) and glycosides (negative).

The results of this study showed a positive secondary metabolite compound with n-hexane solvent, namelyflavonoids and steroids, while the results of the qualitative test through phytochemical screening conductedby (Abdulmumeen et al., 2016) on samples of Japanese papaya leaves from Mr. Bolu Ajayi of the Department of Plant Biology, University of Ilorin, Nigeria, used n-hexane as a solvent, with positive results on glycosides, steroids, flavonoids, and terpenoids. So, in these two studies, there were similar secondary metabolites, only that they differed in glycosides and terpenoids. In this study (Abdulmumeen et al., 2016) glycosides (+) and terpenoids (+) were detected, while in this study, glycosides (-) and terpenoids (-) were not found. The negative results produced on secondary metabolites can occur because secondary metabolites can be influenced by environmental factors such as soil fertility, land height, climate, temperature, and pollution (Rudianti, 2018). Other factors include the nature of chemical compounds, solvents used, and available tools (Azizah et al., 2018).

The relationship between the results of phytochemical screening and the yield contained in a sample is that if the number of yield samples increases, the number of secondary metabolites contained in the samplealso increases. In this study, the most screening results were obtained in 70% ethanol and aqua distillate solvents which contain secondary metabolites of alkaloids, flavonoids, saponins, triterpenoids, and tannins.The secondary metabolites detected in each extract are compounds that can provide antioxidant, antibacterial, and antidiabetic activity (Hasan *et al.*, 2021)

CONCLUSION

Based on what has been described, it can be concluded that:

1. Macroscopic appearance on the leaves of Japanese Papaya (*Cnidoscolus aconitifolius* Mill.) on leaf morphology is 16-17.5 cm long, 14.4-30 cm wide, green, round leaf shape, leaf tip (apex) is tapered, Leaf base is notched/cut, leaf margin is jagged, leaf surface texture is (slightly shiny, smooth, spreadingleaf veins and single leaf arrangement and from powder organoleptic test, powder shape/texture, green, characteristic odor, and bitter taste), whereas in the organoleptic test the extracts, namely 70% ethanol and aqua distillate, had a thick extract from, blackish-brown color, and a characteristic odor, and in n-hexane, it had a thick extract from, blackish green color, and a distinctive smell.
2. The content of secondary metabolites of Japanese Papaya leaves from a variety of solvents, namely in aqua distillate solvent positive containing alkaloids, flavonoids, saponins, triterpenoids, and tannins, 70% ethanol solvent positive containing alkaloids, flavonoids, saponins, triterpenoids, and tannins, and while in positive n-hexane solvent contains flavonoids and steroids.

ACKNOWLEDGMENTS

The author would like to thank STIKes Mitra Keluarga Bekasi for the support during the author'sresearch.

REFERENCES

Abdulmumeen, A., Stephen, O., & Arvind, S. (2016). Chemical constituents, antibacterial, antifungal and antioxidant activities of the aerial parts of *Cnidoscolus aconitifolius*. *Ife Journal of Science*, *18*(2), 561–571.

Agustina, E., Andiarna, F., Lusiana, N., Purnamasari, R., & Hadi, M. I. (2018). Identifikasi Senyawa Aktif dari Ekstrak Daun Jambu Air (*Syzygium aqueum*) dengan Perbandingan Beberapa Pelarut pada Metode Maserasi. *Biotropic : The Journal of Tropical Biology*, *2*(2), 108–118. https://doi.org/10.29080/biotropic.2018.2.2.108-118

Azizah, Z., Zulharmita, & Wati, S. W. (2018). Skrining Fitokimia Dan Penetapan Kadar Flavonoid Total Ekstrak Etanol Daun Pare (*Momordica charantia* L.). *Jurnal Farmasi Higea*, *10*(2), 163–172.

Depkes RI. (2008). Farmakope Herbal Edisi I 2008. *Kementrian Kesehatan Republik Indonesia*, 1–276.

Erlina, D. V., & Hesturini, R. J. (2018). Perbandingan Aktivitas Antibakteri Ekstrak Daun Srikaya Hujau Dan Merah (*Annona squamosa* L.) Terhadap Bakteri Staphylococcus aureus Comparison of Antibacterial (*Annona squamosa* L.) Activity Leaf Extract Green and Red Against Bacteria *Staphylococcus aureus*. *Jurnal Prosiding Seminal Nasional Sains*, 129–135.

Fatimah, B. (2016). *Potensi Daun Afrika (Vernonia amygdalina), Daruju (Acanthus ilicifolius L.), Pepaya Jepang (Cnidoscolus conitifolius) Sebagai Bahan Tambahan Pakan Ternak Ruminansia Dalam Mereduksi Gas Metana (CH4) Secara In Vitro*. Skripsi. Fakultas Sain dan Teknologi. Universitas Islam Negeri Alauddin Makasar.

Harahap, L. H. (2017). Studi Biologi Dan Penyebaran Papaya Ringspot Virus-P (PRSV-P) Di Sumatera

Utara. *Jurnal Pembangunan Wilayah & Kota*, *1*(3), 82–91.

Hasan, H., Akuba, J., & Wilkinson, B. N. (2021). Efek Afrodisiaka Ekstrak Kulit Batang Sanrego (*Lunasia amara* Blanco) Terhadap Mencit Jantan (*Mus musculus*). *Indonesian Journal of Pharmaceutical Education*, *1*(3), 152–157. https://doi.org/10.37311/ijpe.v1i3.11375

Hasibuan, A. S., & Edrianto, V. (2021). Sosialisasi Skrining Fitokimia Ekstrak Etanol Umbi Bawang Merah (*Allium cepa* L.). *Jurnal Pengmas Kestra (Jpk)*, *1*(1), 80–84. https://doi.org/10.35451/jpk.v1i1.732

Kemit, N., Widarta, I. W. R., & Nocianitri, K. A. (2017). Pengaruh Jenis Pelarut Dan Waktu Maserasi Terhadap Kandungan Ssenyawa Flavonoid Dan Aktivitas Aantioksidan Ekstrak Daun Alpukat (*Persea Americana* Mill). *Jurusan Ilmu Dan Teknologi Pangan, Fakultas Teknologi Pertanian Universitas Udayana*.

Lusiyaningrum, T. A. (2021). *Uji Aktivitas Antioksidan Ekstrak Etanol Bunga Pepaya (Carica papaya L.) Dengan Metode ABTS (2,2 azinobis (3-etilbenzotiazolin)-6-asam sulfonat)*. Karya Tulis Ilmiah. Program Studi DIII Farmasi. Sekolah Tinggi Ilmu Kesehatan Nasional Surakarta.

Nyam, M. A., Abdullahi, U. I., Atsen, E., Itelima, J. U., & Daniel, V. (2020). Studies on The Antibacterial Effect of The Ethanolic Extracts of *Cnidoscolus aconitifolius* (Miller) (Hospital Too Far), Piliostigma thonningii (Schum) (Camels Foot) And Lantana camara (Linn) (Lantana). *Journal of Medical Biomedical and Applied Sciences*, *8*(2), 343–351. https://doi.org/10.15520/jmbas.v8i2.212

Obichi, E., Monago, C., & Belonwu, D. (2015). Effect of *Cnidoscolus aconitifolius* (Family Euphorbiaceae) Aqueous Leaf Extract on Some Antioxidant Enzymes and Haematological Parametersof High Fat Diet and Streptozotocin Induced Diabetic Wistar Albino Rats. *Journal of Applied Sciences and Environmental Management*, *19*(2), 201. https://doi.org/10.4314/jasem.v19i2.5

Ogbu, H. I., & Igboanusi, E. C. (2019). Bacterial isolates from surgical wound infection and their susceptibility reaction to *Cnidoscolus aconitifolius* leaf extract and honey. *Journal of Medical Plants Studies*, *7*(2), 163–170.

Rahmawati, L. (2018). *Pengaruh Ekstrak Etanol Daun Kates Jepang (Cnidoscolus aconitifolius) Terhadap Hiperkolesterolemia Pada Tikus Putih (Rattus norvegicus) Dan Pemanfaatannya Sebagai Buku Non Teks*. Skripsi. Program Studi Biologi. Universitas Jember.

Rudianti, S. (2018). *Pengaruh hipoksia sistemik kronik terhadap aktivitas spesifik enzim katalase (ec 1.11.1.6) darah dan otak tikus sprague dawley setelah diberi ekstrak buah maja (aegle marmelos)*. Skripsi. Fakultas Kedokteran. Universitas Tarumanagara Jakarta.

Wijaya, H., Jubaidah, S., & Rukayyah. (2022). Perbandingan Metode Esktraksi Maserasi Dan Sokhletasi Terhadap Rendemen Ekstrak Batang Turi (Sesbania Grandiflora L.) Comparison. *Indonesian Journal of Pharmacy and Natural Product*, *05*, 1–11.