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RASĀYAN *J. Chem.*
Vol. 17 | No. 4 |2075-2079| October - December | 2024
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
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COMPREHENSIVE STUDY OF CEPLUKAN FRUIT (*Physalis angulata L.*) USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)

Sajaratud Dur[1], Leni Widiarti[1], Anes Octaviani[1], Rony Abdi Syahputra[2] and Risti Arianti[1]

[1]State Islamic University of North Sumatera, 20353, Indonesia
[2]University of North Sumatera, 20155, Indonesia
✉ Corresponding author: leniwidiarti@uinsu.ac.id

This study explores the potential therapeutic benefits of Ceplukan Fruit (*Physalis angulata L.*) and its derivatives, focusing on their secondary metabolite content. The fruit a meticulous drying process to preserve its secondary metabolites, followed by extraction and phytochemical screening to identify alkaloid compounds. Results indicate the presence of alkaloids, known for their antimicrobial and immune-stimulating properties. Further analysis via FT-IR spectroscopy reveals the functional groups present in the methanol extract, confirming the presence of carboxylic acids, aldehydes, esters, and alkanes. Additionally, GC-MS analysis identifies dominant compounds, including those with molecular formulas indicative of alkaloids, naphthalene, and azulene. Azulene and its derivatives exhibit promising pharmacological actions in dermatological therapy, such as anti-inflammatory and photoprotective effects. Overall, this study underscores the potential of Ceplukan Fruit and its derivatives in herbal medicine and dermatological treatments, highlighting the need for additional research to elucidate their mechanisms of action and ensure their safety and efficacy.

Keywords: *Physalis angulata L.*, FT-IR, GC-MS, Alkaloid, Flavonoid, Saponin, Phenol.
RASĀYAN *J. Chem.*, Vol. 17, No. 4, 2024

INTRODUCTION

Ceplukan (*Physalis angulata L.*) is widely recognized in global traditional medicine, particularly in Indonesia, for its versatile therapeutic applications. Recent studies, encompassing phytochemical screening, secondary metabolite isolation, and biological activity assays, highlight its rich secondary metabolite profile, including physalins, withanolides, and flavonoid glycosides, showing promising pharmacological effects. Despite incomplete toxicity data, it exhibits a favorable safety profile, indicating its potential as a natural remedy. Ceplukan is a rich source of secondary metabolites, notably physalins, with leaf and fruit extracts demonstrating potent antioxidant properties. A deeper understanding of its anatomy and biochemistry will guide future research, particularly focusing on organs rich in antioxidants and steroids. With various phytochemical compounds identified, there are opportunities for medicinal development after isolation and pharmacological investigation.[1,2,3] FT-IR is a technique used to analyze infrared radiation intensity as a function of frequency or wavelength. The analysis of R. humilis L. fruit extract revealed the presence of various secondary metabolites like terpenoids, alkaloids, flavonoids, and tannins.[4,5] The employment of GC-MS[5] as an analytical tool provides valuable insights into the bioactive constituents found in medicinal plants such as Nut Grass Tuber (Cyperus rotundus L.) and Cleome gynandra. Through the process of extraction and analysis, a diverse array of compounds with potential therapeutic effects have been identified. Further investigation and exploration of their bioactive constituents hold promise for the development of novel pharmaceuticals or herbal remedies.[5,6,7] The objective of this study is to identify and analyze the chemical compounds present in the fruit of Physalis angulata L. using the gas chromatography method. Building on previous research, the author aims to further develop the findings obtained from this study.

EXPERIMENTAL

Material and Methods

The equipment utilized comprises standard glassware, a GR-300 analytical balance, an Equitron water bath, ATR-FTIR Brucker, GC-MS ThermoScientific TRACE1310/ISQ7000. The primary material used is the

Ceplukan Fruit extract, along with various chemical reagents such as 95% Methanol, Alcohol 96%, Magnesium powder, Hydrochloric Acid (HCl), Dragendorff reagent, Mayer's reagent, Bouchardat's reagent, Iron(III) Chloride ($FeCl_3$) from Merck, and distilled water from CV. Bratachem.

Preparation of Ceplukan Fruit Extract

The process begins by placing the powdered sample into a plastic bottle. Extraction is carried out using 98% methanol with a ratio of 1 part sample to 10 parts methanol by weight per volume. This extraction is conducted at room temperature for 3 days. Subsequently, the extract is filtered using filter paper and then concentrated using a water bath at 70°C. The resulting concentrated extract is then stored in a light- and air-tight container until needed.

Testing for Alkaloids, Flavonoids, Tannins, Saponins, and Phenols

In the alkaloid test, the initial test tube receives 2 to 3 drops of Mayer's reagent along with HCl. A positive result, indicating the presence of alkaloids, there will be a color change from white to yellowish. Then, in the subsequent test tube, 2 to 3 drops of Dragendorff's reagent and chloroform are added. If the result is positive for alkaloids, the color transition will be shades of orange-brown, red, or orange. For flavonoid analysis, the methanol extract of Ceplukan Fruit is combined with magnesium powder and concentrated hydrochloric acid until a change in color to red, yellow, or orange denotes a positive outcome. Saponin examination involves the incorporation of hot water, followed by vigorous shaking and subsequent addition of hydrochloric acid to induce foam formation. The presence of saponins is verified if the foam remains stable within a specified duration. Phenol assessment includes mixing the methanol extract of Ceplukan Fruit with iron(III) chloride solution to observe a color alteration to dark greenish-blue, indicative of phenol presence.

Functional Group Analysis with FT-IR

The analysis of functional groups is performed utilizing the Bruker ATR-FTIR system, where the frequency range spanning from 3800 cm-1 to 800 cm-1 is identified.

Mass Analysis using GC-MS

Mass analysis utilizing GC-MS is undertaken at the organic chemistry laboratory of UIN Sumatera Utara Medan. The analysis employs the Gas Chromatography-Mass Spectrometry (GC-MS) equipment manufactured by Thermo Scientific, comprising the Trace 1310 gas chromatography unit coupled with the ISQ 7000 single Quadrupole Mass Spectrometer. The gas flow rate is adjusted to 1 ml per minute, while the column temperature is initially set to 60°C and maintained for 5 minutes before being gradually increased at a rate of 4°C per minute until reaching 220°C, where it is held steady for 20 minutes.

RESULTS AND DISCUSSION

Preparation of Ceplukan Fruit Extract

The fresh Ceplukan Fruit is air-dried indoors, away from direct sunlight, to preserve the integrity of its secondary metabolite content. Subsequently, the dried fruit is ground into a fine powder, a process that can be achieved manually by using a mortar and pestle. Figure-1 illustrates the finely powdered dried simplisia. Figure-2 shows the results of the methanol extract of the Ceplukan Fruit which has been macerated for three days.

(a) (b)

Fig.-1: (a)Ceplukan Fruit's powder, (b) Ceplukan Fruit

Phytochemical Screening of Methanol Extract of Ceplukan Fruit

The variance in solvent types influences the quantity of extract yielded. Qualitative phytochemical screening serves as a preliminary test conducted on the extract and fractions of Ceplukan Fruit with the aim of detecting the presence of secondary metabolite contents using color reagents. The preliminary screening conducted in this study encompasses tests for flavonoid, alkaloid, saponin, and tannin compounds. The phytochemical screening results indicate that Ceplukan Fruit contains alkaloid compounds.

Table-1: Phytochemical Screening Test Result of Ceplukan Fruit Methanol Extracts

No.	Test	Result
1	Alkaloid	+ + +
2	Flavonoid	-
3	Saponin	-
4	Phenol	-

The results of the qualitative analysis of secondary metabolites can be observed in Table-1, indicating a positive outcome for alkaloid compounds. Alkaloids, derived from plants, possess significant therapeutic potential due to their antimicrobial and antifungal properties, along with their ability to stimulate the immune system and combat various pathogens and cancer cells. Their mechanism of action involves inhibiting key enzymes and processes essential for microbial survival, leading to membrane damage and cell death in fungi. Overall, alkaloids represent promising candidates for the development of novel pharmaceutical agents targeting infectious diseases and cancer.[8] This suggests that the methanol extract of Ceplukan Fruit contains secondary metabolites that are potentially beneficial in the field of herbal medicine and traditional treatments.

Identification of Extract Compound using FT-IR

Figure-3 shows the FT-IR spectrum analysis depicting transmittance (%) versus wavenumber (cm^{-1}) for the methanol extract of Ceplukan Fruit. There are seven peaks representing various absorption bands at different wavenumbers. Based on the results obtained from the functional group analysis using FT-IR, the wavenumber at 3200 - 3300 cm^{-1} indicates a wide and clearly defined O-H stretching absorption, originating from the carboxylic acid group -COOH. This is supported by the wavenumber at 1649 cm^{-1}, which corresponds to the C=O stretching absorption band. The wavenumber at 2945 cm^{-1} shows the C-H stretching absorption band from an aldehyde group, further supported by the absorption bands at 2836 cm^{-1}, which also represent C-H stretching. The wavenumber at 1450 cm^{-1} indicates a C-H bending absorption band from alkanes. The wavenumber at 1015 cm^{-1} indicates C-O bending absorption from esters carboxylic acid. For further confirmation, an analysis of ceplukan fruit components was conducted using Gas Chromatography-Mass Spectrometry (GC-MS).

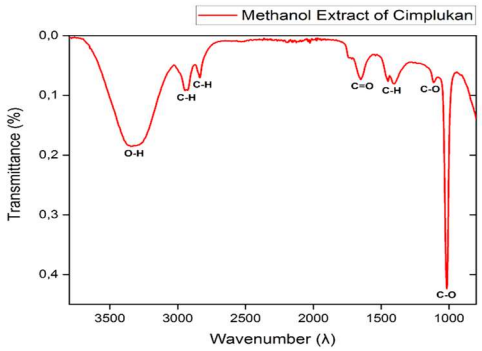

Fig.-2: FTIR Spectrum of Methanol Extract of Ceplukan Fruit

Identification of Extract Compound using GC-MS

Eight dominant peaks appear in the methanol extract of Ceplukan Fruit. The compounds corresponding to these peaks can be seen in Table-2 below. The majority of the compounds detected are those with molecular formulas C_9H_{12} and $C_{10}H_{14}$, which are indicated as alkaloid compounds, confirmed by the presence of

naphthalene and azulene compounds. Based on the table, it can be confirmed that the Ceplukan Fruit contains naphthalene compounds, which belong to the group of secondary metabolites. Consistent with previous research, which stated that Naphthylisoquinolines are a structurally diverse group of secondary metabolites, consisting of naphthalene and isoquinoline groups.[10,11,12] Azulene is an organic compound and an isomer of naphthalene. While azulene is dark blue, naphthalene is colorless. Azulene has lower stability compared to naphthalene.

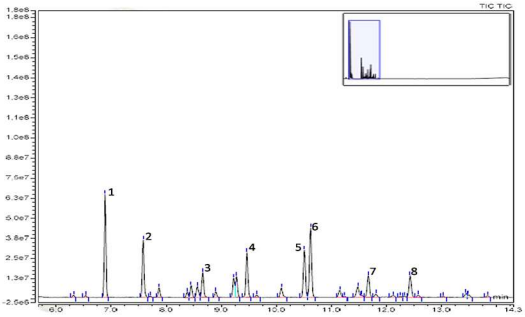

Fig.-3: GC-MS Spectrum Fraction of Ceplukan Fruit

Table-2: The Compounds Identified by GC-MS Analysis of Methanol Extract of Ceplukan Fruit

Peak	Real-Time	Hit 1	Hit 2	Hit 3	Ret. Area (%)	Molecular Formula
1	6,89	Benzene, 1-ethyl-4-methyl-	Benzene, 1,2,4-trimethyl-	Benzene, 1-ethyl-3-methyl-	11,49	C_9H_{12}
2	7,58	Benzene, 1-ethyl-3-methyl-	Benzene, 1-ethyl-4-methyl-	Benzene, 1,2,4-trimethyl-	7,29	
3	8,67	Benzene, 1-ethyl-2,4-dimethyl-	Benzene, 1-methyl-3-(1-methylethyl)-	o-Cymene	3,46	$C_{10}H_{14}$
4	9,47	Benzene, 1-methyl-3-(1-methylethyl)-	Benzene, 1-ethyl-2,4-dimethyl-	o-Cymene	6,22	
5	10,51	Benzene, 1,2,4,5-tetramethyl-	Benzene, 1,2,3,4-tetramethyl-	Benzene, 1,2,3,5-tetramethyl-	7,01	
6	10,63	Benzene, 1,2,4,5-tetramethyl-	Benzene, 1,2,3,5-tetramethyl-	Benzene, 1-ethyl-2,4-dimethyl-	10,35	
7	11,67	Benzene, 1,2,4,5-tetramethyl-	Benzene, 1,2,3,4-tetramethyl-	Benzene, 1,2,3,5-tetramethyl-	3,35	
8	12,43	Azulene	Naphthalene	1,2-Benzenedicarbonitrile	3,72	

Azulene and its derivatives in the therapy of dermatological diseases, showcasing various pharmacological actions such as anti-inflammatory, anticancer, and photoprotective effects, and the management of atopic dermatitis. Despite its promise, the unmodified use of azulene presents limitations such as photodecomposition and the formation of reactive oxygen species under UV radiation. Further research is required to ensure its long-term safety and efficacy. Additional preclinical and clinical studies are recommended to fully understand the mechanisms of action of azulene, thereby opening new avenues for the treatment of dermatological disorders.[12]

CONCLUSION

The Ceplukan Fruit, contains a variety of secondary metabolites, particularly alkaloids, as confirmed by qualitative phytochemical screening and FT-IR analysis. These alkaloids hold significant therapeutic potential, exhibiting antimicrobial, antifungal, and immunostimulatory properties, making them promising candidates for the development of herbal medicines and traditional treatments. Additionally, the presence of naphthalene compounds further enriches the secondary metabolite profile of Ceplukan Fruit, aligning with previous research on Naphthylisoquinolines. Azulene, offers potential therapeutic benefits in dermatological diseases, although its unmodified use presents challenges related to photodecomposition and reactive oxygen species formation. Therefore, further research, including preclinical and clinical studies, is essential to explore the safety and efficacy of azulene and its derivatives for the treatment of

dermatological disorders. Overall, the findings highlight the pharmacological potential of Ceplukan Fruit and underscore the importance of continued investigation into its medicinal properties.

ACKNOWLEDGMENTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

Sajaratud Dur https://orcid.org/0000-0002-6063-8271
Leni Widiarti https://orcid.org/0000-0002-1020-7510
Anes Octaviani https://orcid.org/0009-0002-0704-0227
Rony Abdi Syahputra https://orcid.org/0000-0003-2016-0151
Risti Arianti https://orcid.org/0009-0003-7679-3828

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