GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Bioactivities of Nek Kara (*Dillenia excelsa* (Jack) Martelli ex. Gilg) leaves from forest medicinal plants of Kutai Ethnic

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GSC Biological and Pharmaceutical Sciences, 2023, 25(03), 024–031

Publication history: Received on 17 October 2023; revised on 28 November 2023; accepted on 01 December 2023

Article DOI: https://doi.org/10.30574/gscbps.2023.25.3.0414

Abstract

The utilization of traditional medicine and treatment within the community of Kutai Kartanegara, East Kalimantan, Indonesia is characterized by a wide range of practices. Medicinal plant extracts containing secondary metabolites, possessing diverse molecular structures and biological activities, exhibit promising potential for the development of medicines targeting various diseases. Phenolic compounds, present in plants, exhibit numerous biological effects, including antioxidant and antimicrobial properties. These secondary metabolite compounds play a crucial role in safeguarding against disease-induced damage. Nek Kara (*Dillenia excelsa* (Jack) Martelli ex. Gilg), a forest plant, has been traditionally employed by the local population for the treatment of diabetes. The objective of this study is to investigate the biological activities of the ethanol extract derived from Nek Kara leaves. The analysis encompasses the examination of phytochemical content, toxicity, as well as antioxidant and antimicrobial activities of the extract. The antioxidant activity is assessed through DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging activity using a spectrophotometer, while toxicity is determined via the BSLT (Brine Shrimp Lethality Test). The antimicrobial activity is evaluated using the diffusion method. The findings reveal the presence of phytochemical content, such as alkaloids, flavonoids, tannins, and steroids, within the ethanol extract derived from Nek Kara leaves. The BSLT results indicate the absence of toxic bioactivities in the ethanol extract. Furthermore, the Nek Kara leaf extract demonstrates potent antioxidant activity and exhibits inhibitory effects on the growth of bacterial and fungal colonies.

Keywords: Antioxidant; Phytochemical; Toxicity; Antimicrobial

1. Introduction

Traditional medicine and treatment practices among the general population exhibit a remarkable diversity. These practices encompass a wide range of drugs and treatments, which can be categorized into two distinct types: those intended for internal ailments or diseases, and those designed for external conditions. Furthermore, in addition to their familiarity with traditional medicine and treatment, the people of Kutai possess a comprehensive understanding of various traditional healers and medical experts [1].

The Indonesian forests are renowned for their exceptional biodiversity, boasting one of the highest species richness in the world. They serve as a habitat to over 400 species of trees that possess significant economic value, as well as an estimated 25,000 species of flowering plants [2]. The emergence of novel diseases has been on the rise, and many of

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the existing drugs are no longer effective in combating these new ailments. Consequently, the quest for innovative drugs persists unabated [3].

Plant ingredients contain a variety of antioxidants, with phenolic compounds being particularly prevalent. These compounds, found in plants, have numerous biological effects. Flavonoids and other phenolics, for example, have been shown to play a protective role against diseases [4][5]. Medicinal forest plant extracts also contain secondary metabolites with diverse molecular structures and biological activities, making them promising candidates for the development of treatments for various diseases.

However, the production of herbal remedies is currently limited, primarily due to a lack of scientific information regarding the efficacy of specific plant species [6]. Therefore, the objective of this study was to investigate the phytochemical content, toxicity level, as well as the antioxidant and antimicrobial activities of Dillenia excelsa (Jack) Martelli ex. Gilg., a plant traditionally used by the people of Kutai.

2. Material and Methods

2.1. Identification, utilization, and description

The initial phase involved conducting a comprehensive survey to gather information regarding the traditional medicinal plants of the Nek Kara variety. This information was obtained from traditional healers who have utilized these plants for their medicinal properties. The objective was to determine the efficacy of these plants in traditional medicine. Subsequently, a process of type validation was undertaken until the scientific name of the plants was ascertained.

2.2. Plant material and extraction

The Nek Kara leaves were procured from Sebulu Modern Village, located in the Kutai Kartanegara Regency of East Kalimantan Province, Indonesia. These leaves were then subjected to a drying process at ambient temperature, following which they were ground into a fine powder [7]. The dried leaves were subsequently extracted using ethanol at room temperature for a duration of 48 hours. The resulting extract was then filtered, and the concentrates were prepared using a rotary vacuum evaporator, maintaining a temperature range of 30-40oC.

2.3. Phytochemical analysis

2.3.1. Alkaloid assay [8]

A volume of 1 mL of Dragendorff's solution was introduced into 5 mL of the extract, followed by the addition of 2 mL of hydrochloric acid (HCl). The appearance, characterized by orange or red colors, signifies the presence of alkaloids.

2.3.2. Flavonoid assay [8]

A volume of 1 mL of the extract was combined with a few drops of diluted sodium hydroxide (NaOH 1%). The presence of a distinct yellow color in the extract solution, which turns colorless upon the addition of diluted acid (HCl 1%), indicates the presence of flavonoids.

2.3.3. Saponin assay [9]

Saponins were assessed by adding 10 mL of hot water to the reaction tube containing 1 mL of the tested sample, which had been dissolved in acetone. The solution was then allowed to cool and shaken for 10 seconds. The formation of a stable foam, persisting for 10 minutes with a height of 1-10 cm and not disappearing upon the addition of 1 drop of hydrochloric acid (HCl 2N), indicates the presence of saponins.

2.3.4. Tannin assay [8]

Tannins were evaluated by pouring 10 mL of the extract solution into a reaction tube and adding a 1% acetate lead solution (CH3COO)2Pb. The presence of a yellow sediment in the reaction confirms the presence of tannins.

2.3.5. Triterpenoid and steroid assay [9]

Triterpenoids and steroids were identified using a mixture of acetic acid anhydride and concentrated sulfuric acid, commonly referred to as the Liebermann-Burchard reaction. A total of 10 drops of acetic acid anhydride and 2 drops of concentrated sulfuric acid were sequentially added to 1 mL of the tested sample, which had been dissolved in acetone.

The mixture was shaken and left for several minutes. The presence of red and purple colors in the reaction indicates the presence of triterpenoids, while green and blue colors indicate the presence of steroids.

2.4. Toxicity testing

In order to assess the toxicity of the leaf extract, the Brine Shrimp Lethality Test (BSLT) was conducted according to the methodology outlined by Meyer [10]. The BSLT method is commonly employed to obtain an approximate measure of the bioactivities of plant materials that are suspected to have medicinal applications. This method is simple to execute, cost-effective, rapid, and can be utilized with small quantities of plant extract [10]. LC50, which refers to the concentration of a compound that is expected to cause the death of 50% of a test population within a specified time period [11], was employed to determine the level of toxicity. The aquatic toxicity criteria defined by Wagner [12] were used to determine the extent of toxicity.

2.5. Analysis of antioxidant activity

The investigation into the antioxidant activity was conducted using the method developed by Arung [13]. A spectrophotometer was employed at room temperature (25°C) with a wavelength of 514 nm. A DPPH solution (1,1-diphenyl-1-picrylhydrazyl radical) and ascorbic acid (Vitamin C) were utilized as positive controls. The concentration of the sample extract required to achieve a 50% inhibition was expressed as the IC50 value for the extract. Three replicate analyses were performed for each extract, and the average results were calculated. The determination of the antioxidant activity of the extract using the DPPH method followed the procedure proposed by Jun [14].

The percentage of inhibitory effect of various concentrations of the extract was expressed relative to the controls in the following manner:

Inhibition % = 100% x (DPHH control absorbance – sample absorbance) / (control absorbance)

An IC50 value represents the concentration required to inhibit free radicals by 50%. This numerical value serves as a parameter for characterizing the antioxidant properties of a plant extract. A plant extract is deemed to possess strong antioxidant effectiveness if its IC50 value is less than 50 parts per million (ppm). The analysis of IC50 values involved the utilization of linear regression analysis to examine the relationship between concentration and inhibition.

2.6. Antibacterial testing

The antimicrobial test was conducted using the diffusion method as outlined by Cappucino and Sherman [15], with certain modifications. In this experiment, 20 mL of Nutrient Broth medium (NB) was poured into a sterilized Petri dish. Subsequently, the medium was solidified and leveled using a sterile cotton swab, under aseptic conditions (utilizing laminar flow). The medium was then allowed to dry for approximately 30 minutes. Wells were created in the medium using a cork borer. These wells were filled with 20 µL of the extract at various concentrations: 25 µg/well, 50 µg/well, 100 µg/well, and 200 µg/well. Chloramphenicol was employed as a positive control.

Bacterial incubation was carried out for 24 hours on a Petri dish containing up to 6 wells. Each well represented a distinct concentration, as well as positive and negative controls. The zone of inhibition, which indicated the diameter (mm) of the extract's inhibitory effect on the growth of fungi and bacteria, was measured around and within each well.

3. Results and Discussion

3.1. Identification, Utilization, and Description

Within the Kutai ethnic community of Desa Sebulu Modern, located in the Kutai Kartanegara Regency of East Kalimantan, there exists a wealth of traditional knowledge regarding medicinal treatments derived from the forest vegetation and medicinal herbs found within. This knowledge has been passed down through generations, preserving the wisdom of their ancestors. However, not all individuals possess the expertise required to identify the specific forest plants that can be utilized as herbal medicines. Only a select few possess this unique ability, and they are typically recognized for their proficiency in creating medicinal remedies.

The findings of the identification and description of the Nek Kara plant species, which is utilized by the Kutai community in Sebulu Modern Village for traditional medical purposes, are presented in Table 1. Additionally, Figure 1 provides a visual representation of the Nek Kara plant.

Table 1 Identification, Utilization, and Description of Nek Kara (*Dillenia excelsa* (Jack) Martelli ex. Gilg.) Type

Local name/ scientific name/ family	**Part used**	**Traditional utilization**	**Plant description**
Nek Kara/ *Dillenia excelsa* (Jack) Martelli ex. Gilg./ Dilleniaceae	Leaf	Diabetes, children skin repair	Medium tree, up to 40 meters in height, branch-free height can reach 20 meters, and up to 75 cm in diameter. Medium to large-sized leaves (15-30 cm in length), shiny green-colored, slightly jagged margins, young leaves are reddish in color.

Figure 1 The Plant Nek Kara (*Dillenia excelsa* (Jack) Martelli ex. Gilg.)

Based on the information and discussions with traditional healers within the Kutai ethnic communities, each successive generation endeavors to advance the knowledge inherited from their ancestors. This encompasses not only a comprehensive understanding of the forest vegetation, but also the techniques employed in the preparation of medicinal remedies through the process of mashing, dissolving, and boiling specific herbs. However, the utilization of such methods sometimes deters individuals from consuming herbal medicines due to their pungent aromas and bitter taste.

The range of medical conditions treated with herbal medicines derived from the forests varies from mild ailments such as influenza, colds, coughs, headaches, and stomachaches to more severe diseases including cancer, strokes, heart attacks, hypertension, constrictions of blood vessels, stomach injuries, kidney stones, and so forth.

3.2. Extraction result and phytochemical content

The resulting plant extract yields and phytochemical content are summarized in Table 2.

Tabel 2 Extract Yields of the Medicinal Plant Nek Kara (*Dillenia excelsa* (Jack) Martelli ex. Gilg.) with the Solvent Ethanol and the Phytochemical Content

Powder weight OD (g)	**Extract weight (g)**	**Yield (%)**	**Phytochemical content**
89.83	20.56	22.89	Alkaloids, Flavonoids, Tannins, Saponins, Steroids

The purpose of the yield calculation was to determine the number of parts that can be extracted from the sample under test. The extract yield value obtained from the extraction results in a test sample is greatly influenced by factors such as water content, sample size, solvent, and extraction techniques [9]. This percentage of yields has a direct impact on the amount of extracted weight from a variety of raw materials used. A high yield percentage indicates an increase in the weight of the extract produced.

Phytochemical testing of plant materials is utilized to identify secondary metabolite compounds. The presence of secondary metabolites in plants, as evidenced by various research results, has a positive effect on human health and can actively contribute to the prevention and treatment of diseases. The plant extracts were prepared from the Nek Kara type and contained secondary metabolite compounds such as alkaloids, flavonoids, tannins, saponins, and steroids. The

presence of these phytochemical compounds in the plant materials suggests a potential medicinal value of their extracts in the prevention and/or treatment of specific diseases.

The current study has revealed that the plant Nek Kara (*Dillenia excelsa* (Jack) Martelli ex. Gilg.) contains various phytochemicals, including alkaloids, flavonoids, tannins, saponins, and steroids. These compounds possess potential medicinal properties and exhibit antioxidant activity. Flavonoids, in particular, act as antioxidants and prevent metabolic damage caused by free radicals [16]. The presence of flavonoids and tannins in plants has been observed to play a crucial role in binding free radicals. Phenolics, which are a group of compounds that act as primary antioxidants in plant metabolic pathways, include flavonoids and tannins [17].

In biological systems, flavonoids exhibit antioxidant activity by inhibiting free radicals and have been found to produce anti-allergic effects, reduce inflammation, and prevent platelet aggregation. Additionally, there are reports of their antimicrobial effects and their ability to inhibit ulcers, tumors, and hepatoxicity [18]. Overall, the analysis of phytochemicals in plants with suspected medicinal benefits is a promising area of research for identifying physiologically active compounds that can protect the human body from various forms of metabolic damage caused by both internal and external factors [19].

3.3. Toxicity

The objective of the toxicity assessment of the plant samples was to determine the presence of any toxic substances. The utilization of the brine shrimp Artemia nauplii as a model species has been proposed for the evaluation of pharmacological activity of ecotoxins and complex compounds [20][21]. For our toxicity test, we employed the related species Artemia salina. The initial tests were conducted using extracts to ascertain whether they would cause mortality in shrimp larvae at a concentration of 1000 ppm. The preliminary test revealed that the sample extracts at that concentration resulted in a mortality rate of 50% or more. The findings of the assay are presented in Table 3.

Table 3 The Rate of Shrimp Mortality during the Toxicity Test

Conc. (ppm)	Replication			Total Mortality	Average	% Mortality
	1	2	3			
1000	1	2	2	5	1.7	17

As per Meyer's [10] analysis, a plant extract is deemed to possess toxic activity if it can cause the death of over 50% of Artemia larvae at a concentration of 1,000 ppm. Consequently, we proceeded to conduct a more detailed examination of the toxic activity of three plant extracts, the results of which are presented in Table 3.

The outcomes of the toxicity testing of the ethanol extract derived from the leaves of Nek Kara (*Dillenia excelsa* (Jack) Martelli ex. Gilg.) revealed that the mortality rate of Artemia salina shrimp was 17%. Based on Meyer's [10] analysis, this figure indicates that the Nek Kara leaf extract is non-toxic since the rate of shrimp mortality at 1,000 ppm does not exceed 50%. Therefore, no further testing was necessary to determine its toxicity in this instance.

In light of the above findings, the use of this plant's leaves as medicine and traditional medicine by the community does not appear to result in any complaints related to its toxic properties. This discovery is of great significance since, regardless of potential benefits such as the antioxidant activity of their phytochemical compounds, it is essential to determine whether the ingredients derived from this species are suitable for human consumption if they are used in traditional medicines by the community. Thus far, there have been no grievances regarding the use of these ingredients as components in traditional medicine. According to McLaughin [22], extracts with an LC50 value of <30 ppm have the potential to serve as anticancer agents.

3.4. Antioxidant activity

The exploration for novel natural antioxidant agents relies significantly on natural resources, particularly plants. In order to assess the antioxidant activity, a comprehensive examination was conducted at various concentrations. The outcomes pertaining to the inhibition against free radicals (DPPH) and the corresponding data analysis are presented in Table 4.

Tabel 4 Antioxidant Activity Test of the Extract of Nek Kara (*Dillenia excelsa* (Jack) Martelli ex. Gilg.) Leaves

Conc. (ppm)	Inhibit. (%)	Regression Equation	IC_{50}(ppm)	Classify.
1.562	33.28	Y = 3.5815x + 28.648	6	Strong
3.125	39.85	R^2=0.9953		
6.250	52.70			
12.50	72.70			
25.00	66.28			
50.00	62.92			

The findings of the study revealed that the tested sample exhibited an increased antioxidant inhibition of free radicals with a corresponding increase in the concentration of the relevant extract. The results demonstrated that the plant extracts possessed a significant antioxidant activity, with IC50 values of less than 50 ppm. These outcomes suggest that the plant species utilized by the community for medicinal purposes possess a noteworthy antioxidant activity, which may contribute to the reputed therapeutic efficacy of plant extracts from this species.

The data analysis to establish the correlation between plant extract concentration (ppm) and free radical inhibition (%) using linear regression yielded an R2 value of 0.9953, with the equation Y = 3.581x + 28.648. The R2 value of 0.9953 indicates a strong correlation between the two variables, as the correlation coefficient is close to 1. Based on the regression equation, the IC50 value of 6 ppm indicates that the Nek Kara plant extract possesses potent antioxidant properties. The graph depicting the relationship between plant extract concentrations and free radical inhibition is presented in Figure 2 below.

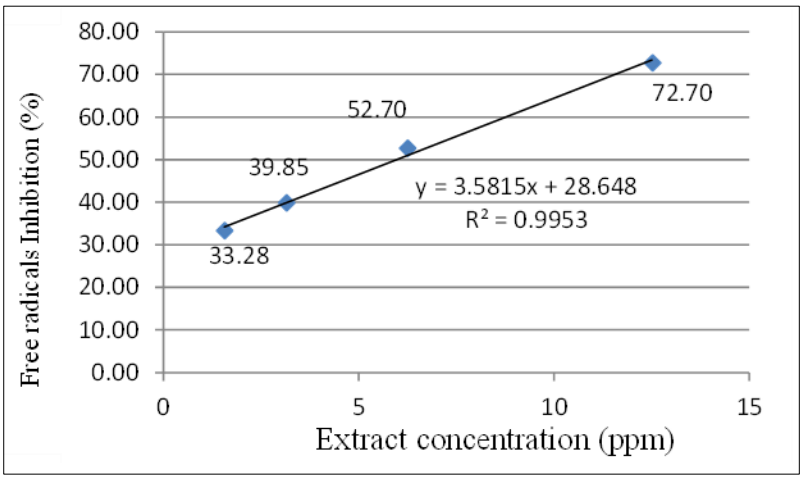

Figure 2 Linear regression between concentrations of Nek Kara extracts (ppm) with free radicals inhibition (%)

3.5. Antimicrobial Activity

In this study, the efficacy of the plant extract in inhibiting the growth of bacteria (*Streptococcus mutans, Streptococcus sobrinus, Escherichia coli, Propionibacterium acne)* and fungi (*Candida albicans*) cultured on a Nutrient Broth medium in Petri dishes was investigated. The plant extract was tested at four different concentrations. The findings of the experiment are presented in Table 5.

Numerous traditional medicines derived from plants have been identified as possessing antimicrobial properties. A commonly employed method for preliminary testing of such antibiotic activity involves assessing whether extracts of the medicinal plant can impede the growth of bacteria and fungi.

The degree of bacterial inhibition zone for each plant extract was found to be greater at higher extract concentrations. A classification of strong inhibitory activity is assigned if the width of the zone of inhibition exceeds 6 mm, while a

classification of moderate inhibitory activity is given if the zone measures between 3-6 mm in width. A classification of weak inhibitory activity is assigned if the zone measures 0-3 mm in extent [23].

Table 5 Results of the Antibacterial dan Antifungi Tests of Nek Kara (*Dillenia excelsa* (Jack) Martelli ex. Gilg.)

Bacterium/ Fungus	**Average Inhibiton Diameter (mm)**				
	Chlor*	25 (µg)	50 (µg)	100 (µg)	200(µg)
Streptococcus mutans	40.22	11.89	14.67	17.56	18.55
Streptococcus sobrinus	38.45	12.78	15.11	16.00	16.89
E. coli	26.78	13.22	14.55	15.11	15.33
Propionibacterium acne	40.11	13.56	13.67	15.67	18.66
Candida albicans	30.44	11.11	11.89	12.66	13.56

*Chlorampenicol (Control +)

The findings suggest that the extracts of Nek Kara (*Dillenia excelsa* (Jack) Martelli ex. Gilg.) leaves possess antibacterial and antifungal potential, likely due to the presence of phytochemically active compounds. It is suspected that the antibacterial activity of the leaf extracts is attributable to the presence of secondary metabolite components such as terpenoids, steroids, saponins, tannins, and flavonoids [24]. The extent of the antibacterial effect may vary depending on the specific extraction method employed and its impact on the stability and efficacy of these active compounds.

4. Conclusion

Traditionally, medicinal plant species have been recognized for their potential to contain bioactive compounds that may be beneficial in the treatment of various human diseases. The majority of extracts from these species have been found to contain phytochemical compounds, including alkaloids, flavonoids, tannins, saponins, and steroids. The sample under investigation has demonstrated significant antioxidant activity, and the results of the toxicity assay indicate that it is non-toxic. Furthermore, antibacterial and antifungal testing has generally revealed that medicinal plants exhibit a potent inhibitory effect on the growth of bacterial and fungal colonies.

Compliance with ethical standards

Acknowledgment

The authors would like to express their genuine gratitude to the Directorate of Research and Community (DP2M) of the Directorate General of Higher Education under the Ministry of Research, Technology, and Higher Education, for their generous funding support, as evidenced by contract number 058/SP2H/LT/DPRM/IV/2017, dated 25 April 2017. Additionally, the authors extend their appreciation to their colleagues and all individuals who have provided assistance in the conduct of this research.

Disclosure of conflict of interest

The author declared no conflict of interest

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