MALAYSIAN JOURNAL OF APPLIED SCIENCES 2024, VOL 9(2): 16-35
E-ISSN:0127-9246 (ONLINE)
http://dx.doi.org/10.37231/myjas.2024.9.2.414
https://journal.unisza.edu.my/myjas

ORIGINAL ARTICLE

Phytochemical and Proximate Composition of *Senna alata* (L.) Roxb.: Nutritional and Medicinal Insights from Different Plant Parts in Besut, Terengganu

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Received: 04/09/2024, Accepted: 29/10/2024, Available Online: 31/10/2024

Abstract

This study was conducted to determine the presence of bioactive compounds in leaves, stems and flowers of *Senna alata,* to characterize the bioactive compound identified from its different parts and to investigate the proximate composition of different parts of *Senna alata* collected in Besut, Terengganu. Samples of *S. alata* leaves, stems, and flowers were collected in Kampung Paya Rawa, Besut, Terengganu. Phytochemical screening and GC-MS analysis were used to determine the chemical composition of different parts of *Senna alata*. The samples were also subjected to proximate analysis to determine their moisture, ash, crude fibre, crude protein, crude fat, and carbohydrate contents. Standard methods were used: moisture content by oven drying, ash content by muffle furnace incineration, crude fibre by acid and alkali digestion, crude protein by the Kjeldahl method, crude fat by Soxhlet extraction, and carbohydrate content calculated by difference. Data obtained from proximate analysis were analysed with one-way ANOVA. The findings show the presence of bioactive compounds in leaves, stems, and flowers of *Senna alata* such as alkaloid, phenol, tannin, saponin, quinone, and terpenoid which shows biological and pharmacological activities that indicates the medicinal properties in *Senna alata*. GC-MS analysis reveals the detection of derivatives compound of azetidine, thiophene, thiazole, and triazole that supports the ethnomedicinal claims of *S. alata* to treat various diseases. The results also reveal significant variations in the proximate composition among the different plant parts. The flower part exhibited the highest levels of moisture (30.88±0.44%), ash (7.49±0.28%), crude fat (6.98±0.03%), and carbohydrates (39.65±0.76%), while the stem contained the highest crude fibre (38.68±0.22%) and the leaves had the highest crude protein content (15.52±0.17%). In summary, the study suggests that *Senna alata* contains bioactive compounds which exhibit biological and pharmacological properties. These findings suggest that different parts of *S. alata* possess varying nutritional values and potential therapeutic benefits, particularly the leaves, which are rich in protein, and the flowers, which are high in energy sources. The results emphasise the possibility of using *S. alata* as a biopesticide to treat ectoparasites infestation in animals, due to the presence of acaricidal chemicals in the plant.

Keywords: Bioactive compound; gelenggang; biopesticide; GC-MS analysis

Introduction

Fleas, lice, mites, and ticks are prevalent ectoparasites infesting various animal species, including cattle, sheep, goats, pigs, dogs, cats, and poultry (Mwangengwa & Rhanga, 2023). Studies have been reported on the infestations of these ectoparasites, for example a recent study by Idris et al. (2023) done in Sarawak indicated that the prevalence of tick infestation in cattle was 62.4%, with a total of 229 ticks identified. A study conducted by Mohamad-Radzi et al. (2021) revealed that the incidence of mite infection in rabbit farms across three districts in Selangor was 51.85%. Syamsul et al. (2020) reported in their study that the most prevalent ectoparasites in small ruminants in Kelantan were lice and ticks, with prevalence rates of 43.64% and 22.98%, respectively. Ectoparasite infestations can provoke allergic reactions, irritation, and inflammation in the animal host, and they are particularly significant as vectors for infections, facilitating the transmission of diseases like as babesiosis, theileriosis, and anaplasmosis (Insyari'ati et al., 2024).In response to control ectoparasites, chemical pesticides are the most widely utilised approach worldwide, despite several challenges such as the emergence of resistance and adverse effects on the environment (Yadav et al., 2017).

Research by Siddiqui et al. (2022) suggests that repeated exposure to certain pesticides can lead to genetic mutations in parasites, resulting in resistance and reduced pesticide effectiveness over time. According to George et al. (2014), there is a growing concern with the use of synthetic chemicals to address parasites in the veterinary field. Pest resistance, product residues, withdrawal of active ingredient, undesirable environmental persistence and unacceptable risks to non-organism are among the issues that leads the researcher to find alternative methods in controlling parasites. Nevertheless, the focus of current research is on the long-term, less severe impacts of continuous pesticide exposure (Sigouin et al., 2021).

To date, knowledge on medicinal plants, their phytoconstituents, and their uses as medications have made up the field of phytomedicine (Igara et al., 2023). Hence, the uses of plant-derived products as biopesticides are rising steadily as a solution to this problem as they are beneficial to limit the development of pest-resistance, minimal toxicity to animals, and short environmental persistence (George et al., 2014). Pavela et al. (2016) explained that throughout history, traditional cultures and communities have depended on plants as a source of active compounds that have repellent and acaricidal effects. In search of effective plant-based alternatives, *Senna alata* has emerged as a promising candidate due to its rich bioactive composition.

Senna alata belongs to the Fabaceae family and is characterized by distinctive pinnate compound oblong-shaped leaves, elongated spikes of bright yellow flowers, and elongated seed pods. The plant is a perennial herb that grows annually and sometimes biannually. Among Malaysians, *S. alata* is very common by the name gelenggang, and in other regions, *S. alata* is also known as *Cassia alata*, Ketepeng Cina, candle bush, and ringworm plant. Recent work by Oladeji et al. (2020) found that the different parts of *S. alata* show biological activities that authenticate the ethnomedicinal claims to treat various diseases such as skin infections, scabies, bacterial infections, and fungal infections. He revealed that the bioactive compound found in different parts of *S. alata* displays pharmacological activities that includes antibacterial, antioxidant, antifungal, anticancer, anti-inflammatory, antidiabetic, antihyperlipidemic, antimalarial, anthelmintic, and antiviral properties. Traditionally, *S. alata* has been employed for the treatment of dermatological conditions such as eczema, athlete's foot, skin irritation, and ringworm (Clement et al., 2021; Oladeji et al., 2020). These medicinal properties are attributed to its bioactive compound as mentioned in a report by Angelina et al. (2021), which are phenolics (rhein, kaempferol, aloe emodin, and glycosides), anthraquinones (alatinone and alatonal), fatty acids (oleic, palmitic, and linoleic acids), steroids, and terpenoids (sitosterol, stigmasterol, and campesterol).

Although *Senna alata* is reported to contain many bioactive compounds beneficial as alternative pesticides, the chemical composition of plants is affected not only by species variations and genetic factors, but also by external variables, such as environmental circumstances, including soil type, precipitation levels, light intensity, and humidity (Angelina et al., 2021). The stability and bioavailability of these bioactive compounds can be affected by their interaction with other components in the plant. For instance, the presence of certain minerals like potassium and magnesium can enhance the absorption of these bioactive compounds in the human body (Abdulwaliyu et al., 2018; Clement et al., 2021). In account of the significant medical applications of *S. alata*, it is necessary to conduct proximate and phytochemical analyses on the plant to gain a deeper understanding of its biochemical composition. Therefore, this study was conducted to determine the presence of bioactive compound in leaves, stems and flowers of *S. alata* and to determine the proximate composition of different parts of *S. alata*.

Materials and Methods

Plant collection

Senna alata was collected in Kampung Paya Rawa, Besut, Terengganu. The samples were prepared according to the method documented by Saha et al. (2020). The samples were separated into three parts: leaves, stems and flowers. The samples for each part were cut into several small pieces and cleaned properly. The plant parts were shade dried at room temperature for seven days. Then, the dried plant parts were grounded using a grinder into coarse powder for analysis and extraction.

Plant extract preparation

The modified methods of Parveen et al. (2016) and Saha et al. (2020) were used. Powdered samples for each part of *Senna alata* were macerated with ethyl acetate using ratio 1:10 at room temperature for 24 hours. The resulting extracts were filtered through using filter paper and then evaporated with rotary evaporator until thick crude extracts are formed. The final crude extracts were then stored in a chiller for storage. The crude extracts were subjected to undergo phytochemical screening test and Gas Chromatography Mass Spectrometry (GC-MS) analysis.

Phytochemical screening

The standard procedures were used for the determination of bioactive compounds presence in different parts of *Senna alata* such as alkaloid, flavonoid, tannin, saponin, phenol, terpenoid, and quinone by using the method adopted from Angelina et al. (2021) and María et al. (2018).

Gas Chromatography Mass Spectrometry (GC-MS) Analysis

The GC-MS analysis of *Senna alata* extracts from the leaves, stems, and flowers was performed using GC-MS Agilent Technologies 7890B. The database of National Institute Standard and Technology (NIST), which has more than 62,000 patterns was used for the interpretation on mass spectrum of GC-MS. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular formula, molecular weight and structure of the components of the test materials were ascertained.

Based on Parveen et al. (2016), the GC-MS analysis of the extracts of the leaves, stems, and flower of *S. alata* was operating at 1000 eV ionization energy, equipped with GC HP-5 capillary column (phenyl methyl siloxane, 25 mm × 0.25 mm) with Helium (He) was used as the carrier gas with split ratio 1:5. Oven temperature was 80°C (2 minutes) to 280°C at 1-40°C/minutes, detector temperature 250-280°C, and carrier gas He (0.9 ml/minutes). 2.0 µl of respective diluted samples was manually injected in the splitless mode, with split ratio and with mass scan of 50-600 amu. Total running time of GC-MS is 40 minutes.

Proximate Analysis

The method used is according to The Association of Analytical Chemists (AOAC).

Determination of Moisture Content

A 5g homogenised sample was placed in a crucible that had been weighed. It was then dried for 4 hours at 105ºC in the oven, chilled in a desiccator, and weighed again. After weighing the sample and recording it, it was put back in the oven to continue drying. The procedure was repeated until a consistent weight was achieved. The moisture loss was calculated and expressed as a percentage of the sample weight examined based on the weight differential. % Moisture= $[(W_2 – W_3)/ (W_2 – W_1)]$ x100. Where W_1= weight of crucible. W_2= weight of crucible + weight of wet sample. W_3= weight of crucible + weight of dried sample.

Determination of Ash Content

The crucible with cover was dried for 4 hours in an oven at 105ºC. The crucible was then cooled in a desiccator and weighed soon after it had attained room temperature. A 5g homogenised sample was weighed into the crucible, and the sample was placed in a muffle furnace at 550ºC and left overnight. The sample was then removed and cooled in a desiccator soon after attaining room temperature. % Ash= $[(W_2 – W_3)/ W_2]$ x100. Where W_1= weight of crucible. W_2= weight of sample. W_3= Weight of crucible + ash.

Determination of Crude Fat

The extraction cup was dried in an oven at 105ºC for 6 hours, cooled in desiccators, and then weighed. Using gloves to avoid errors, 3g of the sample was accurately weighed and wrapped in a piece of filter paper before being placed into the extraction thimble. The opening of the thimble was loosely plugged with cotton. Using a volumetric cylinder, 150 ml of petroleum ether was measured and poured into the extraction cup. The extraction thimble was then inserted into the thimble holder and placed into the extraction cup. The extraction cup was attached to the Automated Soxhlet Fat Extractor, the desired program was selected, and the start button was pressed. % Fat = $[(W_3 – W_2)/ W_1]$ x100. Where W_1= weight of sample. W_2= weight of extraction beaker. W_3= weight of extraction beaker + fat.

Determination of Crude Fibre

The empty fibre bag was weighed, then 1g of the sample was added and reweighed. A glass spacer was placed in the bag, which was inserted into the carousel. For samples with more than 10% fat, defatting was done by immersing the carousel three times into 100 ml of 40/60 petroleum

ether, moving it up and down. The fibre bag was dried for 2 minutes. The carousel was placed into the axis carousel and inserted into a glass container on the hotplate, and the program method was started. After the analysis, the fibre bags were removed and placed into a crucible. The fibre bags and crucible were dried for 4 hours or overnight at 105ºC, then cooled in a desiccator for 30 minutes. The crucible and dried fibre bag were weighed, then placed in a furnace at 550ºC for 4 hours. Once cooled to room temperature in a desiccator, the crucible with ash was weighed. For the blank value, the empty crucible was weighed and then reweighed with the ash of the empty fibre bag. % Crude Fibre = $[(W_3 – W_1)/ (W_4 – W_5)] \times 100/ W_2$. Where W_1= weight of fibre bag. W_2= weight of sample. W_3= weight of crucible and fibre bag after digestion. W_4= weight of crucible and ash. W_5= weight of blank value of the empty fibre bag. W_6= weight of crucible. W_7= weight of crucible and ash of the empty fibre bag.

Determination of Crude Protein

1g of the sample was weighed into a digestion tube, and 2 catalyst tablets plus 15 ml of concentrated H_2SO_4 were added. The tube was shaken and placed in a digestion block set to 400ºC until the solution turned clear green/blue (60-90 minutes), then cooled for 10-20 minutes. For distillation, 25 ml of a receiver solution (2% boric acid with 5 drops of protein indicator) was added to a conical flask. The digestion tube was placed in the distillation unit, which added 70 ml of distilled water and 50 ml of 32% NaOH. The distillation took about 4 minutes, turning the receiver solution green. The distillate was titrated with 0.1 N hydrochloric acid until it turned pink/red, and the volumes of HCl used for the sample and blank were recorded. The N and protein content was calculated as N %= [A× (T−B) ×14.007×100]/[Weight of sample (g)×1000] and % Crude protein= % N×F . Where T= the volume of acid for the sample. B= the volume of acid for the blank. A= is the normality of HCl. F= the protein factor (6.38).

Determination of Carbohydrate

To determine carbohydrate content, the total sample was first weighed. The amounts of proteins, fats, moisture, and ash were then measured and subtracted from the total weight. The carbohydrate content was calculated by subtracting the sum of these components from the total weight, representing the percent remaining after measuring all other components. % Carbohydrates = [100 - % (Moisture – Ash – Crude Protein – Crude Fibre – Crude Fat)].

Statistical analysis

The programme Minitab was used. The average plus or minus standard deviation was shown for the data. The Tukey test was performed for pairwise comparisons at $P<0.05$, and one-way analysis of variance (ANOVA) was used to evaluate the study's data for mean differences among the various extracts.

Results and Discussion

Phytochemical screening

This study revealed the type of phytochemicals present in leaves, stems, and flowers of *Senna alata* found in Besut, Terengganu and they are summarized in Table 1. The result of qualitative

phytochemical screening shows the presence of alkaloid, phenol, quinone, tannin, saponin, and terpenoid in leaf of *S. alata.* For stems, this study found the presence of phenol, tannin, and saponin. This study shows the presence of phenol, tannin, and terpenoid in the flowers of *S. alata.*

Table 1. Phytochemical qualitative profile of *S. alata* leaf, stem and flower crude extracts

Bioactive compound	Leaf	Stem	Flower
Flavonoid	−	−	−
Alkaloid	+	−	−
Phenol	+	+	+
Quinone	+	−	−
Tannin	+	+	+
Saponin	+	+	−
Terpenoid	+	−	+

+ indicates presence and −indicates absence of phytochemicals in each part

GC-MS Analysis

The data presented in Table 2, Table 3, and Table 4 show the GC-MS analysis results of ethyl acetate extracts of *Senna alata* leaves, stems, and flowers obtained in Besut, Terengganu. Figure 1, Figure 2, and Figure 3 show the GC-MS chromatogram of ethyl acetate extract of different parts of *S. alata.*

Table 2. Bioactivity of phyto-components identified in the ethyl acetate extracts of the leaves of *S. alata*

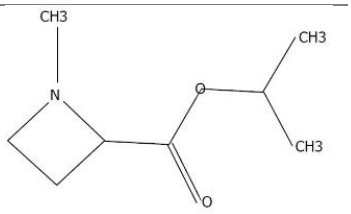
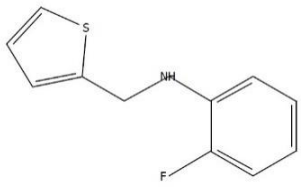
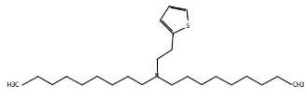
Name of compounds	Retention time (RT) (min)	Molecular formula	Molecular weight (MW)	Structure
N-Methyl-2-isopropoxycarbonylazetidine	2.112	$C_8H_{15}NO_2$	157	
Thiophen-2-methylamine, N-(2-fluorophenyl)-	39.338	$C_{11}H_{10}FNS$	207	
Ethylamine, N, N-dinonyl-2-(2-thiophenyl)-	39.522	$C_{24}H_{45}NS$	379	

Table 3. Bioactivity of phyto-components identified in the ethyl acetate extracts of the stems of *S. alata*

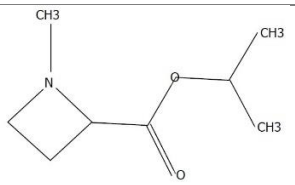
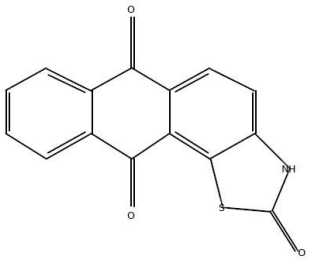
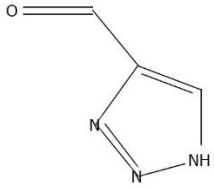
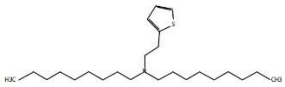
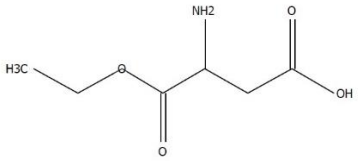
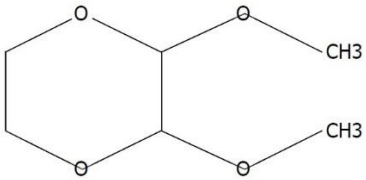
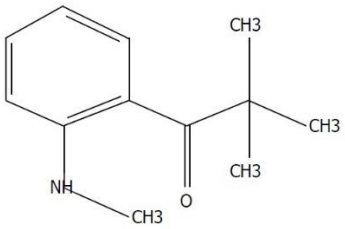
Name of compounds	Retention time (RT) (min)	Molecular formula	Molecular weight (MW)	Structure
N-Methyl-2-isopropoxycarbonylazetidine	1.984	$C_8H_{15}NO_2$	157	
1,2-Dihydroanthra[1,2-d] thiazole-2,6,11-trione	39.112	$C_{15}H_7NO_3S$	281	
1H-1,2,3-Triazole-4-carboxaldehyde	39.443	$C_3H_3N_3O$	97	
Ethylamine, N, N-dinonyl-2-(2 thiophenyl)-	39.574	$C_{24}H_{45}NS$	379	

Table 4. Bioactivity of phyto-components identified in the ethyl acetate extracts of the flowers of *S. alata*

Name of compounds	Retention time (RT) (min)	Molecular formula	Molecular weight (MW)	Structure
.alpha. -Ethyl aspartate	2.051	$C_6H_{11}NO_4$	161	
1,4-Dioxane, 2,3-dimethoxy-	2.081	$C_6H_{12}O_4$	148	
1-Propanone, 2,2-dimethyl-1-(2'-methylamino phenyl)-	39.835	$C_{12}H_{17}NO$	191	

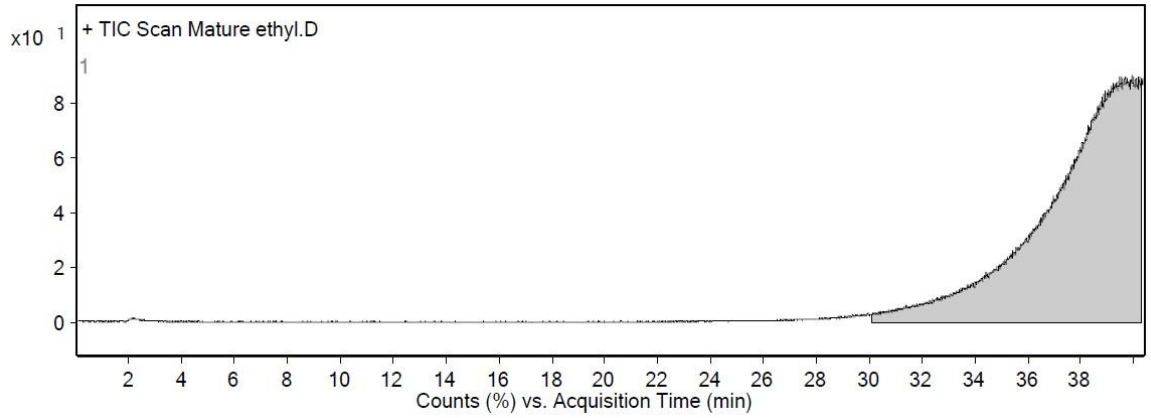


Figure 1. GC-MS chromatogram of ethyl acetate leaves extract of *Senna alata*

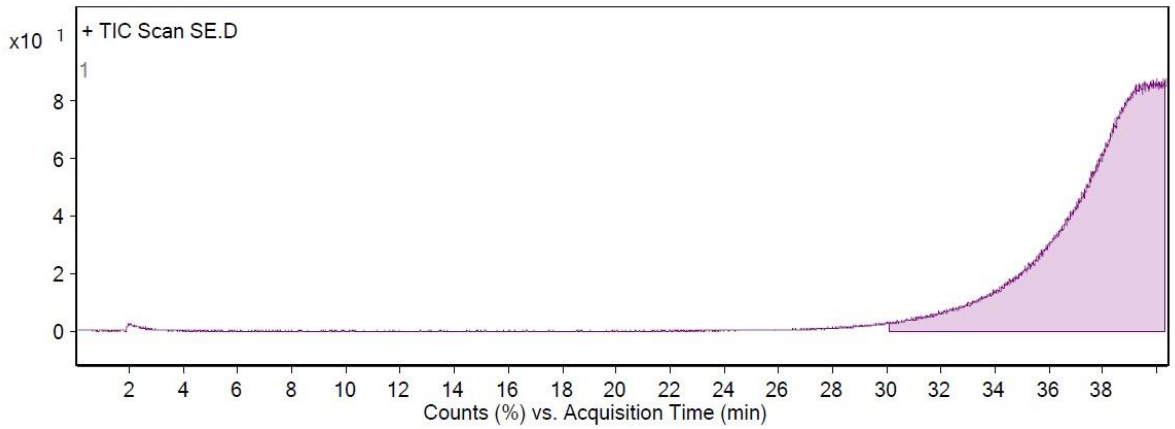


Figure 2. GC-MS chromatogram of ethyl acetate stems extract of *Senna alata*

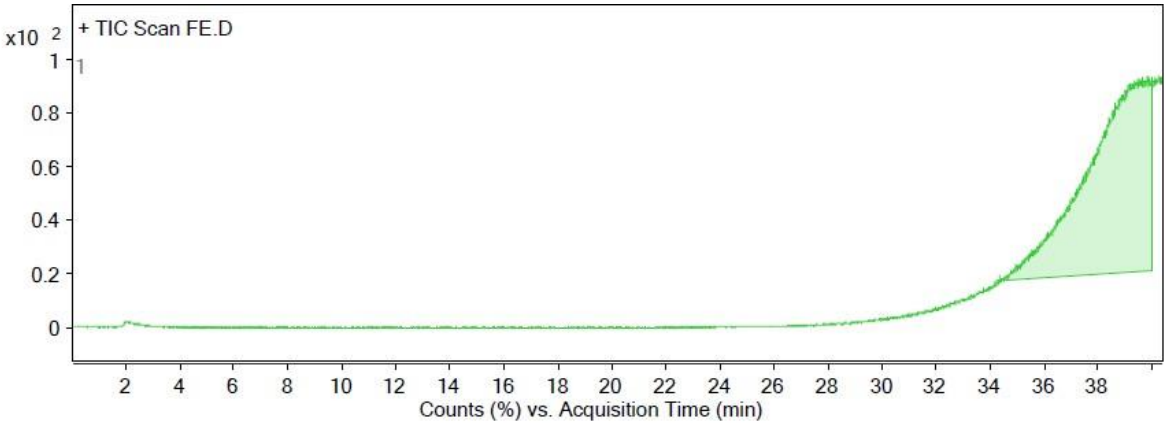


Figure 3. GC-MS chromatogram of ethyl acetate flowers extract of *Senna alata*

Proximate analysis

The data in Table 5 provides a detailed analysis of the nutritional composition of different parts of *Senna alata*, specifically its leaves, stems, and flowers. This analysis evaluates key parameters such as moisture content, ash content, crude fibre, crude protein, crude fat, and carbohydrate levels.

Table 5. Nutritional composition of different parts of *Senna alata*

Parameters	Leaves (%)	Stems (%)	Flowers (%)
Moisture	8.06±0.19^{c}	20.87±0.18^{b}	30.88±0.44^{a}
Ash	6.48±0.21^{b}	4.69±0.09^{c}	7.49±0.28^{a}
Crude Fibre	28.80±0.37^{c}	52.16±0.38^{a}	30.88±0.62^{b}
Crude Protein	19.38±0.18^{a}	5.84±0.44^{c}	8.13±0.08^{b}
Crude Fat	4.58±0.05^{a}	0.76±0.17^{c}	1.58±0.15^{b}
Carbohydrate	40.76±0.40^{b}	36.56±0.59^{c}	51.93±0.68^{a}

Note: Values are presented as mean ± SE

Moisture Analysis

Figure 4 shows the moisture content of the leaves, stems and flowers of *Senna alata*. Results show a significant difference in moisture content between the three parts. Flowers exhibiting the highest moisture content with 30.88%, stems at a moderate 20.87%, and leaves at the lowest level with 8.06%.

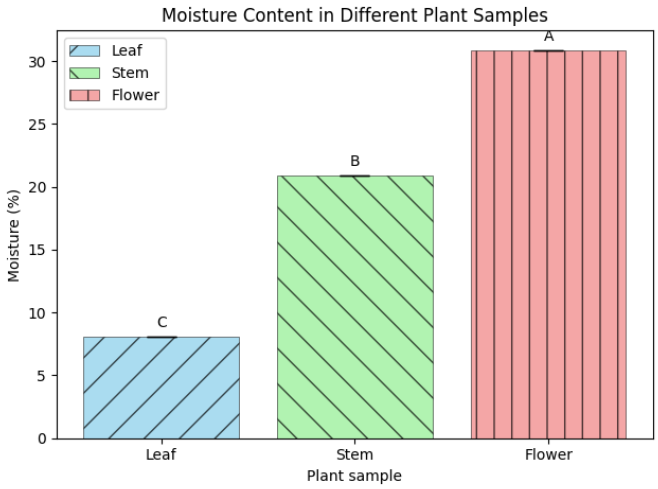


Figure 4. Moisture content in different parts of *Senna alata*

Ash Analysis

Figure 5 illustrates the ash content in the leaves, stems, and flowers of *Senna alata*. The results indicate a significant variation in ash content among these plant parts. The flowers exhibit the highest ash content at 7.49%, followed by the leaves with a moderate 6.48%, and the stems with the lowest level at 4.69%.

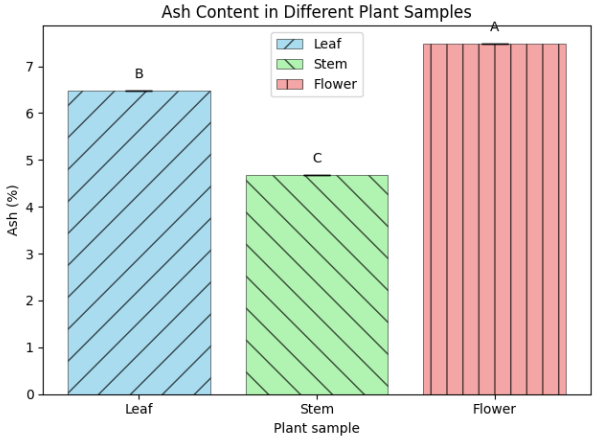


Figure 5. Ash content in different parts of *Senna alata*

Crude Fibre Analysis

Figure 6 illustrates the crude fibre content in the leaves, stems, and flowers of *Senna alata*. The results indicate a significant variation in crude fibre content among these plant parts. The stems exhibit the highest crude fibre content at 52.16%, followed by the flowers with a moderate 30.88%, and the leaves with the lowest level at 28.80%.

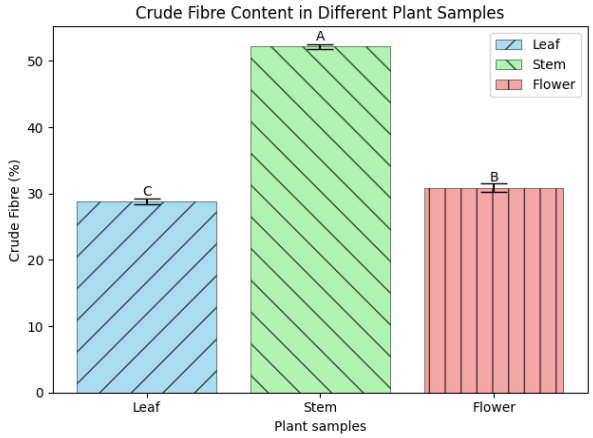


Figure 6. Crude fibre content in different parts of *Senna alata*

Crude Protein Analysis

Fig 7. illustrates the crude protein content in the leaves, stems, and flowers of *Senna alata*. The results indicate a significant variation in crude protein content among these plant parts. The leaves exhibit the highest crude protein content at 19.38%, followed by the flowers with a moderate 8.13%, and the stems with the lowest level at 5.84%.

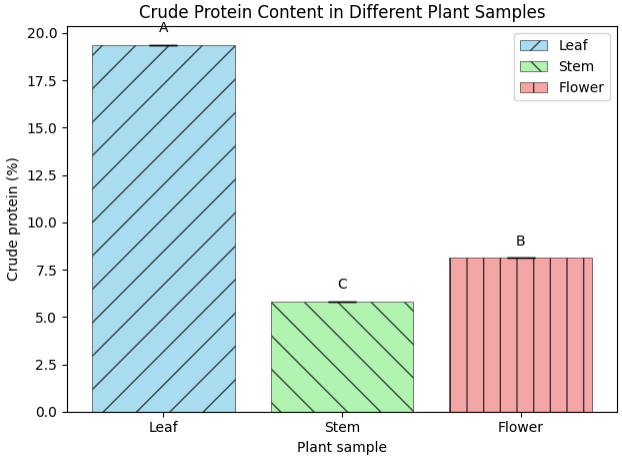


Figure 7. Crude protein content in different parts of *Senna alata*

Crude Fat Analysis

Figure 8 depicts the crude fat content in the leaves, stems, and flowers of *Senna alata*. The results indicate a significant variation in crude fat content among these plant parts. The leaves exhibit the highest crude fat content at 4.58%, followed by the flowers with a moderate 1.58%, and the stems with the lowest level at 0.76%.

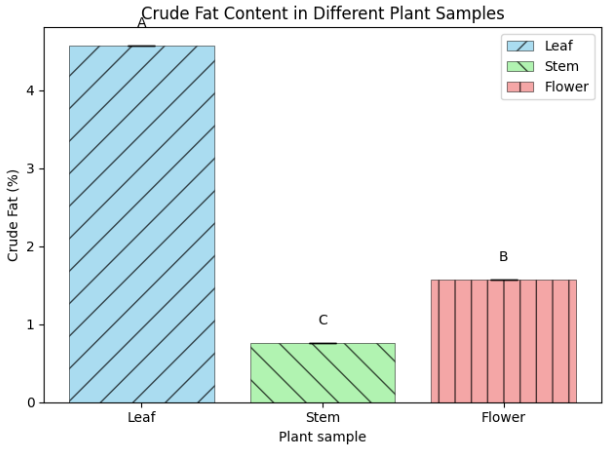


Figure 8. Crude fat content in different parts of *Senna alata*

Carbohydrate Analysis

Figure 9 depicts the carbohydrate content in the leaves, stems, and flowers of *Senna alata*. The results indicate a significant variation in carbohydrate content among these plant parts. The flowers exhibit the highest carbohydrate content at 51.93%, followed by the leaves with a moderate 40.76%, and the stems with the lowest level at 36.56%.

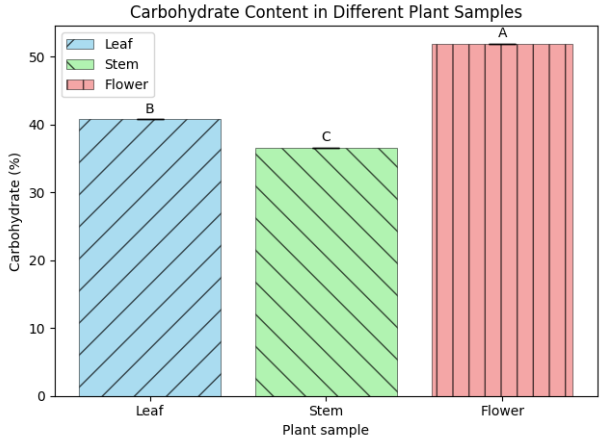


Figure 9. Carbohydrate content in different parts of *S. alata*

Phytochemical screening

The result of qualitative phytochemical screening shows the presence of alkaloid, phenol, quinone, tannin, saponin, and terpenoid in leaf of *Senna alata.* This result obtained are in accordance with the study by Saha et al. (2020) which showed that the bioactive compounds contained in leaves extract of *S. alata* were alkaloid, phenol, tannin, saponin, and terpenoid. However, flavonoid present in ethyl acetate leaves extracts were not detected in this study, contrary to the report by Saha et al. (2020). For stems, this study found the presence of phenol, tannin, and saponin which shows similarity to the study by James et al. (2022) that found the presence of tannin and saponin in *S. alata* stem extracts, although he reported to have also found alkaloid and flavonoid. This study shows the presence of phenol, tannin, and terpenoid in the flowers of *S. alata* that agreed with the findings reported in the study by Isah et al. (2015) and Suriya et al. (2023).

The findings in this study is not abnormal as a similar study by Ndukwe et al. (2020) in another circumstances did not detect the presence of some bioactive compound in *S. alata.* Emmanuel et al. (2023) explained that differences in detection of phytochemicals were caused by variations in geographical location due to concentration of soil mineral. There have been variations in the detection of bioactive compound detection in the plants belonging to the same species. These variations are likely a result of different methods used in the extraction of the plant biomolecular or secondary metabolites or might be due to differences in plants' bio-responsiveness to oestrogen levels.

In addition, the difference in the detection of bioactive compounds in three different parts of *S. alata* which are leaves, stems, and flower may be varies according to their respective biological functions. As described by Li et al. (2020), leaves are used as a synthetic and storage organ for secondary metabolites, while stem is used for transportation of compounds from roots to other parts of plant, hence do not store large amount of secondary metabolite and flower have an aromatic smell mainly composed of terpenes and aromatic compounds.

These bioactive compounds present in *S. alata* extracts of three different parts have been reported to exhibit a broad range of biological and pharmacological activities such as antimicrobial, antifungal, anticancer, and anti-inflammatory (Saha et al., 2020; James et al., 2022; Suriya et al., 2023). Alkaloid is responsible for the antimicrobial, anti-inflammatory, antioxidant, and anticancer activities (Oladeji et al., 2020). Phenol is reported to show anthelmintic, antioxidant, and antimicrobial activities (Karki et al., 2023; Oladeji et al., 2020). Quinone derivatives like anthraquinone is reported to exhibit acaricidal activities against mites (Jeon et al., 2012; Zhang et al., 2017). As described by Saha et al. (2020), tannins can exhibit cytotoxic and antitumor activities. A study by (Chew et al., 2022) described that terpenoid could promote to wound healing recovery.

GC-MS Analysis

N-Methyl-2-isopropoxycarbonylazetidine which is found in leaves and stems of *Senna alata* (RT = 2.112 and 1.984) is a derivative of azetidine. Azetidine is a four-membered saturated cyclic amines, and its derivatives are believed to have an extensive range of pharmacological properties such as antimicrobial activities against various bacterial and fungal species like *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae fungi, Aspergillus niger, Aspergillus flavus,* and *Candida albicans* (Shankar, 2023).

Thiophen-2-methylamine, N-(2-fluorophenyl)- (RT= 39.338) in leaves is a combination of a thiophene ring and a methylamine group, with a fluorine atom attached to the phenyl ring. Ethylamine, N, N-dinonyl-2-(2 thiophenyl)- in leaves and stems (RT= 39.522 and 39.574) is an organic molecule classified as an amine due to the presence of an ethylamine group. It contains long alkyl chains (nonyl groups) and a thiophene ring, making it part of the larger class of

heterocyclic compounds with sulphur. Similarly, both of the compound contain thiophene ring, which have antibacterial, analgesic and anti-inflammatory, antihypertensive, and anticancer properties when used in medicine (Shah & Verma, 2018). Another study by Zainal Abidin et al. (2024) stated that thiophene has been employed as anticancer, antimicrobial, anti-inflammatory, antidepressant, analgesic, and anticonvulsant agents.

The compound 1,2-dihydroanthra[1,2-d] thiazole-2,6,11-trione (RT= 39.112) in stem is structurally related to anthraquinone is reported to have acaricidal effect on mites (Konstantinova et al., 2013; Shang et al., 2018). According to Ripain & Ngah (2021), thiazole is a 5- membered ring moiety containing nitrogen and sulphur at position 1 and 3, and the derivatives were reported to demonstrate anti-inflammatory, antifungal, anticancer, antibacterial, anticonvulsant, antiviral, and antitumor activities.

1H-1,2,3-Triazole-4-carboxaldehyde (RT= 39.443) is a triazole derivative present in the stem of *S. alata* to be effective as antifungal and antibacterial (Gupta & Jain, 2015). Triazole is a five membered basic compound, containing two carbon and three nitrogen atoms having molecular formula $C_2H_3N_3$. According to Asif (2017), triazole derivatives possess wide variety of pharmacological activities such as antifungal, antibacterial, antiviral, anticancer, anticonvulsant, anti-inflammatory, antioxidant, anti-tubercular, anti-malarial, anti-nociceptive.

1-Propanone, 2,2-dimethyl-1-(2'-methylaminophenyl)- (RT= 39.835) that is found in the flower of *S. alata* is an organic compound that contains both a ketone functional group (propanone) and an amine functional group (methylamino). This compound belongs to the class of aromatic ketones due to the presence of a phenyl ring attached to the ketone group. According to Mete et al. (2014), compounds with aromatic ketone structures can exhibit various biological activities, including antimicrobial, antifungal, and anti-inflammatory properties. Mete et al. (2014) also stated that the compound methylamino has also been proven to exhibit cytotoxicity and anticancer activity.

Proximate analysis

Moisture Analysis

The findings of this study similar from those reported by Abdulwaliyu et al. (2013). The study reported that the moisture content of *Senna alata*'s flower is higher with 6.16 g/100 g than the leaves with only 4.49 g/100 g. Moreover, a study reported an even higher moisture content in *S. alata* flowers, at 8.49%, further supporting the variation observed in different studies (Adebayo et al., 2021). Different moisture levels can affect the concentration of bioactive compounds, which is critical for the plant's use in medicinal and nutritional applications. Flavonoids and phenolic acids in *S. alata* are key bioactive substances known for their antibacterial and antioxidant properties (Adelowo, 2017). Flavonoids and phenolic acids are water-loving compounds that easily interact with and hold onto water. This ability helps them retain moisture, as they can attract and absorb water vapour from the air (Dias et al., 2021).

Ash Analysis

A study by Adebayo et al. (2021) found similar ash content in the flowers of *Senna alata*, which contain a high ash content of 7.06%. Additionally, the ash content of *S. alata* leaves in this study aligns closely with findings from previous research by BM et al. (2017), which reported a total ash content of 6.00% in the leaves, reflecting their mineral content. The high value of total ash content in a sample indicates the presence of minerals, as highlighted by Angelina et al. (2021). Flowers

have been found to contain higher total ash and a greater concentration of most minerals, except for magnesium (Młynarczyk et al., 2020).

According to Oladeji et al. (2020), *S. alata* flowers are rich in various bioactive compounds such as flavonoids, phenolic compounds, anthraquinones, tannins, and steroids. These bioactive compounds, including flavonoids, phenolic acids, and anthocyanins, can interact with inorganic minerals like calcium, phosphorus, potassium, and magnesium. For instance, flavonoids can enhance the absorption of minerals such as calcium and iron, while anthocyanins can interact with potassium and magnesium to boost their antioxidant properties. Consequently, these interactions significantly impact the ash content of the flowers (Młynarczyk et al., 2020). The ash content can also vary based on factors such as soil type, climate, and the distribution of minerals within the plant (Gołąb-Bogacz et al., 2021).

Crude Fibre analysis

A study reported that the crude fibre content of 41.36% in the stem bark of *Cassia nigricans* was significantly higher than leaves with 20.64% (Ayo, 2013). This is expected as stems typically have more structural components like cellulose, hemicellulose, and lignin, which contribute to higher fibre content (Núñez-Gómez et al., 2023). High crude fibre content in stems can have significant implications for their use in biopesticides (Gupta et al., 2023). The rigidity from cellulose and lignin can make extracting bioactive compounds more difficult, potentially reducing yield and potency. However, the high fibre content also helps with the stability and slow release of these compounds, improving the biopesticide's effectiveness and duration (Kumar et al., 2021). This can be particularly useful for biopesticides that have a short duration of action or poor stability, as the fibres can help to extend their effectiveness and improve their overall performance (Hazafa et al., 2022).

Crude Protein Analysis

The leaves of *S. alata* possess the highest crude protein content among its various parts. This finding aligns with other study which reported that the crude protein content in the leaves was significantly higher at 18.89%, compared to 14.22% in the stems (James et al., 2022). Similarly, Abdulwaliyu et al. (2013) found high levels of crude protein content in the leaves of *Senna alata* with 18.23 g/100 g of crude protein, whereas the stems had only 13.14 g/100 g. The leaves of *S. alata* are known for their rich protein and amino acid content, which directly enhances the plant's overall crude protein levels (Oladeji et al., 2020). Reflecting these findings, there is also a study that confirmed the leaves are abundant in crude protein, reporting a content of 17.50% (BM et al., 2017). Additionally, the presence of other bioactive compounds such as tannins, alkaloids, flavonoids, and saponins can also influence the crude protein content through their interactions with other nitrogenous compounds (Adelowo, 2017). Tannins can precipitate proteins by forming insoluble complexes, a process influenced by factors such as solution concentration, chemical structure, and pH (Adamczyk et al., 2017). The ability to form multiple hydrogen bonds facilitates the formation of tannin complexes with these nitrogenous compounds.

Crude Fat Analysis

Abdulwaliyu et al. (2013) found that *Senna alata* leaves had a significantly higher crude fat yield at 3.91 g/100 g compared to the flowers, which contained only 1.81 g/100 g. Subsequent research by Clement et al. (2021) supports these results, revealing that leaves exhibit the highest crude fat content at 9.67% while the flowers at the lowest level with 1.99%. The abundant crude fat in the

leaves highlights their richness in bioactive compounds, particularly valuable for potent biopesticide formulations. Previous study also reported that the leaves of *S. alata* contain higher crude fat content with 9.36% and stems with only 5.46% (James et al., 2022). The crude fat content can affect the composition and concentration of fatty acids in *S. alata*, such as saturated, monounsaturated, and polyunsaturated fatty acids (Rahmawati et al., 2022). *Senna alata* may contain plant sterols or phytosterols, and the crude fat content can influence their presence and concentration (Oladeji et al., 2020).

Carbohydrate Analysis

According to a previous study by Abdulwaliyu et al. (2013), the carbohydrate content was found to be higher in the *Senna alata*'s flowers at 57.04 g/100 g compared to the leaves, with only 47.73 g/100 g. This suggests that flowers of *S. alata* may serve as a richer source of carbohydrates compared to the leaves, highlighting potential differences in nutrient composition across different parts of the plant. Another study highlights the nutritional composition of *S. alata* flowers, revealing a substantial carbohydrate content of 45.630%, thereby underscoring the high carbohydrate presence in the flowers and their potential as a significant energy source (Adebayo et al., 2021). This finding emphasizes the importance of *S. alata* flowers in dietary applications, given their notable carbohydrate levels. Higher carbohydrate availability in *S. alata* flowers can increase the production of flavonoids such as quercetin and kaempferol derivatives (Kumar and Pandey, 2013).

Conclusion

In conclusion, this study indicates that *Senna alata* shows the presence of bioactive compounds such as alkaloid, phenol, tannin, saponin, quinone, and terpenoid that possess the biological and pharmacological activities. Leaves have the most presence of bioactive compounds based on the phytochemical screening test. The results from GC-MS analysis shows the presence of azetidine, thiophene, thiazole, and triazole derivatives can contribute to the pharmacological properties of *S. alata* such as acaricidal, antioxidant, anti-inflammatory, antibacterial, and antifungal activities. *Senna alata* has historically been utilized for the treatment of different ailments in human; nevertheless, its application in veterinary medicine remains little explored.

Additional research is required to validate the efficacy and assess the safety of *S. alata* as biopesticide in managing rabbit ear mites and other ectoparasites in other animals. Additional clinical trials are necessary to determine whether *S. alata* possesses therapeutically advantageous qualities for the treatment of particular conditions. The dosage, mode of administration, and adverse effects must be thoroughly assessed. Research focused on the mechanisms of bioactive substances is also required. Investigations into the domestication and sustainable management of *S. alata* for extensive cultivation must be conducted to evaluate its potential as a suitable substitute for synthetic pesticides.

Acknowledgments

The authors would like to offer particular thanks to the Universiti Sultan Zainal Abidin (UniSZA) for the facilities provided for this study. This project is supported by Universiti Sultan Zainal Abidin (UniSZA) under Dana Penyelidikan Universiti 1.0 (UniSZA/20222/DPU1.0/05).

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How to cite this paper:
Nik Mahalim, N.N.S.I., Mohd Yuseri, I.A., Md Yusof, N.A. (2024). Phytochemical and Proximate Composition of Senna alata (L.) Roxb.: Nutritional and Medicinal Insights from Different Plant Parts in Besut, Terengganu. *Malaysian Journal of Applied Sciences,* 9*(*2), 16-35.