

Influence of extraction procedure and sample preparation on the bioactive compositions observed from the extract of the of *Solanum melongena* and *Pandan amaryllifolius*

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ABSTRACT

Background: Successful extraction of bioactive compounds from plant material depends largely on the type of solvent used. However, extraction procedure and sample preparation are often overlooked. The influence of drying and extraction methods on yield and chemical composition of *Solanum melongena* and *Pandan amaryllifolius* are yet to be investigated. **Objective:** This study was undertaken to evaluate the effects of substantial variations in the extraction techniques and solvents on observed bioactive compounds of *Solanum melongena* L and *Pandan amaryllifolius* extracts. **Methods:** Three different methods of extraction (Extraction by Soxhlet Extractor, extraction by soaking pulverized plant material and extraction following drying of the plant in liquid nitrogen) and three different solvents were employed to study. The influence of sample preparation on the bioactive compounds of *Solanum melongena* and *Pandan amaryllifolius*. **Results:** Methanol was observed to yield significantly higher ($P < 0.05$) dry weight of crude extract as well as the percentage of yield compared to chloroform and ethanol for *S. melongena* while chloroform was observed to yield significantly higher crude extract and percentage of yields for *Pandan amaryllifolius*. Similarly, high content of the bioactive compounds of *Solanum melongena* and *Pandan amaryllifolius* were detected with methanol compared with chloroform and ethanol in terms of dry weight and percentage yield. **Conclusion:** The interaction ($P > 0.05$) occurred between type of solvent and extraction of methods for both plant in terms of influencing the yield of crude extract. However, based on the results of the phytochemical screening and UV – vis spectral, the second methods appeared to be better method. It is therefore recommended that it be taken into consideration in preparing for crude extracts from *S. melongena* and *Pandan amaryllifolius* leave extract.

KEYWORDS: Chloroform, Ethanol, Extraction method, Methanol, Soxhlet Extractor, Yield

INTRODUCTION

Eggplants (*Solanum melongena* L.) are the second most vital solanaceous fruit crop after tomato. Both eggplants and tomato belong to the large and species-rich genus *Solanum* L. (Solanaceae), as well as potato (*S. tuberosum* L.). *Solanum* is among the 10 most species-rich genera of flowering plants [13]. About 1400 species from this genus are found in all continents except Antarctica, in a wide variety of habitats ranging from deserts to mountain slopes high above treeline. The common or brinjal eggplant (*Solanum melongena* L.) is a member

of the Leptostemonum Clade (commonly known as “spiny” solanums). Unlike several other genus, the eggplant and its relatives belong to the Old World and most eggplant’s wild relatives are from Africa [17]. The three cultivated eggplants are the gboma eggplant *Solanum macrocarpon* L. and the scarlet eggplant, all of Old World origin. *S. aethiopicum* L. are principally grown locally in Africa but are also cultivated in other regions as minor crops while the brinjal or common eggplant, *S. melongena* L. is cultivated worldwide [9,30].

Solanum melongena Linn. is described as a herbaceous plant, whose leaves are coarsely lobed (Figure 1), with white to purple flowers and berry fruit and are cultivated primarily for food and medicinal purposes. The plant contains glycoalkaloids, flavonoids, tropane, lanosterol, arginine, gramisterol, aspartic acid as vital constituents. The plant has been reported possess analgesic, antioxidant, anti-inflammatory, hypotensive, antiasthmatic, hypolipidemic, antipyretic, antiplatelet, intraocular pressure reducing, central nervous system (CNS) depressant and anaphylactic reaction inhibitory activities [10]. Sample preparation of herbs for crude extract is often overlooked and is frequently considered as just a process of obtaining the crude extracts, ignoring the possibility of substantial influence of the extraction techniques and the solvents on the bioactive compounds [19].



Fig. 1: *Solanum melongena* L.

On the other hand, *Pandanus amaryllifolius* is a member of the family Pandanaceae consisting of a group of plants generally referred to as „screw pines. Pandan leaf (*Pandanus amaryllifolius* Roxb.) is a tropical plant of the *Pandanus* genus. The plant is an erect green plant with fan-shaped sprays of long, narrow, bladelike leaves and woody aerial roots which are about 4 inches (10 cm) long. It is a source of natural flavoring that is widely used in various parts of Asia including Malaysia, Thailand, India and Indonesia. The leaves of this plant are used medicinally in South East Asia for refreshing the body, reducing fever, and relieving indigestion and flatulence scented [20,29]. The leaf of the plant which is often referred to as pandan, is commonly used to add a refreshing, fragrant flavour to south-east Asian dishes [6].



Fig. 2: *Pandanus amaryllifolius* L.

Even though *Solanum melongena* and *Pandanus amaryllifolius* are widespread in several countries and their biochemical compounds are known, the influence of drying and extraction methods on yield and chemical composition of these herbal plants is yet to be investigated. There is an increasing interest in the bioactive compounds of these plants which could be associated with their nutritional incidence and their role in health and disease. In view of their used and widely perceived medicinal value, the need to investigate the influence of drying and extraction methods on their biochemical composition is thus imperative. Hence, this study aimed at investigation the influence of drying and different extraction methods on the crude extract yield and biochemical composition of *Solanum melongena* and *Pandanus amaryllifolius*.

MATERIALS AND METHODS

2.1 Collection of plant samples:

Fresh plant samples (*Solanum melongena* and *Pandan amaryllifolius*) that were used in this study were collected from January to June 2015 around Kampung Sungai Siput, Lubok China Malacca, Malaysia (2.442324, 102.108539). The plant material was identified and authenticated by a botanist of Department of Science and Technology at University Kebangsaan of Malaysia (UKM). A voucher specimen has been deposited at UKM herbarium with the code number of (40308 & 40309) (UKMB).

The plant was then thoroughly washed with tap water and subjected to three different drying methods namely freeze drying, shade drying and oven drying. In freeze drying, leaves of the plant were dried using liquid nitrogen [1,8]. In shade drying, the plant was kept in a shade for a period of 9-24 days at room temperature (28-38 °C) [23]. For oven drying, the plant material were placed in an oven tray and heated at 30°C overnight as described earlier [22]. The dried plant materials were mechanically ground into fine powder using a commercial electric stainless steel table grinder. The finely powdered plant materials were kept in air tight containers for further analysis.

2.2 Extraction of plant materials:

2.2.1 Extraction by Soxhlet Extractor (SE) (First Method):

In the first method (1st), extraction was performed using "Soxhlet Extractor" [23]. A total of 500 g of the powdered plant material was measured and placed in a paper thimble and then placed in the extractor. Subsequently, 700 mL Ethanol, Methanol and Chloroform were added via the receiving flask, using a sequence of solvents of increasing polarity (Methanol, Chloroform and Ethanol) (boiling point range 60-80 °C). The process of extraction lasted for about 10 h. The extracts were then filtered over Whatman No. 1 filter paper. The crude extracts were obtained following complete removal of the solvents, using a rotary evaporator at temperature of 40 °C. The concentrated extracts were transferred to glass petri dishes and dried in a drying oven at 20 °C and the clear residue was used for the study.

2.2.2 Extraction by soaking (ESS) (shade dryer sample) (Second Method):

In the second method (2nd), extraction was performed by soaking method, according to the methods described by [26]. A total of 500 g of pulverized plants were soaked in 400 mL of each solvent separately in 1000 mL flask for 24 hours. The mixture was vigorously shaken for 3 min. at 3 hours intervals to ensure even soaking of the powder. After 24 hours of soaking, the extract was decanted and the liquid extract was filtered using Whatman No. 1 filter paper, and the plant were soaked again in 200 mL of each solvent for another 24 hours. Following filtration, the liquid extracts were combined. The extracts were soaked again for another 24 hours in 100 mL of solvents. After filtering with filter paper, the last liquid extracts were combined with the previous extracts. Crude extract was obtained after complete removal of the solvents with vacuum evaporation at temperature > 40 °C.

2.2.3 Extraction by soaking (ESF) (freeze-dried samples) (Third Method):

In this procedure, plants were dried in the liquid nitrogen and extracted by soaking method. A total of 500 g of powdered plants in 400 ml of each solvent were placed in 1000 ml flask for 24 hours. The mixture was shaken for 3 hours. After 24 hours the extract was filtered and the maceration was re-extracted by the same process until plant materials were exhausted. The extracts were freeze-dried with Savant Refrigerated Vapor Trap after removal of the solvents with vacuum rotary evaporator at 40 °C. Each extracts were kept in freeze until used.

Each extraction procedure was performed in Triplicate under the same operating conditions and the extract was subjected to preliminary phytochemical tests (figure 3).

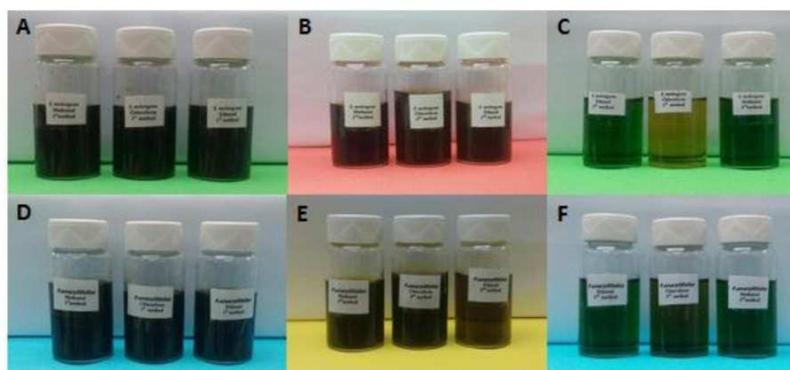


Fig. 3: Crude extract of *Solanum melongena* (above) in three type extraction of method, (A) 1st method (ES), (B) 2nd method (ESS) and (C) 3rd method (ESF) by different solvents, and (bottom) Crude extract of *Pandan amaryllifolius* in three type extraction of method (D) 1st method, (E) 2nd method and (F) 3rd method by different solvent

2.3 Determination of extract yield:

The amount of crud extract contained in a known weight of fine plant powder was calculated following [2]. The percentage yield of the extract was determined gravimetrically using the dry weight of extract (x) and soaked samples material (y) as follows:

$$\text{Percentage yield} = x/y \times 100$$

Qualitative Screening of Bioactive Compounds in plants used in the study:

2.4.1 Phytochemicals Screening of Plants extracts:

The total crude extractor that obtained from the plants were subjected to phytochemical analysis. Phytochemical screening of active plant extracts were performed according to the standard method for the qualitative analysis of various Phytochemicals such as alkaloids, saponins, flavonoids, tannins, steroids and Terpenoids [21,14,5,16].

Flavonoids Test:

Few pieces of magnesium metals were added to extract solution and concentrated hydrochloric acid was carefully added. The formation of orange or crimson colour indicates presence of flavonoids.

Terpenoids Test:

A total of 500 mg of extract was dissolved within 5 ml of chloroform and filtered. Ten drops of acetic anhydride were added to the filtrate followed by two drops of concentrated acid. Presence of pink colour at the interphase was an indication of the presence of Terpenoids.

Saponin Test:

A total of 500 mg of the extract was dissolved within 5 ml of distilled water and filtered. Persistent frothing observed when the filtrate was shaken vigorously indicates the presence of Saponins.

Tannins Test:

A total of 500 mg of the extract was stirred with 10 mL of distilled water and filtered. The diluted extract was then added with 5% ferric chloride reagent and filtrated. A Blue-black precipitate indicates the presence of tannin.

Alkaloids Test:

A total of 500 mg of the extract were taken and dissolved in dilute hydrochloric acid and filtered. The filtrate was then treated with iodine in potassium iodide solution until brown/red colour was observed.

Glycosides Test:

A total of 500 mg of the extract was dissolved in 2 mL of chloroform. Concentrated Sulphuric acid was carefully added to form a lower layer. A reddish-brown coloration at the interphase indicates the presence of a steroidal ring of glycoside.

2.4.2 UV- visible Spectra Analysis:

Qualitative determinations of the main constituents of plants extract was monitored by measuring the UV-Vis spectrum of the reaction medium. The UV-Vis spectral analysis of the sample was carried out using a Shimadzu UV-2450PC Series UV-Vis spectrophotometer at room temperature (Figure 3.2) and operated at a resolution of 1nm between the range of 200 and 900 nm. We used it to see if the components of the plants extract (prepared on the same day), extracted using different solvents by using of a double beam UV-Vis spectrophotometer (UV-2450PC S., from Electronics, India Ltd) with the wave length range of 200–900 nm, at room temperature operated at a resolution of 1nm and a resolution of 0.5 nm for 15 min, three times.

In particular, the maximum value is calculated within a moving window of width (m/z) /R Pest for each m/z value of the representative spectrum; the set of these maxima forms a “maximal curve”. A local maximum of the maximal curve is defined as the central point of a region starting where the derivative passes from a positive value to zero and ending where the derivative passes from zero to a negative value. The spectrophotometer gives us the wavelengths absorbed by the extracts and different compounds absorb UV and visible light differently. We used these readings as a qualitative basis of comparison for the extracts. Then taking out the solution and running a spectrophotometry test on the cuvette to compare the wavelengths absorbed by the residue with those absorbed by the soaking solution. Both absorption wavelength and peak width increases as the particle size increase.

Statistical Analysis:

Data were subjected to normality test using MiniTab software. Percentage yields of crude were subjected to analysis of variance (ANOVA 2-way; methods and solvents as independent variables). If ANOVA result is significant the treatment means were separated using Tukey's test at $P < 0.05$) and analysis were run on MiniTab software version 14.

RESULTS AND DISCUSSION**3.1 Dry weight and yield of *Solanum melongena* crude extract using different methods and solvents:**

The extraction was performed to separate the biologically active portions of plant organic chemicals using selective solvents through standard procedures as described earlier. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity [33]. In this study we found that the mean dry weights of *S. melongena* crude extracts and the percentage yield using different solvents in the 1st, 2nd and 3rd methods is shown in Table 1. When methanol was used as the extraction solvent, the dry weight of *S. melongena* were 26.12g, 12.81g and 21.99 g for the 1st, 2nd and 3rd methods of extraction, respectively. The yield on the other hand was 5.22%, 2.56 % and 4.40%, respectively. This result shows that higher dry weight of the crude extract as well as the percentage yield were obtained on the 1st method of extraction as compared to the 2nd and 3rd methods of extraction using methanol as solvent. Statistical analysis showed significant difference among the solvent in three different methods of extraction, in the 1st methods ($F = 16.25$, $df = 2$, $P < 0.05$), 2nd method ($F = 8.06$, $df = 2$, $P < 0.05$), and 3rd ($F = 114.91$, $df = 2$, $P < 0.05$). This finding is in accordance with previous report [33] who reported high yield of extract with methanol used solvent compared to other solvents, irrespective of methods of extraction employed.

Table 1: Mean Dry weight (+ SE) crude extract of *Solanum melongena* crude extract using different extraction methods and solvents

Solvent used	Dry weight of crude extract (g)		
Ist method	2nd method	3rd method	
Chloroform	17.07 ± 1.94a	8.89 ± 0.17a	10.03 ± 0.47a
Ethanol	22.77 ± 0.26b	10.46 ± 0.28b	19.03 ± 0.12b
Methanol	26.12 ± 0.21c	12.81 ± 1.15c	21.99 ± 0.89c

*Ist method: Extraction by Soxhlet Extractor, 2nd method: extraction by soaking (shade dryer sample) and 3rd method: Extraction by soaking (Nitrogen dryer sample)

**Means ± standard error with different solvents in the same Extraction of methods are significantly different ($P < .05$) from each other

However, when chloroform was employed as the extraction solvent, the dry weight of the crude extract were 17.07g, 8.89g and 10.03 g for the 1st, 2nd and 3rd methods, respectively. The yields were 3.41%, 1.78% and 2.01 % respectively. The 1st method of extraction still appeared to give more dry weight of the crude extracts compared to the 2nd and 3rd extraction methods. For ethanol, in the 1st methods, the dry weight of the crude extract was 22.77g, 10.46g and 19.03g for the 3 methods respectively. The percentage yields were 4.56%, 2.09% and 3.81% respectively. The 1st method of extraction proves to be a better one as it yielded both higher dry crude extract and percentage yield even though the yield is relatively less than the yield obtained from methanol. This result is in accord with earlier findings by Yi and Wetzstein [32] who equally observed that ethanol yields significantly less crude extract than methanol using some selected herbs. However, comparing the 3 solvents used in this study, methanol was observed to be a better solvent for the extraction of *S. melongena* extracts.

3.2 Dry weight and yield of *Pandanus amaryllifolius* leaves crude extract using different methods and solvents:

The mean dry weights of *P. amaryllifolius* leaves crude extracts and the percentage yield in methanol, chloroform and ethanol solvents in both 1st, 2nd and 3rd methods is shown in (Table 2). The dry weight of *P. amaryllifolius* leaves in methanol in the 1st, 2nd and 3rd methods of extraction were 25.32, 16.45 and 9.83 g respectively. It was observed that methanol in the 1st method of extraction yielded the highest dry weight compared to methanol in the 2nd and 3rd methods of extraction. The heat dry weight in methanolic extract of *P. amaryllifolius* was found in the 3rd method of extraction and this was significantly than both the 1st and the 2nd methods. The percentage yield for both the 1st, 2nd and 3rd method in methanol were 5.06%, 3.29% and 1.97 percent respectively.

Table 2: Mean Dry weight (+ SE) crude extract of *pandanus amaryllifolius* using different extraction methods and solvents

Solvent used	Dry weight of crude extract (g)		
Ist method	2nd method	3rd method	
Chloroform	53.90 ± 2.09a	16.34 ± 1.06a	21.15 ± 1.30a
Ethanol	11.50 ± 0.60b	5.81 ± 0.23b	13.95 ± 1.40b
Methanol	25.32 ± 2.21c	16.45 ± 0.56c	9.83 ± 0.49c

Ist method: Extraction by Soxhlet Extractor, 2nd method: extraction by soaking(shade dryer sample) and 3rd method: Extraction by soaking(Nitrogen dryer sample)

**Means ± standard error with different solvents in the same Extraction of methods are significantly different ($P < .05$) from each other

Unlike in the extraction of *P. melongena*, Chloroform solvent produced significantly higher dry weight compared to methanol especially in the 1st and 2nd methods, where the yields were as high as 50.1g and 16.9g respectively. The percentage yield were also significantly higher than those from methanol in both the 1st and 3rd methods. This result has demonstrated that dry weight of *P. amaryllifolius* crude extract as well as the percentage yield were higher when chloroform was used in the 1st method of extraction as against the 2nd and the 3rd methods of extraction using chloroform. Ethanol on the other hand yielded the lowest both in dry weight and the percentage yield in all the three extraction methods as against the yields in methanol and chloroform. The yield of *P. amaryllifolius* was also significantly affected by the solvent in three different methods of extraction, in the 1st methods ($F = 145.99$, $df = 2$, $P < 0.05$), 2nd method ($F = 74.33$, $df = 2$, $P < 0.05$), and 3rd ($F = 12.96$, $df = 2$, $P < 0.05$).

3.3 Yield of crude extract of *S. melongena* leave plant in the three different types of solvent:

The interactions between type of solvent and extraction methods effect on the yield of crude extract of *S. melongena* plant ($F = 7.320$; $df = 4$ & 18 ; $P = 0.001$; Table 3) was significant. The yield of crude extract of *S. melongena* leave in the three different solvents is shown in figure 4. The use of methanol in the 1st method of extraction yielded the highest extract (5.22%) of *S. melongena* followed by methanol in the 3rd method of extraction which recorded a value of 4.40%. The least extract yields for methanol solvent was obtained from the 2nd method where the extract was as low as 2.56% and this was significantly than the extract obtained from the 1st and the 3rd methods. This findings agrees with a report in a previous related study by Das et al., [11] where methanol, chloroform and petroleum ether were used in *S. melongena* crude extraction.

Table 3: Analysis of Variance for *Solanum melongena* yields% by different methods of Extract obtained by different solvents

Source of variation	DF	Mean Square	F-value	P-value
Method	2	11.48	136.315	($P < .05$)
Solvent	2	6.40	76.051	($P < .05$)
Method* solvent	4	0.62	7.320	($P < .05$)
Error	18		0.08	
Total		26		

**Different letters in yield shows significant difference

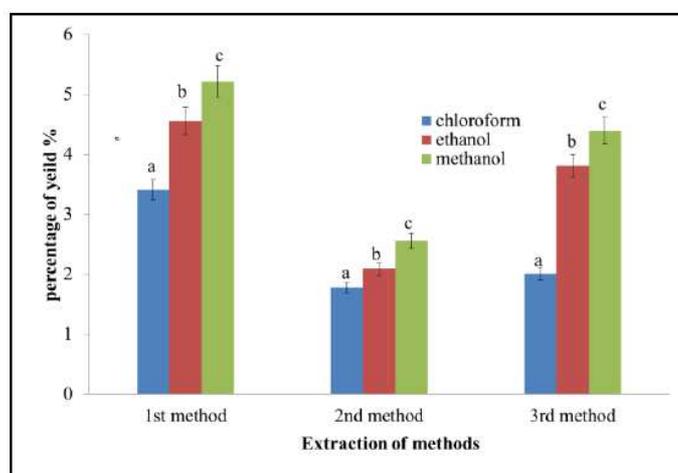


Fig. 4: Yield of crud extract of *Solanum melongena* leaves plant in Type of Extraction of Methods by different solvents

Ethanol as a solvent yielded crude extracts slightly lower than that of methanol in both the 1st and 2nd methods of extraction where the values were 4.56% and 3.81% for 1st and 3rd methods respectively. The chloroform solvent yielded the least crude extract in all the three methods of extraction with the values of 4.56%, 2.09% and 3.81% respectively and these findings is also in accord with the findings of Das et al., [11]. Generally, the 1st method of extraction appears to be the best method for *S. melongena* extraction followed by the 3rd method of extraction. The 2nd method of extraction was the least based on the results obtained in this study.

3.4 Yield of crud extract of *Pandanus amaryllifolius* leaves plant in Type of Extraction of methods by different solvents:

The percentage yield of crude extract of *Pandanus amaryllifolius* leave in the three different solvents is depicted in figure 5. Unlike in the case of *S. melongena*, Chloroform solvent produced the highest *Pandanus*

amaryllifolius crude extract in both the 1st and the 3rd methods of extraction. The interactions between type of solvent and extraction of methods effect on yield of crude extract of *Pandanus amaryllifolius* plant ($F = 59.359$; $df = 4 \& 18$; $P = 0.000$; Table 4) was significant. However, In the 1st methods, Chloroform yielded 10.78% while yielding 4.05% in the 3rd method. This is followed by methanol which yielded significantly higher percentage in both the 1st and the 2nd methods (5.06% and 3.29% respectively) compared to ethanol. However, in the 3rd method of extraction, here was no significant difference between methanolic and ethanolic extracts of *Pandanus amaryllifolius* leave. The generality of the results showed that the chloroform of extraction was better than the methanol and the ethanol solvent especially in the 1st and 3rd method of extraction, Our results disagree with the study by Al-Alwani [4]. that suggested is the ethanol is the best solvent of extraction compare with the six type of solvents including chloroform. Ngadi and Yahya [24] revealed that the ethanol was the best solvent to extract 2AP from Pandan leaves comparison to methanol as higher 2AP peak arises from ethanol chromatogram. However there is no 2 AP detected when propanol was used as solvent.

Table 4: Analysis of Variance for *Pandanus amaryllifolius* yields% by different methods of Extract obtained by different solvents

Source of variation	DF	Mean Square	F	P
Method	2	32.83	140.947	($P < .05$)
Solvent	2	36.23	155.514	($P < .05$)
Method* solvent	4	13.83	59.359	($P < .05$)
Error	18		0.23	
Total		26		

**Different letters in yield shows significant difference

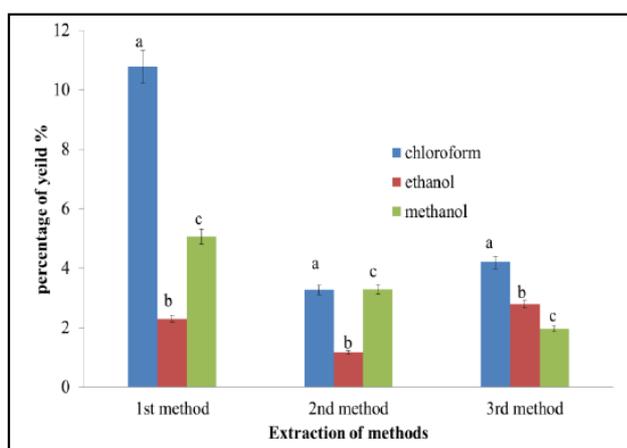


Fig. 5: Yield of crud extract of *Pandanus amaryllifolius* leaves plant in Type of Extraction of Methods by different solvent

3.5 Qualitative Phytochemical Screening of *Solanum melongena* extract:

The qualitative phytochemical screening of *S. melongena* for the 3 solvent used in this study is shown in table 5. Six bioactive compounds of *S. melongena* were tested on the 3 extracts obtained from methanol, chloroform and ethanol. For methanol, In the 1st method, all the bioactive compounds observed were present in varying amount from high to low quantities, with Saponins having the highest quantification score of 3 but with no terpenoids detected 10 which was only observed in the 2nd methods in which methanol was the solvent with glycosides having the highest quantification score of 3 but with no saponins. Whereas, In 3rd method only 3 bioactive compounds out of six were present and these were (saponins, tannins and alkaloids). This result of ours agrees with earlier studies by Yi and Wetzstein [32]; Yin et al. [33] where methanol was reported to produce the highest crude extract yield and it proof by the detection of the presence of bioactive compounds such as flavonoids, terpenoids, saponins, tannins and alkaloids. While, the acetone was the best solvent for the extraction of total phenolics, flavonoids and tannins from *S. melongena* Comparison with metanol and ethanol [7]. In addition, Eddy et al. [12] suggested that the leaves of *S. melongena* is richer in saponin, terpenes, tannins, flavonoid, phlobatanins, anthraquinones, cardiac glycoside and alkaloid when he ethanol was as solvent.

Table 5: Qualitative phytochemical Screening of *Solanum melongena* extract

Bioactive Compounds	Methods of extraction								
	1st Method			2nd Method			3rd Method		
M	Ch	E	M	Ch	E	M	Ch	E	
Flavonoids	+	-	++	+	-	+	-	-	
Terpenoid	-	-	-	+	-	-	-	-	

s									
Saponins	+++	++	-	-	+	+	+	++	+++
Tannins	+	++	-	+	+	++	++	-	+
Alkaloids	+	-	-	++	+	+	+	++	-
Glycosides	+	-	-	+++	+	+	-	-	-
+++ : Present at high concentration, ++: Present at moderate concentration, +: Present at low concentration, -: Absent or present at negligible concentration. M: methanol, Ch: Chloroform, E: ethanol.									

For chloroform solvent, only 2 of the 6 tested bioactive compounds were present in 1st and 3rd method and these were saponins, tannins and saponins, alkaloids, respectively. While, in the 2nd method 4 of the 6 tested bioactive compounds were present and these were Saponins, Tannins Alkaloids and Glycosides. For ethanol solvent, only flavonoids was present out of the 6 compounds tested in the 1st method and Tanins and Saponin in the 3rd method, respectively. However, in the 2nd method was 4 of the 6 tested bioactive compounds were present and these were Saponins, Tannins Alkaloids and Glycosides. This implies that the methanol solvent used in this study is a better than chloroform and ethanol as solvent for the extraction of crude extracts for phytochemical screening. Therefore, the findings in this study suggest that the choice of solvent plays a significant role in obtaining the highest extracts yield as previously reported [33].

3.6 Qualitative Phytochemical Screening of *Pandanus amaryllifolius* extract:

The qualitative phytochemical screening of *Pandanus amaryllifolius* for the three solvents in all the methods of extraction exploited in this study is shown in table 6. Six bioactive compounds of *P. amaryllifolius* the 3 extracts obtained from methanol, chloroform and ethanol in both 1st, 2nd and 3rd methods of extraction were tested. For methanol, only three bioactive compounds (saponin, tannin and glycosides) tested positive in the 1st method.

Table 6: Qualitative phytochemical Screening of *Pandanus amaryllifolius* extract

Bioactive Compounds	Methods of extraction								
	1st Method			2nd Method			3rd Method		
	M	Ch	E	M	Ch	E	M	Ch	E
Flavonoids	-	-	-	+	+	-	+	-	-
Terpenoids	-	-	-	+++	+	+++	+	-	-
Saponins	++	-	-	+++	++	+	-	+	+
Tannins	+	+	-	++	++	+	+++	+	+
Alkaloids	-	+	+	++	+++	+	++	-	-
Glycosides	+	-	-	+++	+	+++	+	-	+++
+++ : Present at high concentration, ++: Present at moderate concentration, +: Present at low concentration, -: Absent or present at negligible concentration. M: methanol, Ch: Chloroform, E: ethanol.									

However, in the 2nd method, all the six bioactive compounds screened tested positive, with terpenoids, saponins and glycosides present in high concentration (+++) while tannins and alkaloids were present in moderate concentrations (++). The high concentration of terpenoids seen in this study is in accord with earlier study (MacLeod and Pieris, 1982) where terpenoids were shown to be present in high concentrations in *P. amaryllifolius*. In the 3rd method, only saponins tested negative out of the six compounds tested and tannins were present in high concentration. For chloroform, only tannins and alkaloids tested positive at low concentration in the 1st method while all the six compounds tested positive in the 2nd method. Unlike the methanol, only alkaloids were present at high concentration while saponins was present at moderate concentration and flavonoids, terpenoids and glycosides were present at low concentrations. In the 3rd method, only saponins and tannins tested positive at low concentration. For ethanol, only saponins were present in low concentration in the 1st method while only flavonoids tested negative in the 2nd methods with terpenoids and glycosides present at high concentrations. In the 3rd method however, only saponins, tannins were present and glycosides present at high concentrations.

The result showed that the extractions were carried out using methanol as solvents is the best for extract bioactive compound of *P. amaryllifolius* plant followed chloroform (Table 6). Mostly methanol is used for extraction various polar compounds but certain group of non polar compounds are fairly soluble in methanol if not readily soluble. Therefore methanol is commonly used for extraction of bioactive compounds. may be due to the properties of polarity of solvent because they contain a hydroxyl group which is hydrophilic [18]. This result of ours agrees with earlier studies by Ghasemzadeh and Jaafar [15] that reported is the *P. amaryllifolius* has a high content of total flavonoids, phenolics and high antioxidant capacity when the methanol was as solvent, While Nur Syazwani [25] suggested that chloroform crude extract *P. amaryllifolius* a large amount of the essential oils comparison with the other solvents including methanol.

3.7 UV-visible absorption spectrum of *Solanum melongena* in 1st, 2nd and 3rd methods of extracts:

The UV – vis absorption spectrum of *S. melongena* in both the 1st, 2nd and 3rd method of extraction is depicted in figure 6. In the 1st methods of extraction, the UV – vis spectrum of *S. melongena* extract demonstrated 3 sharp peaks at 359, 207.5 and 416.5 nm. However, in the 2nd method of extraction, 8 sharp peaks were seen at 306, 359, 207.5, 285, 416.5, 12, 14 and 16 nm while only 2 sharp peaks at 207.5 and 285 nm were observed in the 3rd method of extraction.

These broad peaks of *Solanum melongena* extract shows the peak areas of separations. Different phytochemicals found to have a broad range of activities, which may help in protection against chronic diseases. The increase in intensity could be as a result of the increase in the number of nanoparticles formed owing to the reduction of gold ions present in the aqueous solutions of the extract [31].

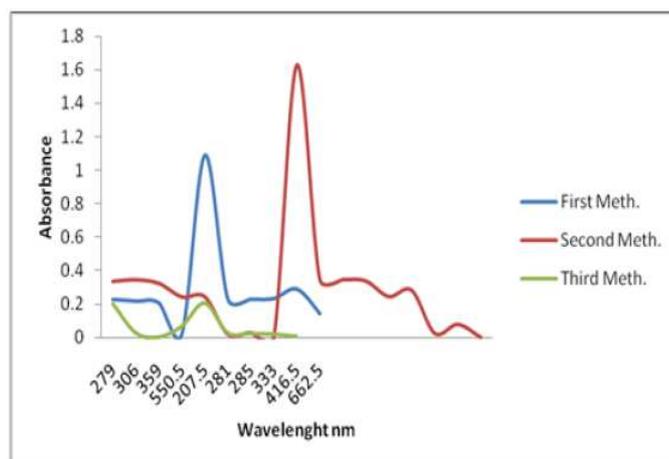


Fig. 6: UV-visible absorption spectrum of Extract of *Solanum melongena* leaves with methanol as solvent in 1st, 2nd and 3rd Method of Extractions 1st (Extraction by Soxhlet Extractor),2nd (Extraction by soaking (shade dryer sample)) and 3rd (Extraction by soaking (freeze-dried samples))

3.8 UV-visible absorption spectrum of Chloroform Extract of *Pandan amaryllifolius* leaves in 1st , 2nd and 3rd Method of Extraction:

The UV – vis absorption spectrum of *Pandan amaryllifolius* in the 1st , 2nd and 3rd methods is depicted in figure 7. In the 1st method, the UV – vis adsorption spectrum of *Pandan amaryllifolius* showed three sharp peaks at 342.5, 276.5 and 414.5 nm while in the 2nd method of extraction, five sharp peaks were seen at 342.5, 582.5, 276.5, 538.5 and 668 nm. Six sharp peaks were however, seen at 315, 525, 276.5, 414.5, 358.5 and 668 nm in the 3rd method of extraction. These wide absorption ranges observed in both the 1st, 2nd and 3rd methods of extraction has been similarly reported in an earlier study by Al-Alwani [4] where *Pandan amaryllifolius* was reported to exhibit a wide absorption spectrum ranging from wavelength of 400 nm to 500 nm.

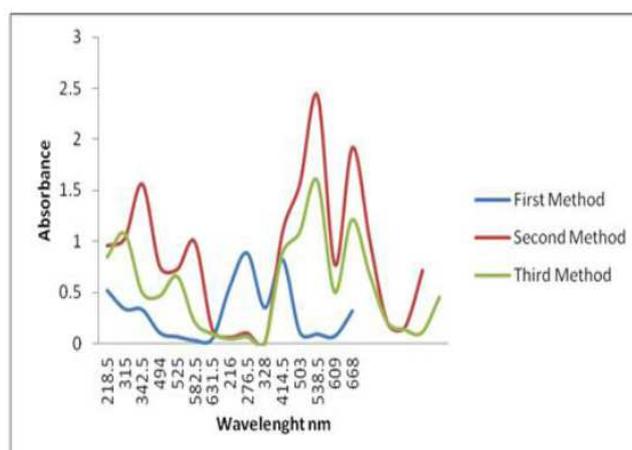


Fig. 7: UV-visible absorption spectrum of Extract of *Pandan amaryllifolius* leaves with methanol as solvent in 1st, 2nd and 3rd Method of Extractions 1st (Extraction by Soxhlet Extractor), 2nd (Extraction by soaking (shade dryer sample)) and 3rd (Extraction by soaking (freeze-dried samples)) 13

3.9 UV-visible absorption spectrum of *Solanum melongena* in chloroform, ethanol and methanol extracts:

The synthesis of silver nanoparticles solution with crude extracts of plants has been demonstrated to be easily observed through ultraviolet-visible (UV-Vis) spectroscopy. The UV – Vis absorption spectral of *Solanum melongena* in chloroform, ethanol and methanol extracts is shown in figure 8. The spectrum of crude extract of *Solanum melongena* in chloroform exhibited 8 sharp peaks. The peaks were sharp and at 257, 271, 442.5, 267, 241, 273.5, 415.5 and 668.5 nm, characteristic of polyphenols and other absorbing components present in the *Solanum melongena* extracts. This finding agrees with earlier reports by Singh et al., [28] where the polyphenol contents of eggplant pulp was investigated using both HPLC, UV –Vis and mass spectral data. The spectrum of ethanol crude extract showed 9 sharp peaks at 257, 271, 340.5, 442.5, 627, 241, 273.5, 415.5 and 668.5 nm. Methanolic extract of *S. melongena* demonstrated five sharp peaks at 257, 310.5, 627, 880 and 241 nm. The absorption characteristics is said to be based on the type of anthocyanins and colour of the extracts [3] and the total anthocyanin content of *Solanum melongena* and pattern characterization via HPLCDAD and LC-MS3 as it has been previously reported [27].

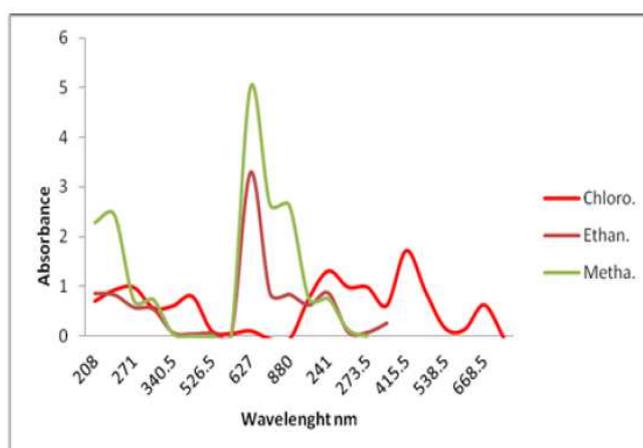


Fig. 8: UV-visible absorption spectrum of *Solanum melongena* leaves in chloroform, Ethanol and Methanol Extract

3.10 UV-visible absorption spectrum of *Pandan amaryllifolius* leaves in Chloroform, Ethanol and Methanol Extract:

The UV – vis absorption spectrum of *Pandan amaryllifolius* in chloroform, ethanol and methanol is shown in figure 9. In the chloroform solvent, *Pandan amaryllifolius* exhibited a total of 7 peaks with five sharp peaks at 232.5, 317.5, 631, 243 and 668 nm and 2 broad peaks between 417 and 424 nm and between 366.5 and 424.5 nm. Ethanolic extract of *Pandan amaryllifolius* however, showed only one sharp peak at 582.5 nm while methanolic extract showed two sharp peaks at 525.5 and 224 nm. The wide absorption range of *P. amaryllifolius* extract observed in this study is in accord with earlier related study of Ghasemzadeh and Jaafar [15] It is well known that similar nanoparticles exhibit yellowish brown colour in aqueous solutions owing to excitation of surface plasmon vibrations in silver nanoparticles.

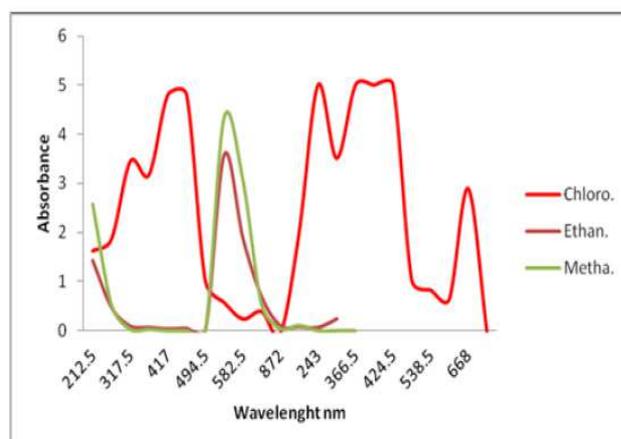


Fig. 9: UV-visible absorption spectrum of *Pandan amaryllifolius* leaves in Chloroform, Ethanol and Methanol Extract

Conclusion:

Diversity of medicinal plants and herbs containing various phytochemicals with biological activity can be of valuable therapeutic significance. Each plant sample needs a special drying method to show the best radical scavenging activity and highest phytochemical content, because of differences in the secondary metabolites.

In general, the drying had effect on the total of secondary metabolites contents which are major contribution activity of *P. amaryllifolius* and *S. melongena* plants. Based on the findings of this study, methanol was observed to yield significantly higher dry weight and percentage yield of crude extract as for *S. melongena* compared to chloroform and ethanol. Similarly, higher content of the bioactive compounds of *Solanum melongena* were observed with methanol compared with chloroform and ethanol. Unlike *P. amaryllifolius*, chloroform appeared to be the solvent of choice as it yielded more crude extract and higher percentages compared to the two other solvents. These findings demonstrated that Extraction by Soxhlet Extractor is the best at least for the *S. melongena* and *Pandan amaryllifolius* as it yielded higher dry weight of crude extract and higher percentage yields. %. The phytochemicals screened was an indication that is these plants could be a beneficial medicinal herb and spice. Methanol for and chloroform for as a solvent had been established to be best for the extraction of phytochemicals in the *S. melongena* and *P. amaryllifolius* respectively. Therefore, sound processed these plant could be used as accessible sources of natural antimicrobial and antioxidants.

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