Special Conference Edition, November, 2019

http://dx.doi.org/10.4314/bajopas.v12i1.67S

Bayero Journal of Pure and Applied Sciences, 12(1): 443 - 449
ISSN 2006 – 6996

TLC ANALYSIS AND ANTIOXIDANT ACTIVITY OF GARDEN EGG LEAVES

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ABSTRACT
The Solanum melongena is used traditionally to treat different diseases such as cancer, atherosclerosis, and inflammation. This study is aimed at investigating the thin-layer chromatography analysis and antioxidant activity of n-hexane, acetone and methanol leaves extract of Solanum melongena. Sequential extraction with solvents of increasing polarity was carried out using the n-hexane, ethyl acetate and methanol. The thin-layer chromatography of the extracts carried out with different solvent system. Antioxidant activity was evaluated quantitatively using DPPH (2, 2-diphenyl-2-picrylhydrazyl) for its free radical scavenging ability. The results of thin-layer chromatography revealed some spots with R_f values in the respective extracts, n-hexane (0.31, 0.42, 0.59, 0.65, 0.72, 0.85, 0.92), acetone (0.97, 0.92, 0.88 and 0.59) and methanol (0.72, 0.83, 0.86, 0.94 and 0.96). The extracts exhibit strong antioxidant activities as radical scavengers, indicating that they have strong proton donating abilities. The results from this research show credence to the traditional application of the plant. Further research is recommended on the isolation and characterization of the antioxidant compounds from the plant.
Key words: DPPH (2, 2-diphenyl-2-picrylhydrazyl), Thin layer chromatography, Reactive Oxygen Species (ROS)

INTRODUCTION

Medicinal plants are vital in the continued existence of living organisms. Nature has been a source of medicinal agents for thousands of years; by using the natural resources, remarkable numbers of drugs have been isolated. Most of these isolations are commonly based on their uses in traditional medicine (Ponnamma *et al.,* 2017). It has been estimated that 14-28% of higher plant species are used medically and 74% of pharmacologically active plants derived components were discovered after following up on ethnomedicinal use of plants (Ncube *et al.,* 2008). Medicinal plants contain a vast combination of phytochemicals such as phenolic acids, flavonoids, tannins and other compounds that such several therapeutic effects (Hassan *et al.,* 2018).

Natural products are important in drug discovery of all major diseases. In order to find new drug in plants, it is necessary to screen plant extracts for biological activities with the aim of obtaining novel compounds. Once novel compounds are expected they are generally isolated in order to have material available for further biological and toxicological testing (Falodun and Obasuyi, 2009). In Africa up to 80% of the population still rely on herbal medicine to treat diseases (Ugwah *et al.,* 2013). Most of the medicinal plants share active secondary metabolites with high antioxidant property, which are playing important role in the prevention of various diseases (Lobo *et al.,* 2010). The antioxidant properties of phenolic compounds such as flavonoids are due to some different mechanisms, such as scavenging of free radicals, chelating of metal ions and inhibition of enzymes responsible for the generation of free radicals (Acker *et al.,* 1996; Belinda *et al.,* 2019).

Solanum melongena (Figure 1) is a specie of the African eggplant or garden egg as it is commonly called in many part of Nigeria where they are use for hospitality in place of kola nuts and with other *Solanum* species in traditional medicine as antioxidants and laxatives. Garden egg (*Solanum melongena*) is cultivated in Nigeria as an annual crop and is usually called "gautan daaci" in Hausa (bitter-garden egg) (Okoh *et al.,* 2010). The color, size, shape of the eggplant fruit vary significantly with the type of cultivar. Fruits are ranked amongst the top ten vegetables in terms of antioxidant capacity due to the fruit phenols and flavonoic constituents (Timberlake, 1981; Singh *et al.,* 2009), which

have been linked to various health benefits Extracts from eggplant are effectual for curative a number of diseases, including high blood pressure, cancer, atherosclerosis, inflammation and hepatosis due to content of anthocyanins (Hung *et al.,* 2004; Umesh *et al.,* 2015).
and strychnine (Magioli and Mansur, 2005). Thus the present experiment was planned to evaluate antioxidant activity and TLC analysis of *Solanum melongena.*

Figure 1: *Solanum melongena* in its natural habitat

MATERIALS AND METHODS

Collection, Identification and Preparation of Plant Sample

Fresh leaves of *Solanum melonogena* were collected from the Kwalkwalawa fadama, Sokoto State Nigeria in February 2019. They were washed under running water to remove earthy impurities, identified and authenticated at the Botany Unit, Department of Biological Science, Usmanu Danfodiyo University Sokoto where a herbarium specimen was deposited and a voucher number UDUH/ANS/071 issued. The plants were air dried for three (3) weeks with occasional turning to prevent rot before they were reduced to fine powder with the aid of an electric milling machine. The powdered sample was stored in clean, air-tight glass container until required for use.

Chemicals and reagents

2,2-Diphenyl-1-picrylhydrazyl radical (DPPH), and ascorbic acid (Sigma Aldrich). Methanol, acetone, n – hexane, sodium carbonate, aluminum chloride and sodium nitrate were of analytical grade.

Extraction and Purification

Preparation of extract

250 g of the powdered leaves was macerated with 750 ml analytical grade n-hexane for 48 hours. Stirring was performed intermittently to enhance the extraction. The n-hexane solvent with its extracted components was decanted and filtered with Whatman filter paper. The filtrate was concentrated under reduced pressure at 45°C in a rotary evaporator and dried at room temperature to constant weight and leveled N-hexane leaves extract (NHE). The same procedure was employed using acetone and methanol to obtain the acetone extract (AE) and methanol extract (ME) respectively.

Thin Layer Chromatography Profile of *Solanum melonogena* Leaves Extracts

Thin layer chromatography was run using silica gel pre-coated plates by ascending manner. Capillary tubes were used to spot the samples on the base line on the 10 cm by 4 cm TLC plates; the spots were developed in an air tight chromatank at room temperature. A preliminary TLC separation of all the extracts were carried out using different solvent systems, the solvent front was allowed to travel at least 75 % height on the TLC plate. Spots were visualized under day light, ultraviolet light (254 nm and 365 nm) and then by spraying with 10 % tetraoxosulphate(VI) acid followed by heating in an oven for 5 minutes at 105 ºC.

The R_f values of distinct spots for each extract of the *Solanum melonogena* leaves were calculated using the formula.

$$\mathrm{Rf} = \frac{\text{Distance travelled by the spot}}{\text{Distance travelled by solvent}}$$

Antioxidant assay using 2, 2-diphenyl-1-picrylhydrazyl (DPPH)

The DPPH assay has been largely used as quick, reliable and reproducible parameters to search for the in-vitro antioxidant parameter of pure isolate as well as plant extracts. The scavenging effect of extracts on DPPH radical was estimated with base on methods described by Ayoola *et al.* (2008) and Siangu *et al.* (2019). The following concentrations of the extracts were prepared, 10, 20, 30, 40 and 50 mg/ml in methanol. Ascorbic acid was used as the standard antioxidant; a blank solution was prepared to contain the same amount of methanol and DPPH but lacks the extract(s) and the standard antioxidant. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 minutes. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid with the same concentrations was used as reference standard. The ability to scavenge DPPH radical was calculated from the following equation:

$$\text{DPPH RSA (\%)} = \frac{Ao - A1}{Ao} \times 100$$

Where RSA = radical scavenging activity; Ao was the absorbance of DPPH radical + methanol, A1 was the absorbance of DPPH radical + sample extract /standard. The IC_{50} of the various extracts were also calculated by plotting a graph of the percentage inhibition against the logarithm to base 10 of the concentration and then extrapolated from the 50 % inhibition.

RESULTS AND DISCUSSION

The percentage yield of the crude extracts obtained from the n-hexane, acetone and methanol extracts are presented in Table 1. From the result, the highest yield was obtained from the methanol extract, which could be attributed presence of high polar substances in the plant material.

The results of the thin layer chromatography (TLC) of *Solanum melonogena* of n-hexane, acetone and methanol extracts are presented in Table 2. The results showed various spots on the TLC plates for all the extracts when viewed with unaided eye, under UV light and when sprayed with 10 % H_2SO_4. Some compounds are UV active while others are not. The R_f values were determined after spraying the plates with 10 % H_2SO_4.

Table 1: Percentage yield of crude extracts of *Solanum melonogena* Leaves Powder

Solvents	Yield of the extracts (g)	Percentage yield (%w/w)
n-Hexane	4.025	1.61
Acetone	5.075	2.33
Methanol	13.275	5.01

Table 2: R_f Values of the Separated Components of *Solanum melonogena* Leaves Extracts

Spots	n-Hexane Extract	Spots	Acetone Extract	Spots	Methanol Extract
A	0.31	A	0.59	A	0.72
B	0.42	B	0.88	B	0.83
C	0.59	C	0.92	C	0.86
D	0.65*	D	0.97	D	0.94
E	0.72			E	0.96
F	0.85				
G	0.92*				

Key: * = UV active.

The percentage yield of the n-hexane, acetone and methanol are increasing with increase in solvent polarity. The result obtained is as a result of the variation in their polarity. The results is in agreement with the report of Halilu *et al*. (2013) who stated that polar solvents is a better extractor of phytochemicals than non polar solvents. The methanol extract yield obtained in this study appears promising for the plant. Plants are known to possess different chemical constituent which varies between plant species. The percentage yields obtained might be attributed to the changeability in the constituents of the plant that have impact on the solubility of the constituents (Hostettman, 1998). The yield obtained is within ranges reported from the literature as stated above and this shows that the leaves of *Solanum*

melonogena possessed an extractable yield of Tables 2 showed the various R_f values of the spots observed when the extracts of the *Solanum melonogena* were chromatographed. The R_f values were different for each spot observed. This suggests that the extracts contain several compounds. The recorded R_f is between 0-1. R_f value can be used to identify an unknown compound. TLC provides semi quantitative information about the main active constituents present in the plant (Banu and Nagarajan, 2014; Okoye *et al.,* 2014). TLC is also used to find a better elution solvent for further purification. The poor solvent system give retention of the majority of components (R_f<0.1), where as a good solvent system allow elution of all of the components of interest (R_f>0.5) and well separeted (Chattopadhyay, 2008; Hassan *et al.,* 2018).

Antioxidant activity

The reduction of DPPH has been used to detect extracts with antioxidant activity, including those considered as free radical scavengers (Gamez *et al*., 1998). From the results (Figure 2) it could be observed that, all the extracts of n-hexane, acetone, and methanol showed a significant activity in reducing DPPH solution. Ideally wide ranges of antioxidant defense mechanism protect the body against the harmful effect of free radicals which are continuously produced as a result of normal metabolic processes in the body (Halliwell *et al*., 1989). Both enzymatic and non enzymatic antioxidants defense systems help to protect the body from the damages caused by ROS like superoxide, hydroxyl, hydrogen peroxide and nitric oxide (Salem *et al*., 2015).

The observed antioxidant activity of *Solanum melonogena* leaves extracts could be attributed to its high levels of total flavanoids and polyphenols (Salem *et al*., 2015; Mezgebe and Shura, 2015). It is well recognized that polyphenols, and specifically flavonoids, behave as inhibitors of ROS generation and could be accountable for the antimelanogenic activity of plant extracts (Amalia *et al.,* 2016).

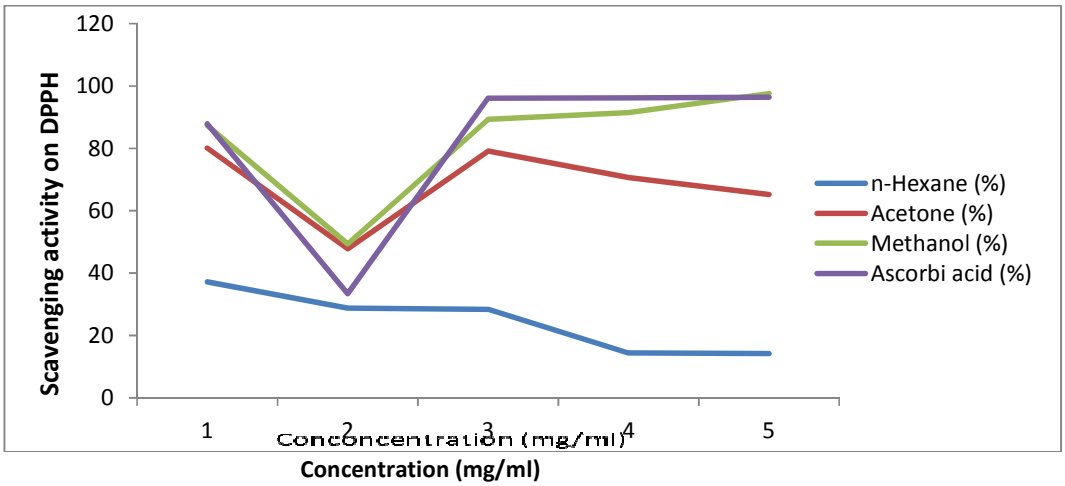

Figure 2: Radical scavenging activity of the extracts of *Solanum melonogena* on DPPH.

The plant extracts showed significant DPPH scavenging activity (14.15 – 97.54%) as compared with values obtained for standard ascorbic acid (96.42%) at 10.00 mg/ml (Figure 2). Methanol extract showed the highest scavenging activity of 97.54% (IC_{50} < 1.21 mg/ml), higher than that of ascorbic acid 96.42% (IC_{50} = 1.14 mg/ml) followed by acetone 80.05% (IC_{50} = 1.09) at 50.00 mg/ml. This was in agreement with the scavenging ability of 84% reported for methanol extract of different parts of *Solanum melongena* by Jung *et al.,* (2011). n-Hexane extracts showed the lowest scavenging activity of 14.15% (IC_{50} = 0.67 mg/ml).

The scavenging activity recorded for the investigated sample was greater than 40.735% reported by Umesh, (2015) for the same plant. The observed variation in the results may be due to geographical location; time of sampling, polarity and solubility of extracting solvent.

CONCLUSION

The study revealed that *Solanum melonogena* leaves are a good source of various metabolites. The extracts of the plant showed promising radical scavenging activity hence the plant could be a potential source of natural antioxidant. Further work could be carried out for possible isolate of potent antioxidant agent for *Solanum melonogena.*

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