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PHYTOCHEMICAL, METABOLITE PROFILE AND ANTIOXIDANT ACTIVITY OF *Azadirachta indica* LEAF EXTRACTS FROM TWO DIFFERENT LOCATIONS

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ABSTRACT
Azadirachta indica commonly known as Neem is widely distributed for more than a thousand years as one of the most versatile medicinal plant having wide spectrum of biological activities. The aim of this work is comparative study on phytochemicals, metabolites profiling and antioxidant activity of Azadirachta indica leaf extracts from two different locations. Samples were collected from Gaya and Nassarawa L. G. As in Kano State, identified, extracted with ethanol and subjected to phytochemical screening using standard methods. Metabolite profiling and antioxidant activity of the crude ethanol extracts were evaluated by TLC analysis and DPPH radical scavenging assay respectively. Phytochemical screening of the crude extracts revealed the presence of flavonoid, phenolic, fat and oil but coumarin, carboxylic acid and emodine were found to be absent. Tannin was found to be present in the sample obtained from Gaya while absent Nassarawa sample. TLC analysis of the extracts revealed the presence of similar compounds but with some variations. The extract of the sample obtained from Gaya was found to be the most active in DPPH radical scavenging assay by revealing the IC_{50} value of 13.70µgml^{-1} relative to other test samples. The plant extract obtained from Nassarawa demonstrated higher antioxidant activity than BHT (IC_{50} 39.95µgml^{-1}) but lower than ascorbic acid (IC_{50} 20.34µgml^{-1}) and Gaya sample. The variation in the antioxidant activity of the two samples can be attributed to the presence of tannin in one sample but absent in the other sample which could be due to geographical variation.
Keywords: Azadirachta indica, Phytochemicals, Metabolites, Antioxidants, Thin Layer Chromatography

INTRODUCTION

Medicinal plants have been used in the treatment of various diseases, as they possess potential pharmacological activities including antineoplastic, antimicrobial, antioxidant, anti-inflammatory, analgesics, anti-diabetic, anti-hypertensive, antidiarrheal and other activities. The phytoconstituents of a plant either individually or in the combination, determine the therapeutic value of a medicinal plant. Alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides and terpenes are some of the important phytochemicals with diverse biological activities (Shaikh and Patil, 2020).

Antimicrobial, antidiabetic, antifertility, antiulcer, antitumor, antifungicidal and antioxidant activity are among the pharmacological properties associated with medicinal plants (Siddiqui and Moid, 2022**:** Atta and Abdelgawad, 2017). According to Junaid and Patil 2020, the pharmacological activity of a plant can be predicted by the identification of the phytochemicals present in it.

During respiration metabolic processes free radicals are formed in the living systems that cause oxidative stress leading to aging and disease progression. Antioxidants in the living systems or from plants origin are responsible for quenching the free radical thereby preventing the body system from oxidative stress (Atta and Abdelgawad, 2017; Mark, 2013; Azlim *et al*., 2010).

Azadirachta indica (*A.indica*) which commonly known as Neem belongs to the family of Meliaceae and has been used in a pyruvic treatment for more than 4000 years (Khatkar *et al.,* 2013, Pankaj *et al.,* 2011).

Neem is known by a variety of names depending on ethnicity, in Hausa it is known as Darbejiya (Muhammad and Ibrahim, 2022), in Tamil it is called as Vembu (Anbarashan *et al.,* 2011), Hindi: Nim, English: Lilac, Margosa tree, Neem tree. Persian name for Neem is *Azad-Drakath-E- Hind* (Khatkar *et al.,* 2013).

Azadirachta indica elaborates a vast array of biologically active compounds that are structurally complex and chemically diverse. Every part of this plant is used as herb (Srinivasa *et al.,* 2014). During the past two decades, the chemical constituents and biological activities of *Azadirachta indica* were intensively investigated in both developing and developed countries. Several published studies revealed isolation of a lot of biologically active compounds and effects of these compounds on insects, and other inhibitory activities such as antitrypanosomal, antiprotozoal and antimicrobial (Wylie and Merrell, 2022; Sultana *et al.,* 2011). Other uses include their applications in fertilizer, poultry and as pesticides (Chandrawathani *et al.,* 2013: Lokanadhan *et al.,* 2012: Musabyimana *et al.,* 2001). Hossain *et al.,* 2014 investigated the antioxidant properties of this plant using DPPH radical scavenging assay in which it displayed significant activity. According to Khattak and Rahman, 2015, the geographical distribution of a plant can determine the distribution of phytochemicals in that plant and conversely their biological activities. The aim of this work is comparative study on phytochemicals, metabolites profiling and antioxidant activity of *Azadirachta indica* leaf extracts from two different locations.

EXPERIMENTAL

Sampling and Extraction

Plant leaves were collected from Gaya town in Gaya LGA and Hotoro in Nassarawa LGA and identified at the Department of Plant Biology, Bayero University, Kano with identification number BUKHAN 0312 as *Azadirachta indica*. The plant material was shade-dried for 14 days at room temperature and ground to powder using a cleaned wooden mortar and pestle. The powdered plant material was stored in a cleaned, dark and sealed polythene bag.

The sample (30g) was extracted using 400 ml ethanol in accordance with (Aboobaker *et al.*, 2019). This was filtered and the filtrate was concentrated to dryness at 40°C using a rotary evaporator, labelled and stored until use.

Thin Layer Chromatography (TLC)

The TLC was carried out by spotting the extracts on a pre-coated aluminium silica gel plate, and developed using a solvent system; ethanol: chloroform (0:100), ethanol: chloroform (50:50) and ethanol: chloroform (30:70) according to (Ginovyan *et al.*, 2020).

DPPH Radical Scavenging Assay

The antioxidant activity of the extract carried out using DPPH radical scavenging assay as described by (Seephonkai *et al.*, 2011; Soher *et al.*, 2016) with slight modification. Each sample of stock solution (10mg/ml) was diluted to final concentrations of 1000, 500, 250, 125, 62.5 and 31.25µg/ml. 200 µL DPPH solution was added to sample solution (100 µL) and incubated at room temperature for 30 min in dark. The absorbance of the mixture was measured at 517nm. Ascorbic acid and butylated hydroxy toluene (BHT) were used as positive controls. The percentage antioxidant activity (% RSA) for each sample was determined using the following relation:

$$\% RSA = \left(1 - \frac{Asample}{Ablank}\right) \times 100$$

Where % RSA is the percentage radical scavenging activity, Asample is the absorbance of the sample (1 mL of sample solution mixed with 2 mL of 0.2 mmolL^{-1} DPPH solution) and Ablank is the absorbance of the blank (1 mL of MeOH mixed with 2 mL of 0.2 mmolL^{-1} DPPH solution). The percentage radical scavenging activity (% RSA) was converted to activity of inhibition concentration at 50 percent (IC_{50}) using SPSS statistical software. The graph of % RSA was plotted against concentration (µgmL^{-1}).

Phytochemical Screening

Crude extracts were analysed for the presence of phytochemicals including tannins, flavonoids, phenolics, carboxylics, emodins, fats and oil (Junaid and Patil, 2020).

RESULTS AND DISCUSSION

Extraction

The percentage yield and appearance of the crude extracts (ethanolic extracts) are presented in table 1

.

Table 1: Yield, Percentage Yield and Appearance of the Samples

Sample	Weight (g)	Yield (g)	% Yield	Color	Texture
S-1	30.0	20.9	69.7	Green	Gummy
S-2	30.0	20.4	68.0	Green	Gummy

S-1 (Sample 1) implies sample obtained from Nassarawa L.G.A. while S-2 (Sample 2) implies sample obtained from Gaya L.G.A.

The texture and colour of the samples were found be gummy and green indicating that the samples could have similar content. The percentage yield of sample 1 (69.7 %) was marginally higher than sample 2 (68.0 %). This suggested both having high content of similar polar compounds.

Phytochemical Analysis

The phytochemical result is presented in table 2 showing phytochemicals distribution of the samples.

Table 2: Phytochemical Screening of the Extracts

Sample	Flavonoid	Phenolic	Tannin	Carboxylic acid	Coumarin	Emodin	Fat & oil
S-1	+	+	-	-	-	-	+
S-2	+	+	+	-	-	-	+

Key: (+) indicates present while (–) indicates absent

Phytochemical screening of the crude extracts revealed the presence of flavonoid, phenolic, fat and oil but coumarin, carboxylic acid and emodin were found to be absent. Tannin was found to be present in sample obtained from Gaya while absent in the Nassarawa sample as presented in table 2 above.

TLC Analysis

TLC analysis was conducted by spotting 20 µL of each extract (200 mg mL^{-1} concentration) on TLC plate. The result presented in table 3 shows the number of spots and R_f values in different solvent gradient system used (Plate 1).

Table 3: TLC Analysis Result of the Extracts

Sample	Solvent system	Composition	No of spots	R_f values
S-1	A EtOH/$CHCl_3$	0:100	1	0.12
S-2			1	0.15
S-1	B EtOH/$CHCl_3$	50:50	1	0.42
S-2			1	0.42
S-1	C EtOH/$CHCl_3$	30:70	5	0.26, 0.28, 0.56, 0.63, 0.79
S-2			8	0.26, 0.29, 0.38, 0.44, 0.49, 0.55, 0.64, 0.81

R_f value = $\frac{Distance\ moved\ by\ compound}{Distance\ moved\ by\ the\ solvent}$

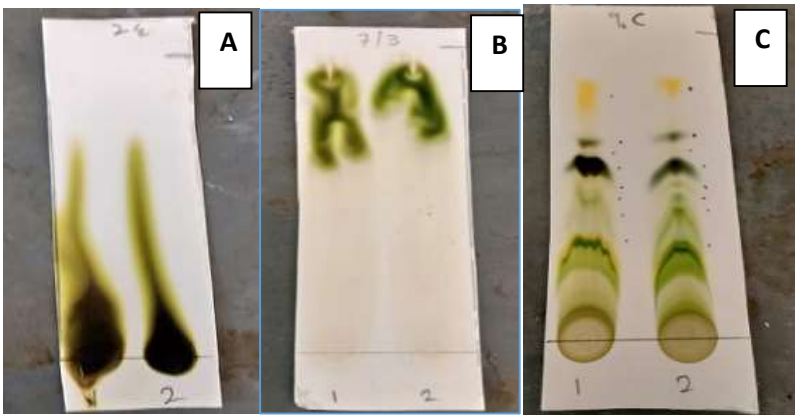

Plate 1: TLC analysis of samples 1 and 2 in different solvent systems showing different compounds with different TLC profiles**.**

Both samples in ethanol:chloroform (0:100 and 50:50) solvent systems were found to display single spot for each with nearly the same or similar R_f value indicating similar chemical contents. The solvent system ethanol:chloroform (30:70) displayed more compounds with better separation in which some have nearly the same R_f values. Sample 1 was found to display five compounds while sample 2 displayed eight compounds. The variation of compounds could be linked to geographic location of the plants and human activities.

DPPH Radical Scavenging Assay

The antioxidant results of the samples are summarised in table 4 and fig. 1 respectively. Inhibition concentration at 50% (IC_{50}) is the concentration at which 50% of the samples are inhibited. The higher the IC_{50} value the lower the activity of the sample and vice versa.

Table 4: Comparative Percentage DPPH Radical Scavenging Activity of the Extracts and Positive Controls

Conc. (µg/ml)	% Inhibition			
	S-1	S-2	VIT.C	BHT
1000	89.37	117.61	98.00	98.54
500	93.64	108.19	97.89	96.76
250	79.29	100.91	96.93	88.68
125	64.87	98.41	96.67	79.85
62.5	51.56	94.43	96.00	62.34
31.25	46.44	69.85	76.46	48.32
15.625	48.50	59.41	30.86	24.93
IC_{50}	31.96	13.70	20.34	39.95

The extract of the sample obtained from Gaya (S-2) was found to be the most active in DPPH radical scavenging assay by revealing the least IC_{50} value of 13.70µgml^{-1} relative to other test samples. The lower the IC_{50} value the higher the activity and vice-versa. The plant extract obtained from Nassarawa (S-1) demonstrated higher antioxidant activity of IC50 31.963µgml^{-1} than BHT (IC_{50} 39.95µgml^{-1}) but lower than ascorbic acid (IC_{50} 20.34µgml^{-1}) and Gaya sample as reflected in both table 4 and figure 1 (a graph of % Radical Scavenging Activity against Concentration). The variation in the antioxidant activity of the two samples can be attributed to geographical location as tannin is present in one sample but absent in the other sample. Tannins are phenolic compounds that precipitate proteins and act as excellent antioxidants (Bhattacharya, 2019; Garg, 2017). The geographical distribution of a plant can determine the distribution of phytochemicals in that plant and conversely their biological activities (Khattak and Rahman, 2015).

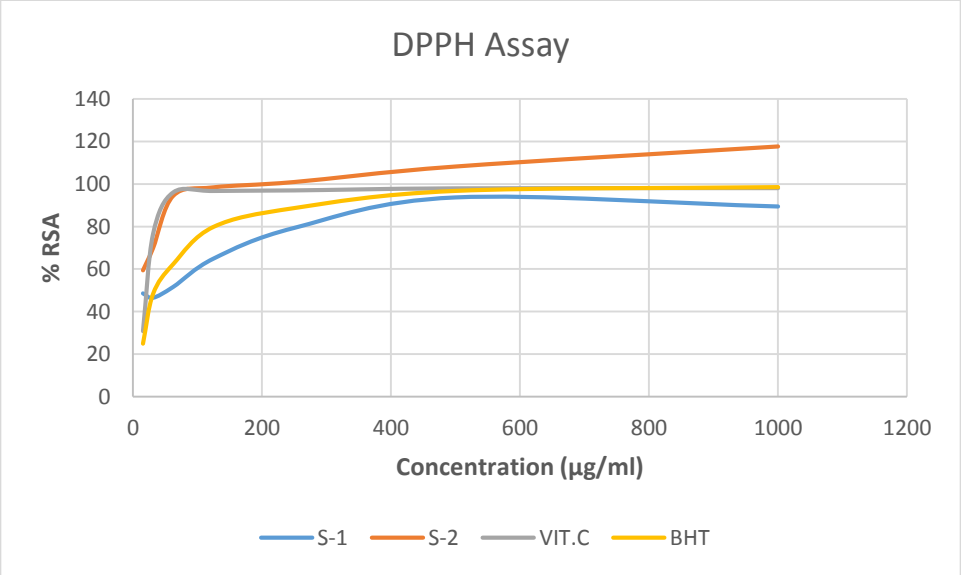

Figure 1: Antioxidant activity of the samples in comparison with the standards.

CONCLUSION

Both the plants demonstrated antioxidant activity with a sample obtained from Gaya revealing the highest activity and even higher than all the standards (Ascorbic acid and BHT). TLC analysis of the extracts revealed the presence of similar compounds but with some variations. Phytochemicals such as flavonoid, phenolic and fat & oil were found to be present in both the extracts.

Tannin was found to be absent in the Nassarawa LGA sample but present in the Gaya LGA sample. Phytochemicals such as carboxylic acid, coumarin and emodin were absent in both samples.

Recommendation

As the world turns to herbal remedies in the treatment of adverse and serious medical conditions, this work recommends further work on these plants. The use of HPLC, LCMS and column chromatographic techniques should be employed towards identification of the tannin present in sample obtained from Gaya area. Elemental analysis on the soil samples from these locations will help in finding out the reason behind the phytochemical variation in these plants.

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