



## **PHYTOCHEMICAL SCREENING AND THIN LAYER CHROMATOGRAPHIC PROFILE OF *Nauclea diderrichii* LEAF EXTRACTS**

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### **ABSTRACT**

The present study investigates the phytochemicals and thin layer chromatographic profile of *Nauclea diderrichii* (Rubiaceae) leaf extracts. Phytochemical in the hexane, ethyl acetate and methanol extracts were determined using standard chemical tests. Thin layer chromatographic techniques were carried out using various solvent systems of varying polarity on these extracts. Preliminary phytochemical screening revealed the presence of alkaloids, glycosides, saponins, phenolic compounds, tannins, phytosterols, carbohydrates, flavonoids and terpenoids. Further screening using thin layer chromatographic technique on the *N. diderrichii* leaf extracts also revealed different phytochemical compounds with different  $R_f$  values. The results obtained in present study indicated that *Nauclea diderrichii* leaf is a rich source of phytochemicals. This could justifies the use of plant species in traditional medicine for treatment of various diseases.

**Keywords:** *Nauclea diderrichii*, Rubiaceae Leaf extracts, Phytochemicals, Retention factor, TLC.

### **INTRODUCTION**

Medicinal plants are of great importance to the health of individuals and communities in general. The medicinal value of such medicinal plants lies in the chemical substances present in the plants that produce a definite physiological action on the human body. The most important of such bioactive constituents are alkaloids, tannins, flavonoids and phenolic compounds (Hill, 1952; Okwu, 2001). Herbs being easily available to human beings and have been explored to the maximum for their medicinal properties, where different parts of the plants like bark, roots, leaves, exudates etc. are used for different medicinal purposes (Perumal and Gopala, 2007). *N. diderrichii* is widely used in local traditional medicine by different communities. The roots are credited with diuretic properties and used for the treatment of anemia. Bark decoctions are taken in Sierra Leone and Ghana against stomach-ache and malaria, and as a foot wash after long walks. In Côte d'Ivoire, the bark is sometimes used to treat fever and jaundice. In Nigeria, bark preparations are used against fever and malaria and as an appetizer and diuretic. In Gabon a bark infusion is drunk against fever. In Congo, bark decoctions are taken or the leaf pulp is rubbed in for the treatment of fever, stomach problems, gonorrhea and menstruation problems, while a bark infusion is taken for the treatment of hepatitis and as a vermifuge. In Guinea, leaf preparations are applied on tumours. In Sierra Leone, leaf decoctions are drunk against diarrhea and as a wash for the treatment of measles and the ripe infructescence is eaten as a cough medicine (Bridson and Verdcourt, 1988; Burkill, 1997; Neuwinger, 2000; Mustafa et al., 2000; Obute and Ekiye, 2008; Addo-Danso et al., 2012). According to survey conducted among Hausa/Fulani tribes in Keffi, Nasarawa State Nigeria, *N. diderrichii* is used for the treatment of skin infections (Alqasim et al., 2013).

Due to the reputation of *N. diderrichii* in folk medicine, especially the leaf being used to cure numerous ailments, this study was carried out to identify the bioactive compounds responsible for these activities.

### **MATERIALS AND METHODS**

#### **Collection of the plant material**

Leaf of *Nauclea diderrichii* was collected in the month of October, 2015 from Kufaina village of Zaria Local Government Area, Kaduna state. The plant's identity was first authenticated by a Botanist in the Herbarium Section, Department of Biological Sciences Ahmadu Bello University, Zaria and it was assigned a voucher specimen number: 16677.

#### **Preparation of the plant extract**

The leaf was dried under the shade and comminuted to powdered form using pestle and mortar. Four thousand grams of the powdered leaf was extracted successively using cold maceration with hexane, ethyl acetate and methanol as extracting solvents. The extracts were filtered and dried at a temperature of 50°C using water bath. The extracts were concentrated and percentage yield was calculated. The dried extracts were properly stored in a desiccator prior to analysis.

#### **Phytochemical Screening**

Preliminary phytochemical tests for the screening and identification of bioactive chemical constituents like alkaloids, carbohydrates, glycosides, saponins, phenolic compounds, phytosterols, flavonoids, and tannins present in the extracts were carried out using standard procedures adopted from Kokate (1994).

#### **Thin layer chromatographic profiling**

The three extracts were subjected to thin layer chromatography (TLC) using dimensional ascending method in silica gel 20x20 (Merck) plate. This was cut into desired sizes and used appropriately. Plate markings were made with ruler and soft pencil.

Glass capillaries were used to spot the samples within a distance of 1 cm at 3 tracks. Two small tanks were used for the development of the chromatograms. The solvent systems used are; Hexane: Acetic acid (9:1); Hexane: Ethyl acetate (9:1); Hexane: Ethyl acetate: Acetic acid (2:7:1); Hexane: Ethyl acetate (7:3); Hexane (100%); Butanol: Acetic acid: water (8:1:1) and Butanol: Acetic acid: water (6:1:1). After pre-saturation with the mobile phase for 20 minutes, the plates were placed in the tanks and chromatographed between 15 to 60 minutes. The chromatograms were dried and sprayed with freshly prepared p-anisaldehyde, this was used as a general reagent to detect the bands and there retention factor ( $R_f$ ) values were calculated using standard method as reported by Rajendra and Estari, (2013). More chromatograms were developed and specific reagents were sprayed for phytochemical profiling.

## RESULTS

### Percentage yield of the extracts of *Nauclea diderrichii* Leaf

Physical appearance of hexane and ethyl acetate extracts were dirty green in color, methanol extract was brown in color. Yields of extracts in grams after

concentration for hexane, ethyl acetate and methanol were 24.28, 18.60 and 139.14. Results were translated into percentages as follows: 0.61, 0.47 and 3.48, Table 1.

### Phytochemical Screening

Preliminary phytochemical screening using different chemical reagents revealed the presence of alkaloids, glycosides, saponins, phenolic compounds, tannins, phytosterols, carbohydrates, flavonoids and terpenoids. Among these phytochemicals, carbohydrates, phytosterols and terpenoids were present in all the extracts whereas the remaining phytochemicals were only present in methanol extract (Table 2).

### Thin layer chromatographic profiling of *Nauclea diderrichii* leaf using general spraying reagent

Six solvent systems were used to attain good resolution, in the end hexane: acetic acid (9: 1) mixture was considered suitable for ethyl acetate extract. Hexane: ethyl acetate (9: 1) mixture was considered suitable for hexane extract. And butanol: acetic acid: water (6: 1: 1) mixture was considered suitable for methanol extract (plates 1, 2 and 3).

**Table 1: Percentage yields of hexane, ethyl acetate and methanol extracts of *Nauclea diderrichii* leaf**

S/No	Solvent	Color of extract	Yield of extract	Percentage yield (%w/w)
1	n- Hexane	Dirty green	24.28 gm	0.6070
2	Ethyl acetate	Dirty green	18.60 gm	0.4650
3	Methanol	Brown	139.14 gm	3.4785

**Table 2: Phytochemical constituents of the leaf of *Nauclea diderrichii***

S/No	Phytoconstituents	Test	Methanol	Ethyl acetate	n- Hexane
1	Alkaloids	Mayers	Present	Absent	Absent
2	Glycosides	Dragendorff	Present	Absent	Absent
3	Saponins	Frothing test	Present	Absent	Absent
4	Phenolic compounds	Lead acetate test	Present	Present	Absent
5	Tannins	Bromine water test	Present	Absent	Absent
6	Phytosterols	Salkowski test	Present	present	Present
7	Carbohydrates	Molisch test	Present	Absent	Absent
8	Flavonoids	NaOH test	Present	Absent	Absent
9	Terpenoids	Liebermann Buccard test	Present	present	Present



Plate 1



Plate 2



Plate 3

**Plate1:** Chromatogram of ethyl acetate extract of *Nauclea diderrichii* leaf developed in hexane: acetic acid (9: 1) for 20 minutes and sprayed with p- anisaldehyde. This revealed 9

spots with  $R_f$  values of 0.16, 0.25, 0.29, 0.32, 0.41, 0.47, 0.51, 0.55 and 0.63.

**Plate 2:** Chromatogram of hexane extract developed in hexane: ethyl acetate (9: 1) for 20 minutes and sprayed with p- anisaldehyde revealed 10 spots with Rf values of 0.67, 0.12, 0.27, 0.31, 0.35, 0.44, 0.48, 0.56, 0.60 and 0.69.

**Plate 3:** Chromatogram of methanol extract developed in butanol: acetic acid: water (6: 1: 1) for 50 minutes and sprayed with p- anisaldehyderevealed 6 spots with  $R_f$  values of 0.24, 0.33, 0.40, 0.53, 0.67 and 0.80.

## **Thin Layer Phytochemical profiling of *Nauclea diderrichii* Leaf Extracts Using Specific Spraying Reagents**

Solvent system hexane: acetic acid (9:1) was used to developed more chromatograms for ethyl acetate extract, hexane: ethyl acetate (9: 1) was also used to developed more chromatograms for hexane extract and butanol: acetic: water (6: 1: 1) was used to develop more chromatograms for methanol extract (plates 4, 5, 6, 7, 8 and 9).



## **Plate 4**



## Plate 5



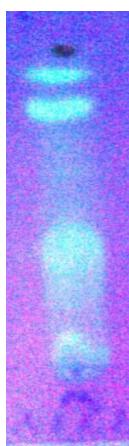
## **Plate 6**

**Plates 4:** Chromatogram of ethyl acetate extract developed in hexane: acetic acid (9: 1) for 20 minutes and sprayed with Liebermann Bucchard reagent revealed the presence of triterpenes and unsaturated steroids, 8 spots were revealed with the following  $R_f$  values: 0.13, 0.16, 0.20, 0.23, 0.29, 0.31, 0.35 and 0.40.

**Plate 5:** Chromatogram of n- hexane extract developed in hexane: ethyl acetate (9: 1) for 20 minutes and sprayed with Liebermann Buccard reagent revealed the presences of

triterpenes and unsaturated steroids, 8 spots were revealed with the following  $R_f$  values: 0.04, 0.07, 0.11, 0.16, 0.21, 0.29, 0.35 and 0.52.

**Plate 6:** Chromatogram of methanol extract developed in butanol: acetic acid: water (6: 1: 1) for 50 minutes and sprayed with Liebermann Bucchard reagent depicted the presence of triterpenes and unsaturated steroids, 5 spots were revealed with the following  $R_f$  values: 0.17, 0.23, 0.43, 0.49 and 0.59.



## **Plate 7**



## **Plate 8**



**Plate 9**

**Plates 7:** Chromatogram of methanol extract developed in butanol: acetic acid: water (6: 1: 1) for 50 minutes and sprayed with Aluminium chloride depicted the presence of flavonoids, 4 spots were visible and the  $R_f$  values are 0.08, 0.37, 0.76 and 0.93.

**Plate 8:** Chromatogram of methanol extract developed in butanol: acetic acid: water (6: 1: 1) for 50 minutes and sprayed with ferric chloride detected the presence of phenolic compounds, depicted 2 spots and the R<sub>f</sub> values are: 0. 36 and 0.71.

**Plate 9:** Chromatogram of methanol extract developed in butanol: acetic acid: water (6: 1: 1) for 50 minutes and sprayed with Dragendorff to detect the presence of alkaloids, shown 3 spots with these R<sub>f</sub> values: 0.12, 0.23 and 0.48.

## DISCUSSION

Most plants produce secondary metabolites such as alkaloids, flavonoids, phenols, terpenoids, steroids and saponins that are used in pharmaceuticals, cosmetics and pesticide industries.

In the present study, phytochemicals in the n-hexane extract showed the presence of steroids and terpenoids; the ethyl acetate was found to contain also steroids and terpenoids in addition to phenolic compounds and the methanol extract was observed to have alkaloids, glycosides, saponins, phenolic compounds, tannins, phytosterols, carbohydrates, flavonoids and terpenoids (Table 2). Plants used in the treatment of disease are said to contain active principles known as phytochemicals with biological activity, some of which are responsible for the characteristic odours, pungencies and colours of plants while others give a particular plant its culinary, medicinal or poisonous virtues Evans(2002).

These results could supplement the folkloric usage of the studied plant which possess alkaloids, triterpens, and  $\beta$ -sitosterolas already observed by Lamidi *et al.*, (2006) and Lamidi *et al.*, (2011). By isolating and identifying these bioactive compounds new drugs can be formulated to treat various diseases and disorders. TLC profiling of all 3 extracts gave an impressive result that direct towards the presence of a number of phytochemicals (Plates 1,2 and 3). The phytochemicals confirmed in methanolic extract are alkaloids, flavonoids, phenolic compounds, triterpens and steroids (Plates 6, 7, 8 and 9). The ethyl acetate and n- hexane extracts confirmed the presence of triterpens and steroids (Plates 4 and 5). These biologically active substances give different  $R_f$  values in different solvent systems (Chromatograms 1, 2 and

3). This variation in  $R_f$  values of the phytochemicals provides a very important clue in understanding their polarity and also helps in selection of appropriate solvent system for separation of pure compounds by column chromatography (Chromatograms 4, 5, 6, 7, 8 and 9). Mixture of solvents with variable polarity in different ratio can be used for separation of pure compound from plant extract. The selection of appropriate solvent system for a particular plant extracts can only be achieved by analyzing the  $R_f$  values of compounds in different solvent system. Different  $R_f$  values of the compound also reflect an idea about their polarity. This information will help in selection of appropriate solvent system for further separation of compound from these plant extracts (Chromatograms 4, 5, 6, 7, 8 and 9).

## CONCLUSION

The extracts of *Nauclea diderrichii* leaf was found to contain phytoconstituents such as alkaloids, glycosides, saponins, phenolic compounds, tannins, phytosterols, carbohydrates, flavonoids and terpenoids, The presence of these compounds could be responsible for the health benefits claimed by traditional medical practitioners. In addition to this, chromatographic profiling of this extracts showed different  $R_f$  values confirming the presence of this phytocompounds.

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