Phytochemical screening of the leaves of *Lophira lanceolata* (Ochanaceae)

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Abstract

Phytochemical screening of the ethanol extract of the leave of *Lophira lanceolata* indicated the presence of flavonoids, anthraquinones, carbohydrate, glycoside, phenols, saponin steroid, tannin, free reducing sugar. However alkaloid were absence in this species. The glycoside after hydrolysis afforded quercetin as the genin and a sugar was identified as glucose. [Life Science Journal. 2007; 4(4): 75 – 79] (ISSN: 1097 – 8135).

Keywords: *Lophira lanceolata*; ash value; sugar; chromatogram

1 Introduction

While the forest is referred to as GOD's own pharmacy (Treben, 1986) many medicinal plants are used in modern medicine where they occupy a very significance place as raw material for important drugs and plants used in traditional system of medicine in pharmaceutical houses are collected from wild sources (Singh *et al,* 2003). Most drugs of plant origin used by medical practitioners are in the form of extract of the whole plant material or part of it. In view of this, local medicinal plants, which show suitable biological effect, could be standardized and similarly utilized. Some of the effects elaborated by extract of plants used in traditional medicine include antiviral, antitumor, antimicrobial, insecticide and central nervous system effect (Sofowora, 1982).

During the past century, the extra ordinary results of research have unquestionably led to success at an exponential rate, which the practitioner of modern medicine now enjoys. However the exclusive use of this research oriented approach with little regard for knowledge acquired through the empirical method has served to delay the application of many potential benefits (Dastur, 1964).

World health organization has estimated that perhaps about 80% of more than 4000 million people on earth rely chiefly on traditional medicine for their primary health care needs (Akerele, 1990), and also can safely be presumed that a major part of traditional therapy involve the use of plant extract or their active principles. Such treatments include the administration of infusion boiled parts, as some of the natural drugs are not usually in the form of tablet or pills (Oliver, 1960). Although the structure of some plant constituents are now known, many compounds that remain undiscovered in plant are beyond the imagination of scientist (Sievers, 1930). The chemical constituents in medicinal plant usually explain the rational for the use of the plants in traditional medicine (Fansworth, 1984).

2 Materials and Methods

2.1 Extraction

Dried and coarsely powdered leaves of *Lophira lanceolata* (350 g) were refluxed with petroleum ether (60 – 80 ºC) for 10 hours. The extract was decanted off and fresh quantity of the petroleum ether was added again and refluxed for another 10 hours.

The defatted leaves were completely dried and extracted with ethanol. The combine ethanol extracts where concentrated on water bath whereby a highly viscous greenish-brown mass was obtained. This was refluxed with petroleum ether (60 – 80 ºC), benzene and chloroform successively until the solvent in each case was almost colourless. The residue left behind was then treated with

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hot water. The water insoluble portion was dissolved in ethanol and dried under reduced pressure. A solid brown residue (30.2 g) obtained respond to usual flavonoid colour tests was marked "A".

The aqueous solution was extracted with ethyl acetate. The process was repeated twice. The ethyl acetate extract where combined and the solvent was recovered under reduced pressure. The semi- solid residue was marked "A_1" and respond to usual flavonoidal test.

2.2 Thin layer chromatographic examination of fraction "A"

Thin layer chromatographic plate (5 × 20 cm) 0.5 mm thickness was prepared by usual method using silica gel G (E.Merck). The sample of 0.1% of "A" was dissolve in alcohol and spotted manually using a capillary tube. The plate was developed in BPF (benzene : pyridine : formic acid, 36 : 9 : 5) as solvent system. After development, on examination of the chromatogram under U.V. light and then sprayed with ferric chloride the presence of one spot with trailing was revealed. This was purified with column chromatography.

2.3 Fraction "A_1"

The ethanol solution of fraction "A_1" was subjected to paper chromatographic analysis using Whatmann paper No.1. In each solvent system, the chromatograms were developed for 10 hours. After drying in fume cupboard, the chromatograms were examined under U.V. light, which revealed the presence of two spots with trailing.

2.4 Hydrolysis of fraction "A_1"

10 mg of fraction "A_1" was dissolved in water and hydrolyzed by refluxing with 2 ml of 0.6 M hydrochloric acid. The hydrolysis appeared to be completed within a few munites, but the heating was continued for 2 hours to ensure complete hydrolysis. After leaving over-night, the aglycone was filtered, washed, dried at room temperature and marked fraction G.

2.5 Chromatographic identification of the sugar

The filtrate (from which the aglycone was removed) was neutralized with aqueous ammonia until it was neutral to litmus paper. It was concentrated under reduced pressure to a syrupy mass. This syrupy mass was chromatographed on whatmann paper No. 1 using n-Butanol : acetic acid : water (4 : 1 : 5) as solvent system with authentic (reference sugar) samples. The chromatogram after development was dried in a fume cupboard sprayed with aniline-H-phthalate reagent and then heated at 110ºC for 5 minutes. After development on examination the chromatogram shows the presence of a spots equivalent to R_f values of authentic glucose were revealed.

2.6 Preparation of authentic sugar

The authentic sugar rhamnose, glucose, galactose, fructose, xylose and arabinose were prepared by dissolving small sample of the sugar in distilled water.

2.7 Quantitative evaluation of the leave of *Lophira lanceolata* leaves.

(a) Determination of total ash value
(b) Determination of acid insoluble ash value
(c) Determination of water-soluble ash value
(d) Alcohol soluble extractive value
(e) Water soluble extractive value (British Pharmacopoeia, 1993).

3 Results

On defatting the powdered leave of the plant material with petroleum ether (60 – 80 ºC), the merc was extracted with ethanol. The ethanol extract was fractionated into fraction "A" and fraction "A_1" both of which gave usual colour test for flavonoid.

3.1 Phytochemical screening

The result of the phytochemical screening of ethanol (fraction "A") (A), chloroform (B) and petroleum ether (C) extracts was summarized in Table 1.

3.2 Determination of ash value of *Lophira lanceolata* leaves

Evaluation of crude powder leave of the plant *Lophira lanceolata* in Table 2.

3.3 Colour test of fraction "A"

The result of various colour reactions conducted on fraction "A" was summarized in Table 3.

3.4 Colour test of fraction "A_1"

The result of various colour reactions conducted on fraction "A_1" was summarize in Table 4.

3.5 Thin layer chromatographic examination of fraction "A" in three different solvent

BPF, 36 : 9 : 5; TPA, 10 : 1 : 1 and B : EA : A, 8 : 5 : 2 gave a good chromatogram, Figure 1.

3.6 Result of hydrolyzed extract G

The aqueous sugar portion after hydrolysis and neutralization was chromatographed with reference authen-

Table 1. The phytochemical screening results.

Group	Test	Observation	Inference
Flavonoids			
(a) Ferric chloride test	Aqueous extract + 10% ferric chloride	A green precipitate	A (+++), B (+++), C (–)
(b) Lead acetate test	Aqueous extract + 10% lead acetate	Buff coloured solution	A (++), B (++), C (–)
(c) NaOH test	Ethanol extract + 10% NaOH + dil HCl	Yellow solution turned colourless on addition of dil HCl	A (++), B (++), C (–)
(d) Shinoda test	Mg chip + alcoholic extract + few drops of conc. HCl	Effeverscence dark brown solution turns red	A (++), B (++), C (–)
Carbohydrate			
General test	Extract + distilled water + H_2SO_4	A dull violet precipitate	A (+), B (+), C (–)
Test for starch	Aqueous extract + 5 ml 5% KOH solution Aqueous extract (filtrate) + 1 ml	A cinary coloured solution	A (++), B (+), C (+)
Barfoed's test for sugar	Barfoed's reagent then heated in a beaker of boiling water	A red precipitate	A (++), B (+), C (+)
Reducing sugar			
Fehling's test	Aqueous extract + 5 ml of equal volume of fehling solution A and B and boiled for 5 minutes	Red precipitate	A (+++), B (+++), C (+)
Combine reducing sugar	Aqueous extract + dil HCl (heat) +NaOH + fehling A and B solution	Red precipitate	A (+++), B (+++), C (+)
Cardiac glycoside			
Steroidal nucleus Liebermann-Burchard test	Chloroformsolution of the plant + acetic anhydride + few drop of conc. H_2SO_4	A colour change from violet to blue	A (+++), B (+++), C (+)
Salkowskii test	Aqueous extract + 2 ml $CHCl_3$ + conc. H_2SO_4 to form a lower layer	A reddish brown colour at interphase	A (+++), B (+++), C (+)
Cardenolide	Extract + pyridine + Sodium nitroprusside + 20% NaOH	Red colour fades to brownish yellow	A (+++), B (+++), C (+)
Keller-Killiani test	Extract 2 ml 3.5% $FeCl_3$ + glacial acetic acid + 2 ml conc. H_2SO_4	Reddish brown ring at interphase	A (+++), B (+++), C (+)
Saponin			
Frothing test	Extract + distilled water was shaken	Frothing persist on warning	A (+), B (+), C (+)
Tanins			
General test	Ethanol extract H_2SO_4 + 5% HCl	A green solution	A (+), B (+), C (+)
Phlonatanins test	Ethanol extract + 5 ml of 1% HCl	Red precipitate	A (+), B (+), C (+)
Anthracene derivatives	Ethanol extract + 5% HCl, filter, filtrate + $CHCl_3$ then 10% NH_3	The aqueous was colourless	A (+), B (+), C (+)
Alkaloids			
General test	Wagner reagent	Yellow colour instead of brown ppt	A (–), B (–), C (–)
	Dragendoff's reagent	Red colour instead of red ppt	A (–), B (–), C (–)
	Mayer's reagent.	Brown colour instead of buff ppt	A (–), B (–), C (–)
	Radulescu test	No colour change	A (–), B (–), C (–)
Morphine alkaloid			
Indole alkaloid	Extract + conc.H_2SO_4 + potassium dichromate	No colour change	A (–), B (–), C (–)
Quinoline alkaloid	Thalleiqiune test	No colour change	A (–), B (–), C (–)
Tropane alkaloid	Vitali – Morin test	No colour change	A (–), B (–), C (–)

Faintly: (+); Moderately: (++); Highly: (+++); Absent: (–).

Table 2. Evaluation of results

	Percentage (%)
Total ash value content	9.5
Acid insoluble ash value	6.8
Water soluble ash value	6.4
Alcohol soluble extractive value	2.4
Water extractive value	4.9

Table 3. Colour test of fraction "A"

Reagent	Colour produced	Inference
(1) Mg- HCl	Orange	++
(2) Alc.Ferric Chloride	Dark green	+ + +
(3) Zn-HCl	Red	+ +

tic sugar. The sample gave a spot with the same R_f value equivalent with that of an authentic (reference) glucose sample.

3.7 Paper chromatography of hydrolyzed sugar

Technique: descending.

Paper: whatmann No 1.

Solvent system : n-butanol : acetate acid : water (4 : 1 : 5) developed for 20 hours.

Visualization: after drying in the fume cupboard, it was sprayed with aniline-H-phthalate and heat at 110 ºC for 5 minutes (Figure 2 and Table 5).

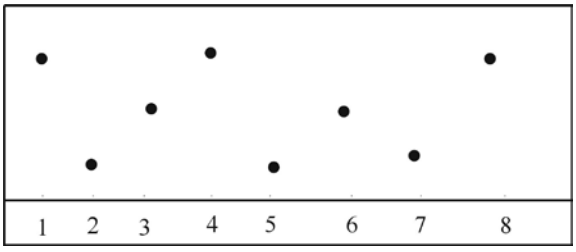

Figure 2. Paper chromatogram of fraction "A" after hydrolysis.

Table 5. R_f value of spot in paper chromatogram of fraction "A"

1	Hydrolyzed Portion B
2	Arabinose
3	Xylose
4	Glucose
5	Galactose
6	Rhamnose
7	Fructose
8	Free sugar A_1

Table 4. Colour test of fraction "A_1"

Reagent	Colour produced	Inference
(1) Mg-HCl	Orange	++
(2) Alc.Ferric Chloride	Dark green	+ + +
(3) Zn-HCl	Red	+ +

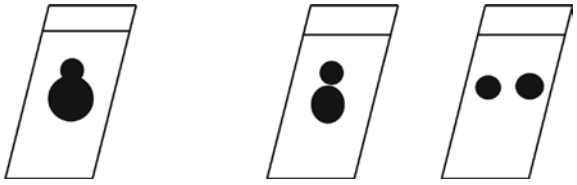

Figure 1. TLC chromatogram of fraction "A" in BPF, TPA and B: EA: A, respectively.

The result of various solvent system used in developing solvent for the TLC on fraction "A" was summarized in Table 6.

Table 6. Solvent system development

Toluene : pyridine : acetic acid	10 : 1 : 1
Toluene : ethanol	19 : 1
Benzene : ethyl acetate : acetic acid	8 : 5 : 2
Ethyl acetate : formic acid : water	10 : 2 : 3
Chloroform : ethanol : water	1 : 2 : 1
Ethyl acetate : methanol : water	10 : 14 : 1
Methanol : acetic acid	9 : 1

3.8 Paper chromatograpm of aglycone before hydrolysis

Technique: descending.

Paper: what mann No 1.

Solvent system: n-butanol : acetate acid : water (4 : 1 : 5) developed for 20 hours.

Visualization: after drying in the fume cupboard, it was sprayed with ferric chloride and heat at 110 ºC for 5 minutes (Figure 3 and Table 7).

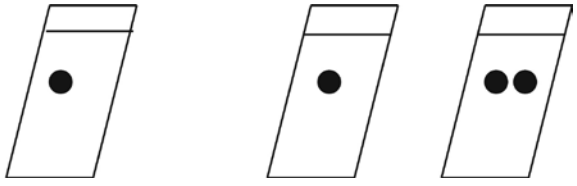

Figure 3. Paper chromatogram of aglycone before hydrolysis.

Table 7. Solvent system for paper chromatogram of aglycone before hydrolysis of fraction "A"

n-butanol : acetic acid : water	4 : 1 : 5
Benzene : pyridine: n-butanol : water	1 : 3 : 5 : 3
Benzene : ethyl acetate : acetic acid	8 : 5 : 2

4 Discussion

The plants ethanol extract was extracted with various organic solvents to afforded a dark brown mass 30.2 g The mass gave dark green colour with alcoholic ferric chloride, orange colour with Mg-HCl and red colour with Zn-HCl. This clearly indicates that this fraction contains flavone nucleus. The extract was treated with hot water and filtered. The hot water insoluble portion afforded a brown residue, which gave various colour tests for flavonoid and was marked "A" (Table 3). The hot water soluble portion was extracted with ethyl acetate to afford a dark brown mass which gave various colour test for flavonoid and was marked "A_1" (Table 4).

Acid hydrolysis of fraction "A" yielded an aglycone. The fraction was co-chromatographed on Whatmann paper No.1, with two solvent systems, against authentic samples of aglycone (Table 7) and where identified as quercetin.

The aqueous portion after neutralization gave a positive test for reducing sugars. This fraction was co-chromatographed on Whatmann paper No.1 with reference sugar sample in different solvent system for 18 hours (Figure 2). The sugar portion gave the same R_f value Table 5 with glucose in two different solvent system (Table 7).

The quantitative evaluation of the dry powdered leave (Table 2) gave a total ash value 9.5%, acid insoluble ash value 6.8%, water soluble ash value 6.4%, alcohol soluble extractive value 2.4%, and water extractive value 4.9%.

5 Conclusion

It has been concluded that the leaf of *Lophira lanceolata* (Ochanaceae) showed the presence of flavonoids, anthraquinones, carbohydrate, glycoside, phenols, saponin steroid, tannin, and a sugars as glucose.

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