Original Research Article

Preliminary Phytochemical Screening and Thin Layer Chromatography Analysis of Stem Bark Extracts of African Mistletoe Parasitic on Vitelaria paradoxa, Pilostigma thonningii and Combretum fragrans

ABSTRACT

The chemistry of African mistletoe is not sufficiently documented. This paper is therefore, aimed at determining the phytochemicals present in the crude extracts of mistletoe parasitic on plants that are commonly seen as hosts. Powdered stem bark of mistletoe was extracted successively with hexane, ethyl acetate and methanol. Preliminary phytochemical screening was carried out the extracts. Thin layer chromatography (TLC) was carried out on silica gel precoated plates in 9:1 (Hex/E.A), 5:5 (Hex/E.A), and 7:3 (E.A/MeOH) mobile phases for hexane, ethyl acetate and methanol extracts respectively. The study revealed the presence of secondary metabolites such as alkaloids, flavonoids, tannins/phenols,cardiac glycosides, steroids and triterpenoids. It was evident from TLC analysis that mistletoes from various plant hosts contain similar chemical profile. We therefore debunk the claim by some herbalists that medicinal values of mistletoes vary due to host plant. This is the first time a study of this kind is reported on mistletoe parasitic on *Vitellaria paradoxa Pilostigma thonningii*, *Combretum fragrans*.

Hex= Hexane; E.A= Ethyl acetate; MeOH= Mehanol

Key words: Mistletoe, Thin layer chromatography, Phytochemicals, Silica gel, Mobile phase

1. INTRODUCTION

African mistletoes are hemiparasitic plants that grow on other plants. The fact that they photosynthesize and derive some salts from host plants; they are regarded as hemiparasitic [1]. Mistletoes grow on many plants in Nigeria including *Vitellaria paradoxa, Pilostigma thonningii*, *Combretum fragrans*, *Parkia biglobosa* and many others. They are also rarely seen to grow on mango, guava, cocoa and kola nut trees etc [2]. It is believed that birds excrete the seeds of mistletoe through feaces on trees upon which they sit. The sticky feaces facilitate the attachment of seeds on tree branches. Almost all trees could have opportunity to host mistletoe but just a few have been seen to do so. In Igboughul District of Bali Local Government Area, Taraba State, almost 9 in 10 *Vitellaria paradoxa* (shea butter tree) and 8 in 10 *Pilostigma thonningii* host mistletoe. This could be due to their thick, freshy and easily penetrated stem barks.

Medicinal plants produce various classes of secondary metabolites such as alkaloids, tannins, steroids, phenols, saponins, flavonoids glycosides, terpenoids and others that are responsible for therapeutic and defence properties [3]. Preliminary phytochemical screening is a useful method of detecting these bioactive principles that are present in medicinal plants and may subsequently in drug discovery and development processes [4].

Mistletoes are highly utilized in traditional medicine to treat different kinds of diseases such as heart diseeases and diabetes etc. However, some traditional practioners claim that the use of mistletoe depends on the type of host plant- mistletoe from specific plants are used to treat specific diseases. In Nigeria for example, mistletos found on bamboo trees and gamba grasses are used to perform rituals especially money making. However, mistletoe on bamboo and gamba grasses is not common.

The chemistry of African mistletoe is not sufficiently documented. However, polysacharides and peptides were isolated and identified structurally [1]. The report published by Ezema *et al*. [5] revealed the presence of cardiac glycosides, steroids, saponins, carbohydrates, and terpenoids in the leaves of mistletoe parasitic on *Parkia biglobosa*. The methanolic extract of mistletoe leaves were shown to contain saponins, alkaloids, phenols, flavonoids and tannins [2].

From the survey of literature, little or no information is reported of the phytochemistry of mistletoe parasitic on *Vitellaria paradoxa, Pilostigma thonningii* and *Combretum fragrans.* This paper is therefore, aimed at determining the phytochemicals present in the crude extracts of mistletoe parasitic on these plants that are commonly seen as hosts in Igboughul District of Bali L.G.A of Taraba State using preliminary phytochemical screening and thin layer chromatography analyses of crude extracts.

2. MATERIALS AND METHODS

2.1 Sample Collection

Mistletoe stem were harvested from *Vitellaria paradoxa, Pilostigma thonningii* and *Combretum fragrans* in Igboughul District of Bali L.G.A, Taraba State in August, 2018. The barks were peeled and allowed to dry under shed. The barks were pulverized using pestle and motar.

2.2 Extraction

Using cold marceration, a powdered sample was (10 g) extraction 50 mL of hexane for 48 hour with intermitent shaking. The marcerated sample was filtered using Whatman filter papper No. 5 and the filtrate allowed to evaporate to obtain crude hexane extract. The residue was allowed to dry for further extraction with ethyl acetate followed by methanol [6].

2.3 Preliminary Phytochemical Screening

Preliminary phytochemical screening of the hexane, ethyl acetate and methanol crude extracts each of the three plants were carried out based on routine practices described by Adawia *et al*. [7]; Sabri *et al.* [8]; Satheesh *et al*. [9].

2.3.1 Test for steroids and triterpenoids (Liebermann-Burchard test)

Approximately 3 mg of an extract was mixed with 3 drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was then added from the sides of the test tube and observed for the formation of a brown ring at the junction of two layers. Green coloration of the upper layer and the formation of deep red colour in the lower layer would indicate a positive test for steroids and triterpenoids, respectively.

2.3.2 Test for cardiac glycosides (Keller-Killiani Test)

About 3 mg an extract was mixed with 3 drops of conc. glacial acetic acid and diluted ferric chloride solution and mixed. Concentrated sulphuric acid was added, and observed for the formation of two layers.

Lower reddish brown layer and upper acetic acid layer which turn bluish green would indicate a positive test for glycosides.

2.3.3 Test for phenolics and tannins (Ferric chloride test)

About 2 mg each of a crude extract was dissolved in 2 mL of solvent of extraction and treated with 4 drops of ferric chloride solution. Formation of bluish black colour would indicate the presence of phenols. Generally, the formation of bluish-black colour would indicate the presence of gallic tannins and bluish-green would indicate the presence of cathechic tannins.

2.3.5 Test for flavonoids (alkaline test)

About 5 mg of an extract was added 5 mL of diluted sodium hydroxide solution. The appearance of yellow colour which would become colourless on addition of few drops of dilute hydrochloric acid would indicate the presence of flavonoids.

2.3.6 Test for saponins

The ability of saponins to produce frothing in aqueous solution and to haemolyse red blood cells was used as screening test for these compounds. Distilled water (5 mL) was added to anextract (5 mg) and strongly shaken in a test tube. Formation of a large amount of froths that would last for about 30 minutes indicated the presence of saponins.

2.3.7 Test for alkaloids

About 3 mL of an extract was mixed with 1 mLof 10% HCl in a test tube and heated for 20 minutes. This was allowed to cool and filtered; 1 mL of the filtrate wastreated with few drops of Mayer's reagent. Appearance of creamy precipitate would indicate the presence of alkaloids.

2.4 Thin Layer Chromatography

Approximately 2 mg of an extract was reconstituted with solvent of extraction and spotted on silica gel precoated plates. The extracts were drawn with capillary tubes and applied as spots on a stationary phase (silica-gel coated plate) about 1 cm from the base. These plates were developed in suitable solvent system of 9:1 (Hex/E.A), 5:5 (Hex/E.A), and 7:3 (E.A/MeOH) for hexane, ethyl acetate and methanol extracts respectively in a TLC tank. The developed plates were then heated at about 120 ^{o}C for charring to have a better vision of possible spot [10].

3. RESULT AND DISCUSSION

From the result of TLC (Table 1), it is shown samples of *V. paradoxa, C. fragrans* and *P. thonningii* contain similar chemical profile. Of all samples, hexane extracts showed about eight spots with R_f that ranged from 0.2 to 0.8. The spots were violet in colour- a characteristic of triterpenoids.

Table 1: Result of TLC Analysis

Extracts	Solvent System	No. of Spots	R_f
Hexane	9:1 (H/E.A)	8	0.2-0.8
Ethyl Acetate	5:5 (H/E.A)	4	0.3-0.6
Methanol	7:3 (E.A/MeOH)	Continous lines	-

Key: H= Hexane, E.A= Ethyl Acetate, MeOH= Methanol

Similarly, the ethyl acetate extracts of all samples cantain four spots of similar R_f values depicting similar phytochemical profile irrespective of the source of sample. However, no distinctive spot was observed on TLC plate of the methanol extracts as heavy dragging was observed (Fig.1)

From the TLC analysis, it is indicative that, hexane, ethyl acetate and methanol extracts of mistletoe contain similar phytochemical profile irrespective of plant host. This study thus, disagrees with the claim that mistletoe from particular host plant has unique medicinal values.

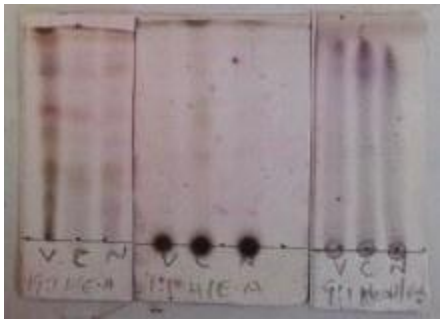

Figure 1: TLC **plates; left=hexane, middle; ethyl acetate, right; methanol extracts**

V= *V. paradoxa*, C= *C. fragrans*, N= *P. thonningii*

Table 2: Phytochemical screening result of mistletoe samples obtained from different plant host

Class of of Compounds	Hexane			Ethyl acetate			Methanol		
	V.P	***C.F***	***P.T***	***V.P***	***C.F***	***P.T***	***V.P***	***C.F***	***P.T***
Steroids/triterpenoids	+	+	+	+	+	+	-	-	-
Cardiac glycosides	-	-	-	+	+	+	+	+	+
Phenols/Tannins	-	-	-	+	+	+	+	+	+
Flavonoids	-	-	-	+	+	+	+	+	+
Saponins	-	-	-	-	-	-	-	-	-
Alkaloids	-	-	-	-	-	-	+	+	+

Key: - = Absence, + = presence

V.P= Vitellaria paradoxa, *C.F= Combretum fragrans*, *P.T= Pilostigma thonningii*

The phytochemical screening result (Table 2) showed that only steroids and triterpenoids were present in the hexane extracts of all the samples. It was shown from the result that ethyl acetate extracts contained steroids and triterpenoids, cardiac glycosides, phenols and flavonoids. The methanol extracts contained similar classes of compounds to ethyl acetate. Alkaloids were present in the methanolic extracts of the samples. Saponins were however, not detected in any of the samples. This result is comparable to that published by Tabe *et al*. [2].

4. CONCLUSION

The stem bark extracts of mistletoe contained alkaloids, flavonoids, steroids, triterpenoids, cardiac glycosides and tannins. Based on similar phytochemical profile possessed by mistletoes obtained from different plants, it can be concluded that the plant may have similar medicinal or biological effects

irrespective of their hosts. Furthermore it can be inferred from this research that, mistletoe do not obtain their phtytochemicals from host plants but rather produce by them since they undergo photosynthesis on their own. Thus, the claim by some herbalists that medicinal values of mistletoes vary due to host plant is debunked. This is the first time a study of this kind is reported on mistletoe parasitic on *Vitellaria paradoxa Pilostigma thonningii*, *Combretum fragrans.*

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