



Phytochemical Analysis and Chromatographic Studies of *Pergularia tomentosa* L. and *Mitracarpus scaber* Zucc

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Author's contribution

This whole work was carried out by author SAS.

Original Research Article

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ABSTRACT

Qualitative phytochemical analysis was carried out on *Pergularia tomentosa* and *Mitracarpus scaber* (leaves), the results revealed the presence of Tannins, Flavonoids, Alkaloids, Saponins, Glycosides, Saponin Glycosides, Cardiacglycosides, Anthraquinone and Steroids. The quantitative phytochemical analysis of the plants indicated high contents of total saponins and flavonoids compounds (4.44g% and 4.32g% saponins and 4.28g% and 4.51g% for flavonoids of *P. tomentosa* and *M. scaber* respectively). Results obtained from the analysis of variance indicated significant difference between the values of different weights of phytochemical components obtained. ($P \leq 0.5$) at 5% confidence level. Post experimental analysis using the least significant difference test (LSD = 0.69) showed that saponins and flavonoids compounds stand out different from other compounds. Extraction of the plants was carried out using organic solvents, (chloroform and N- Hexane). Column chromatographic fractionation of the active extracts of the plants revealed five fractions in each of the extracts, (CHL1 to CHL5 and HX1 to HX5). The phytochemical analysis of the active fractions indicated Saponins and Flavonoids compounds in large amount. Thin layer chromatography (TLC) of Saponins and Flavonoids compounds revealed the retention factor values (Rf) as 0.869 and 0.92 when compared with the standards gymnemic acid and quercetin as 0.862 and 0.92 respectively.

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1. INTRODUCTION

The word phytochemistry is the study of the chemical components of plant. The subject of phytochemistry to plant chemistry has developed in recent years as a distinct discipline, somewhere in between natural product, organic chemistry and plant biochemistry and is closely related to both [1]. It is concerned with the enormous varieties of organic substances that are elaborated and accumulated by plant and deals with chemical structure of these substances, their Biosynthesis, turnover and metabolism, their natural distribution and biological functions [2]

The main aim of phytochemical screening is to identify the nature of the compounds present in a given plant extract, which may be responsible for the observed biological effect. Medicinal action of some species of plant is as a result of the effect of the plants constituents on some of the organs of human body. They clear up residual symptoms or destroy the cause of the disease in most cases infectious microorganisms. They increase the body's resistance to disease, retard or ease the process of natural ageing. These components are responsible of a green therapeutic effect and they frequently serves as model for the synthetic of new medicine [3]

Phytochemical screening of the active principle and compounds contained in plants as a result were able to discover compounds such as Tannis Quinine, Flavonoids, Alkaloids, Saponins, Steroids, Scopolamine, Coumarins and Glycosides. *Pergularia tomentosa*, (milk weed) belongs to the family Asclepiadecea, it is a perinnial plant, and found mostly in the Sahara region [4]. The local name of the plant is "Fatakko" or "Malaiduwa" in Hausa, and it is mostly found in northern part of Nigeria. Information gathered from traditional healers of the northern part of Nigeria has shown that for over twenty years the milk extract from the plant leaves has been used in the treatment of skin infections, such as *Tinea capitis*. A number of researches have also been carried out on *Pergularia tomentosa* which was first discovered in Saudi Arabia, the antifungal activity of this plant was tested and results obtained shows that the plant has antifungal effect against *Aspergillus niger*. The plant was reported to have molluscidal activity and Persistent hypoglycemic effects, its isolated cardenolides have been shown to cause apoptotic cell death of Kaposis sacorma cells [5].

Mitracarpus scaber belongs to the family Rubiaceae popularly known as Madder family belonging to the Gentianales order, recently called Rubiales order. The family consists of about 500 genera and 6000 species distributed all over the world. The leaves are short and green with parallel lines, the leaves secretes watery substance which is painful when applied to the skin. The parts of the plant that are normally used is the aerial part and the leaves [6]. The medicinal uses of the plant are that the plant is an effective antifungal and also revitalizes areas of hypo pigmentation and hyper pigmentation [19]. The juice of the palnt is applied to ring worm and other fungal diseases. The crushed leaves are used as dressing for fresh cuts, wounds and ulcers. The leaf extracts of *Mitracarpus scaber* is widely used in traditional medicine practices in West Africa for the treatment of headache, toothache, amenorrhoea, dyspepsia, hepatic diseases, veneral diseases as well as leprosy. It is claimed to have antifungal and antibacterial activities [7].

Medicinal plants are of great importance to the health of individuals and communities. Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases. Plants provide abundant resources of antimicrobial compounds and have been used for centuries to inhibit microbial growth [8]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, Tannins, Flavonoids, Saponins, Glycosides and other phenolic compounds. This work was therefore, designed to determine the phytochemical compounds of *P. tomentosa* and *M. scaber* and also to separate the phytochemical compounds which appeared in large amount into fractions, and to determine the quantitative phytochemical content of each fraction using chromatographic studies [9].

2. MATERIALS AND METHODS

2.1 Sample Collection

Fresh leaves of *Mitracarpus scaber* Zucc and *Pergularia tomentosa* L. were collected around Usmanu Danfodiyo University (permanent site) Sokoto. The plants were identified and confirmed at Usmanu Danfodiyo University, Sokoto Herbarium (Botany unit, Department of Biological Science). Voucher specimens were deposited in the Herbarium. The plant materials (fresh leaves) were air dried, pulverized into a fine powder.

2.2 Extraction and Fractionation Procedure

Extraction and fractionation of the plants extract was carried out by activity guided fractionation. The procedure was carried out using ethanol-water (1:1 v/v) and different organic solvents, (Hexane, Petroleum ether and Chloroform).

Forty grams of the powdered plant materials were extracted using percolation process in 250ml distilled water and 250ml ethanol at 35°C overnight. The extract was filtered and the filtrate was evaporated to dryness and then partitioned with 250ml hexane. The extract was separated by filtration. The hexane filtrate was evaporated to dryness at 40°C to obtain residue. The remaining water ethanol extract was further partitioned with petroleum ether and chloroform using the same procedure above. The last remaining water ethanol extract was also evaporated to dryness to yield residue. The dried extracts were reconstituted in water at different concentrations of 10, 20, 40, 80 and 160 mg/ml respectively. Another extraction was carried out using 40g of procured plant material with 500 ml distilled water at 35°C overnight. The extract was filtered and evaporated to dryness, and residues were obtained in gram.

2.3 Phytochemical Screenings of the Plant Extracts

Qualitative and quantitative phytochemical analysis of different phytochemical compounds (Flavonoids, Tannins, Saponins, Alkaloids, Glycosides, and Cardiac Glycosides, Saponin glycosides, Anthraquinones, Steroids and Volatile oil) were carried out in accordance with the procedures of [10,11,12].

2.4 Column Chromatography of the Plants Extracts

This was carried out on the two most active plants (*P. tomentosa* and *M. scaber*)

Column preparation: The lower end of a glass column 10cm long and 1.5cm in internal diameter was plugged with glass wool. The plant material was poured on to the glass wool and air bubbles released was trapped with the flat end of a packed rod. The column was packed with wet silica gel by pouring the silica gel into the column in a stepwise manner. The side of the column was taped gently with a glass rod for compaction of the particles. As the silica gel settles, the column outlet was adjusted. Two (2g) of each sample was drawn onto the adsorbent and eluted with distilled water.

2.5 Determination of Quantitative Phytochemical Compounds of the Most Active Column Fractions

Quantitative phytochemical analysis of the most active column fractions was carried out according to the procedure above (2.3).

5. RESULTS

The results of phytochemical analysis of aqueous extracts of *P. tomentosa* and *M. scaber* (leaves) are shown in Table 1. The aqueous extracts of *P. tomentosa* revealed the presence of all the phytochemical constituents tested except volatile oil. Flavonoids and Saponins were present in large quantities more than the entire phytochemical constituents' detected. The amount of residues realized after the quantitative phytochemical analysis of some purified compounds of *P. tomentosa* was obtained as saponins 30.23g, alkaloids 15.3g, tannins 10.50g, cardiac glycosides 20.53g and flavonoids 31.6g

Phytochemical analysis of *M. scaber* extracts revealed the presence of all the phytochemical constituents except Cardiac glycosides and Volatile oil. Flavonoids and Saponins compounds were present in large quantities more than all the phytochemical compounds detected. The amount of residues obtained after the quantitative phytochemical analysis the plant was obtained as Saponins 35.7g, Alkaloids 10.4g, Tannins 22.21g, Cardiacglycosides 25.60g and Flavonoids 30.3g.

The phytochemical analysis of organic solvents extracts of *P. tomentosa* and *M. scaber* are shown in Table 2. The phytochemical analyses of organic solvent extracts were determined. The chloroform and hexane extracts of all the plants revealed the presence of all the phytochemical compounds screened except volatile oil. Saponins and Flavonoids compounds were present in large quantities.

Table 1. Phytochemical contents of aqueous extracts of *P. tomentosa* and *M. scaber* (Leaves)

Plant extracts	Phytochemical compounds									
	Tannins	Flavonoids	Alkaloids	Saponins	Glycosides	Saponin glycosides	Cardiac glycosides	Anthraquinones	Steroids	Volatile oil
<i>P. tomentosa</i>	++	+++	++	+++	++	+	+	+	+	-
<i>M. scaber</i>	+	+++	+	+++	++	+	-	+	+	-

Key: - = absent, + = Trace amount, ++ = presence, +++ = presence in large amount

Table 2. Phytochemical contents of organic solvents extracts of *P. tomentosa* and *M. scaber* (leaves)

Plant		Phytochemical compounds									
		Tannin	Flavonoids	Alkaloids	Saponins	Glycosides	Saponin glycoside	Cardiac glycoside	Anthraquinone	Steroids	Volatile oil
<i>P. tomentosa</i>	HX	++	++	+	+++	+	++	+	+	+	-
	PE	+	+	+	+	+	+	+	-	+	-
	CHL	++	+++	+	+++	+	++	+	+	++	-
<i>M. scaber</i>	HX	++	+++	+	+++	+	++	+	+	+	-
	PE	+	+	+	+	-	+	+	-	+	-
	CHL	++	++	+	+++	+	++	+	+	++	-

Key: - = Absence, + = Trace amount, ++ = presence, +++ = presence in large amount, HX = Hexane, PE = Petroleum ether and CHL = Chloroform

The phytochemical analysis of Hexane and chloroform extracts of *P. tomentosa* is presented in Table 3. The chloroform fraction four (CHL4) revealed the presence of all phytochemical compounds except volatile oil. Saponins and Flavonoids compounds were present in large amount with 4.44 and 4.28g % respectively. Analysis of variance (one way) Anova conducted on the results indicated that there is a significant difference between the values of different weights of phytochemical components obtained from active fractions of the column chromatography ($P \leq 0.5$) at 5% confidence level. Post experimental analysis using the least significant difference test (LSD= 0.69) showed that Saponins and Flavonoids compounds stand out different from other compounds. No significant difference ($P \geq 0.5$) was observed between Saponins and Flavonoids. No significant difference ($P \geq 0.5$) between flavonoids and glycosides. However, difference was observed ($P \leq 0.5$) between saponins and glycosides. Table 4 indicated the phytochemical analysis of Hexane and chloroform extracts of *M. scaber*. The chloroform extract of *M. scaber* revealed the presence of all the phytochemical compounds tested except volatile oil. Saponins and Flavonoids compounds were present in large amount (4.32 and 4.51g %). Similarly, analysis of variance (one way) was carried out and the results from all the extracts of indicated that there is a significant difference ($P \leq 0.5$) on various weights of the samples. Post experimental analysis using least significant difference test (LSD = 1.15) showed that the weight of Flavonoids and Saponins have outweighed all other components of the plant. However, no significant difference ($P \geq 0.5$) on weight changes was observed between Flavonoids and Saponins, Flavonoids and Tannins and Saponins and Tannins.

All the fractions revealed the presence of all the tested phytochemical compounds except volatile oil, which was absent completely. Saponins and Flavonoids compounds in all the fractions were present in large amount more than any detected phytochemical component of the plants. The thin layer chromatograph of the isolated saponins and flavonoids compounds of *Pergularia tomentosa* and *Mitracarpus Scaber* is presented in Table 5. The RF values of the compounds were 0.869 and 0.92 respectively compared with the standards Gymnemic acid (0.862) and Quarcetin (0.92) respectively.

Table 3. Quantitative phytochemical analysis of the active column chromatographic fractions of *Pergularia tomentosa* (Ingram)

	Alkaloid	Tannins	Flavonoids	Saponins	Steroids	Cardiac glycosides	Glycosides	Saponin glycoside	Anthraquinone
HX2	1.33	0.57	1.43	2.06	ND	1.54	2.44	ND	0.77
HX4	1.28	0.88	1.08	1.58	ND	1.38	1.33	ND	1.42
CHL1	1.82	1.47	3.43	3.11	ND	1.38	0.67	ND	2.30
CHL4	2.51	3.10	4.28	4.44	ND	2.09	3.20	ND	2.06

Key = 0.01-2g = Trace amount, 2-3g = present, 3-4 = present in large amount, HX2 & HX4 = Hexane fractions

Table 4. Quantitative phytochemical analysis of the active column chromatographic fractions of *Mitracarpus scaber* (Ingram)

Column fractions	Alkaloids	Tannins	Flavonoids	Saponins	Sateroids	Cardiac glycosides	Glycosides	Saponin glycoside	Anthraquinone
HX4	0.58	1.49	2.22	2.46	ND	0.36	0.48	ND	1.51
CHL1	2.03	1.88	4.51	4.32	ND	2.03	1.51	ND	1.31
CHL2	1.77	2.34	2.40	2.00	ND	0.49	2.05	ND	0.54
CHL4	1.61	1.32	2.09	1.33	ND	1.21	0.29	ND	0.91

Key = 0.01-2g = Trace amount, 2-3g = present, 3-4g = present in large amount.

HX4 = Hexane and CHL 1, 2 and 4 = Chloroform fraction of *M. scaber*, ND = Not detected

Table 5. The RF values of TLC of the purified active fractions of *Pergularia tomentosa* and *Mitracarpus scaber*

Phytochemical Compounds	RF values
Flavonoids	0.92
Quercetin (Standard)	0.92
Saponins	0.869
Gymnemic acid (Standard)	0.862

6. DISCUSSION

The phytochemical analysis of all the screened plants was determined. Both extracts of *P. tomentosa* and *M. scaber* revealed the presence of all the tested phytochemical compounds in large amount except volatile oil. Phytochemical compounds are known to possess antimicrobial properties as reported by [13,14,15]. Findings from this work agreed with the work of [16,17] who reported that plants containing Flavonoids Triterpenoids and other phenolic compounds are reported to have antimicrobial activity [3]. Showed that Saponins complexes from extracts of the climbing – ivy, *Hedera helix* L., was active against dermatophytes and yeasts. Anthraquinones and Flavonoids are used as antiseptics in certain skin diseases, example dry eczema and other fungal skin infections. This statement is in support of what was obtained in this work, where flavonoids compound happened to be one of the active components in the active plants [18].

The hexane and chloroform extracts of *P. tomentosa* and *M. scaber*, were further fractionated into different fractions using column chromatography. Both hexane and chloroform were fractionated into five (5) different fractions as (Hx1 to Hx5 and CHL1 to CHL5). The thin layer chromatography of the isolated Saponins and Flavonoids compounds of *Pergularia tomentosa* and *Mitracarpus Scaber* revealed RF values of the compounds as 0.869 and 0.92 respectively. When compared with the standards, according to [19]. Gymnemic acid (0.862) and Quercetin (0.92) respectively.

7. CONCLUSION

The results obtained for both qualitative and quantitative phytochemical analysis of the screened plants revealed the presence of all the tested phytochemical compounds. Saponins and Flavonoids compounds were present in large amount in the most active plants and this may be responsible for high activities exhibited by the two plants. Column chromatography fractionation of the active plants revealed the active fractions as chloroform fraction four and chloroform fraction one for *P. tomentosa* and *M. scaber* respectively. There was no difference between the RF values of the thin layer chromatography of the isolated Saponins and Flavonoids compounds and that of the standard values.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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