

# Antidermatophytic activities of column chromatographic fractions and toxicity studies of *Pergularia tomentosa* L. and *Mitracarpus scaber* Zucc used in the treatment of dermatophytoses

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## ABSTRACT

This research was conducted on antidermatophytic activities of column chromatographic fractions and toxicity studies of *Pergularia tomentosa* and *Mitracarpus scaber* used in the treatment of dermatophytoses. Column chromatographic fractionation of *Pergularia tomentosa* and *Mitracarpus scaber* (leaves) was investigated. The column revealed five (5) fractions, each of N- hexane and chloroform extracts. These fractions were HX1 to HX5 and CHL1 to CHL5 respectively. The antidermatophytic activities of the column fractions was tested, showed that (CHL4) and (CHL1) were active against *T.rubrum*, *T. mentagrophyte* and *M. gypseum* at 10 mg/ml. The results of toxicity studies of the plants carried out on experimental rabbits indicated that the infected rabbits treated with hexane and chloroform extracts of the two plants were effective on treatment at concentration of 10 mg/ml. A very slight erythema was observed only on rabbits treated with hexane extract of *M. scaber*. From the results it can be concluded that the two extracts of *P. tomentosa* and *M. scaber* exhibited strong antidermatophytic activities against most of the isolates tested more than the conventional antifungal drug (Griseofulvin). The high quantities of saponins and flavonoids components of the two plants might be responsible for their activities. The results of animal testing showed that the plants (leaves) extracts are safe for topical application on the skin as claimed by traditional healers.

**Keywords:** Antidermatophytic activities, column chromatography, toxicity studies, plants, dermatophytoses.

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## INTRODUCTION

Dermatophytes are fungi capable of parasitizing only keratinized epidermal structures like the superficial skin, hair and nails. They are the most common agents of fungal infections worldwide (Robert et al., 2004; Wu et al., 2009). Dermatophytes cause infection of the skin, hair and nails due to their ability to obtain nutrient from keratinized material.

Dermatophytic infections have been considered to be a major public health problem in many parts of the world. The infections are common in the developing countries, and are of particular concern in the tropics and subtropics regions where the environment is humid and warm (Guest and Sam, 1998). The reported peak incidences of dermatophytic infections occur in school aged African

and American children (Hebert, 1988). The dermatophytes belong to a group of morphologically and physiologically related molds fungi that causes superficial mycoses. The infections are commonly referred to as ring worm or tinea infections, a name which is qualified by the site affected, viz: tinea capitis (head), tinea cruris (groin) tinea unguium (nails) and that of the foot as tinea pedis (Gupta et al., 1997).

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 (Hernandez et al., 2000). Plants provide abundant resources of antimicrobial compounds and have been used for

centuries to inhibit microbial growth (Gupta et al., 2004). 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 (Rojas et al., 1992). *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 (Abere et al., 2007). The local name is Harwatsi or gudadal in Hausa, Irawo ile in Yoruba and Obuobwa in Igbo. The leaves are short and green with parallel lines, the leaves secrete watery substance which is painful when applied to the skin. The part of the plant that is normally used is the aerial part and the leaves. The medicinal uses of the plant are that the plant is an effective antifungal and also revitalizes areas of hypopigmentation and hyperpigmentation (Van-wyk et al., 1997). The juice of the plant is applied to ring worm and other fungal diseases. The crushed leaves are used as dressing for fresh cuts, wounds and ulcers (Gill, 1992). The leaf extracts of *M. scaber* is widely used in traditional medicine practices in West Africa for the treatment of headache, toothache, amenorrhoea, dyspepsia, hepatic diseases, venereal diseases as well as leprosy. It is claimed to have antifungal and antibacterial activities (Abere et al., 2007).

*Pergularia tomentosa* (milk weed) belongs to the family Asclepiadecea, it is a perennial plant, and is found mostly in the Sahara region (Gill, 1992). 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 *P. 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* (Gill, 1992). The plant was reported to have molluscicidal activity and persistent hypoglycemic effects (Hussein et al., 1999; Shabana et al., 1990) and its isolated cardenolides have been shown to cause apoptotic cell death of Kaposi's sarcoma cells (Hamed et al., 2006).

The growing interest in medicinal plants demands toxicity risk assessment of various indigenous preparations used in the treatment of diseases. Thus this work was designed to investigate the activities of *P. tomentosa* and *M. scaber*, by testing different fractions of the plants against organisms causing dermatophytoses. The selection of these plants for evaluation was based on ethnomedical information obtained from traditional healers in Sokoto.

## MATERIALS AND METHODS

### Sample collection

Fresh leaves of *Mitracarpus scaber* Zucc and *Pergularia tomentosa* L. were collected around Usmanu Danfodiyo University (permanent site) Sokoto, Nigeria. 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.

### Phytochemical screenings of the plant extracts

Qualitative and quantitative phytochemical analysis of different phytochemical compounds (flavonoids, tannins, saponins, alkaloids, glycosides, cardiac glycosides, saponin glycosides, anthraquinones, steroids and volatile oil) were carried out in accordance with the methods of (El-Olemyl, 1994; Harbone, 1973; Wall et al., 1954).

### Determination of the phytochemical compounds in large quantities

#### Determination of saponins

**Procedure:** From the powdered plant extract, 50 g was placed in a 500 ml flask containing 300 ml of 50% alcohol. The mixture was boiled under reflux for 30 min and was immediately filtered while hot through a coarse filter paper.

Two grams (2 g) of charcoal was added, the content was boiled and filtered while hot. The extract was cooled (some saponins may be separated) and an equal volume of acetone was added to complete the precipitation of saponins. The separated saponins were collected by decantation and dissolved in the least amount of boiling 95% alcohol and filtered while hot to remove any insoluble matter. The filtrate was allowed to cool to room temperature thereby resulting in the precipitation of saponins. The separated saponins were collected by decantation and suspended in about 20 ml of alcohol and filtered. The filter paper was immediately transferred to a desiccator containing anhydrous calcium chloride and the saponins were left to dry. They were weighed with reference to the weight of the extract used.

#### Determination of flavonoids

**Procedure:** Ten (10 g) of plant samples were extracted separately with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper number 42. The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed (Trease and Evans, 1978).

$$\% \text{ Flavonoid} = \frac{\text{Weight of flavonoid residue}}{\text{Volume taken}} \times 100$$

### Column chromatography of the plants extracts

This was carried out on *P. tomentosa* and *M. scaber* extracts. 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 grams (2 g) of each sample was drawn into the adsorbent and eluted with distilled water. Five fractions were obtained each from chloroform and hexane extracts. Phytochemical analysis of the eluents was carried out (Brain and Tunner, 1975).

#### Antidermatophytic activity of the most active column fractions

The antidermatophytic activity of the most active column fractions (CHL4 and CHL1) of *P. tomentosa* and *M. scaber* was carried out using agar incorporation method (dilution on solid medium) according to procedure of Zacchino et al. (1999).

#### Toxicity study

This was carried out to test for skin irritation as a consequence of the topical application of the plants extracts. The method adopted was in accordance with Anonymous (1982).

A total number of seventeen (17) Albino rabbits (young male and female rabbits) were used for the test. The animals were sourced from Sokoto Veterinary Clinic. The rabbits were caged differently under four (4) groups, five in each of the three cages and two in one cage. They were kept under experimental housing and feeding conditions in one of the breeding cages of Biological Sciences Department of the Usmanu Danfodiyo University Sokoto, for a period of five (5) days prior to experiment. The animals had free access to water and standard diet. A period of 24 h before testing, fur was removed by shaving the dorsal area of the trunk of the rabbits. The shaved experimental rabbits were infected with the isolated organisms (dermatophytes) that showed sensitivity with different extracts of the active plants (leaves), for the establishment of infections. The adjacent areas of untreated skin of each animal served as the negative control for the test. The test organisms were applied to a small area (approximately 6 cm<sup>2</sup>) of skin and covered with a gauze patch which was held in place with non-irritating tape. The patch was loosely held in contact with the skin by means of a suitable semi-occlusive dressing for the duration of four hours. At the end of the exposure period residual test substance was removed using water.

The experimental rabbits that indicated signs of infections were treated twice daily with the most active extracts (Hexane and Chloroform) of *P. tomentosa* and *M. scaber*. The extracts were applied at 10, 20, 40, 80 and 160 mg/ml. Griseofulvin was also applied at 10 and 160 mg/ml. The test substance was covered using the same procedure above. Each group of rabbits was observed for signs of erythema and oedema and response were graded at 30 to 60 min, and then at 48 and 72 h after patch removal. Dermal irritation was also graded and the results recorded. In addition to the observation of irritation, corrosivity was also observed and other histopathological findings.

## RESULTS

Column chromatographic fractionation of *P. tomentosa* and *M. scaber* (leaves) was investigated. The column revealed five (5) fractions, each of N-hexane and chloroform extracts. These fractions were HX1 to HX5 and CHL1 to CHL5 respectively. Only the active fractions were presented in the tables. The antidermatophytic activities of column chromatographic fractions of Hexane and chloroform extracts of *P. tomentosa* (leaves) are indicated in Table 1. The CHL4 fraction of the column of *P. tomentosa* was the most active fraction, the fraction

was active against the tested organisms, *T. rubrum*, *T. mentagrophyte* and *M. gypseum*) at all the concentrations used. The HXp2 and HXp4 fractions inhibited the growth of *T. rubrum* at all the concentrations used.

Table 2 showed the antidermatophytic activities of column chromatographic fractions of organic solvents extracts of *M. scaber* (leaves). Only the active fractions of the column were shown. Each fraction was tested for antidermatophytic activities at different concentrations of 10 mg/ml to 160 mg/ml, the CHL1 fraction of *M. scaber* showed significant antidermatophytic activity on the growth of some organisms tested, by showing total inhibition of growth of *T. rubrum*, *T. mentagrophyte* and *M. gypseum* from the least concentration used 10 mg/ml. CHL2 fraction of column chromatography also showed activity against *T. rubrum* and *M. gypseum*. In this study, both CHL4 and CHL1 fractions of *P. tomentosa* and *M. scaber* was more active than Griseofulvin.

Table 3 indicated the minimum inhibitory concentrations of the most active chloroform fractions (CHL4p and CHL1m) of *P. tomentosa* and *M. scaber* respectively. Both fractions of the column revealed low MIC values of 10 mg/ml against *T. rubrum*, *T. mentagrophyte* and *M. gypseum*. Griseofulvin indicated low MIC value of 10 mg/ml against only *T. rubrum*. The results of minimum fungicidal concentration MFC followed the same pattern with that of MIC (Table 4). Table 5 indicated the results of antidermatophytic activities of flavonoids and saponins contents of the most active column fractions (CHL4 and CHL1) of *P. tomentosa* and *M. scaber* (leaves).

Table 6 indicated the results of experimental rabbits infected with *T. rubrum* and treated with the Hexane and chloroform extracts of *P. tomentosa* and *M. scaber* at different concentrations of 10, 20, 40, 80 and 160 mg/ml, respectively. Both extract were active on rabbits infected with *T. rubrum* from the least concentration of the extracts used 10 mg/ml to the highest 160 mg/ml. Griseofulvin the control showed positive results on the treatment of all the rabbits infected with the organisms at concentrations of 10 and 160 mg/ml.

Table 7 indicated the results of experimental animals (rabbits) infected with *T. mentagrophyte* and treated with both hexane and chloroform extracts of *P. tomentosa* and *M. scaber* (leaves). At concentrations of 10 to 160 mg/ml, the extracts were able to treat infections on rabbits infected with *T. mentagrophyte*, from the least concentration of 10 mg/ml to the highest concentration 160 mg/ml. No response to treatment noticed on rabbits treated with Griseofulvin (positive control) at all the concentrations used.

The results of experimental rabbit infected with *M. gypseum* and treated with hexane and chloroform extracts of *P. tomentosa* and *M. scaber* is shown in Table 8. At concentrations of 10 to 160 mg/ml the extracts were able to treat infections on rabbits infected with *M. gypseum* from the least concentration 10 mg/ml to the highest concentration, 160mg/ml. There was no response to

**Table 1.** Antidermatophytic activities of column chromatographic fractions of *P. tomentosa*.

Plant leaves	Organic solvents extracts / controls	Extract conc. (mg/ml)	Growth		
			<i>T. rubrum</i>	<i>T. mentagrophyte</i>	<i>M. gypseum</i>
<i>P. tomentosa</i>	HXp2	10	-	+	+
		20	-	+	+
		40	-	+	+
		80	-	+	-
		160	-	+	-
	HXp4	10	-	+	+
		20	-	+	+
		40	-	+	+
		80	-	+	+
		160	-	+	+
	CHLp1	10	+	+	+
		20	+	+	+
		40	+	+	+
		80	-	-	+
		160	-	-	+
	CHLp4	10	-	-	-
		20	-	-	-
		40	-	-	-
		80	-	-	-
		160	-	-	-
Gs (positive control)	10	-	+	+	
	160	-	-	+	
Water -ve control			+	+	+

Key: - = Presence of growth, + = Absence of Growth, HXp2 and HXp4 = Hexane fractions 2 and 4, CHLp1 and CHLp4 = Chloroform fractions 1 and 4 of *P. tomentosa*, GS = Grisiolfulvi.

**Table 2.** Antidermatophytic activities of column chromatographic fractions of *M. scaber*

Plant leaves	Fractions/ controls	Extract conc. (mg/ml)	Growth		
			<i>T. rubrum</i>	<i>T. mentagrophyte</i>	<i>M. gypseum</i>
<i>M. scaber</i>	HX4m	10	+	+	+
		20	+	+	+
		40	+	-	+
		80	+	-	-
		160	+	-	-
	CHL1m	10	-	-	-
		20	-	-	-
		40	-	-	-
		80	-	-	-
		160	-	-	-
CHL2m	10	+	+	+	
	20	+	+	+	

**Table 2.** Continues.

	40	+	+	+
	80	+	-	+
	160	+	-	+
CHL4m	10	+	+	+
	20	+	+	+
	40	+	+	+
	80	+	-	-
	160	+	-	-
CHL5m	10	+	+	+
	20	+	+	+
	40	+	+	+
	80	+	+	+
	160	+	+	+
GS (positive control)	10	-	+	+
	160	-	-	+

Key - = Presence of growth + = Absence of Growth, HXm4 = Hexane fractions 4, CHLM 1, 2, 4 and 5 = Chloroform fractions of *M. scaber*, GS = Griseofulvin.

**Table 3.** Minimum inhibitory concentration (MIC) in mg/ml of the most active column fractions CHL4 and CHL1 of the column chromatographic fractionation of *P. tomentosa* and *M. scaber*.

Fractions	MIC values (mg/ml)/plant		
	<i>T. rubrum</i>	<i>T. mentagrophyte</i>	<i>M. gypseum</i>
CHL4p	10	10	10
CHL1m	10	10	10
Griseofulvin	10	80	-

Key: - = No MIC value, CHL4p & CHL1m = Chloroform fractions 4 & 1 of *P. tomentosa* and *M. scaber*.

**Table 4.** Minimum fungicidal concentration (MFC) of the most active column fractions CHL4 and CHL1 of *P. tomentosa* and *M. scaber*

Fractions	MFC values (mg/ml)/plant		
	<i>T. rubrum</i>	<i>T. mentagrophyte</i>	<i>M. gypseum</i>
CHL4p	10	10	10
CHL1m	10	10	10
Griseofulvin	10	80	-

Key: - = No MFC value, HL4p & CHL1m = Chloroform fractions 4 & 1 of the column chromatography of *P. tomentosa* and *M. scaber*.

treatment noticed on rabbits treated with Griseofulvin at all the concentrations used.

Grading of skin reactions due to oedema, erythema and eschar formations on infected experimental rabbits treated with different extracts of *P. tomentosa*, *M. scaber* and Griseofulvin (positive control) is shown in Table 9. Various grades of erythema, and oedema formations

such as, very slight erythema, well defined erythema, severe erythema, very slight oedema, slight oedema, moderate oedema and severe oedema were observed. From the results obtained there was no sign of erythema, eschar and oedema formations on all the experimental rabbits subjected to treatment with different extracts of the plants and griseofulvin except on rabbit treated with

**Table 5.** Antidermatophytic activities of flavonoids and saponins contents of the most active column fractions (CHL4 and CHL1) of *P. tomentosa* and *M. scaber* (leaves) growth.

Phytochemical compounds	Extract/ dug conc. (mg/ml)	<i>T. rubrum</i>	<i>T. mentagrophyte</i>	<i>M. gypseum</i>
<i>P. tomentosa</i>				
FLV	10	-	-	-
SAP	10	-	-	-
<i>M.scaber</i>				
FLV	10	-	-	-
SAP	10	-	-	-
Gs (positive control)		-	+	+

Key: FLV = flavonoids, SAP = Saponin, GS = Griseofulvin. - = Absence of growth + = Presence of growth.

**Table 6.** Effect of *P. tomentosa* and *M. scaber* extracts on rabbits infected with *T. rubrum*.

Plant extracts	Plants	Extract conc. (mg/ml)				
		GRP 1 (Rab1)	GRP 1 (Rab 2)	GRP 1 (Rab 3)	GRP1 (Rab 4)	GRPI (Rab 5)
		10	20	40	80	160
Hexane	<i>P. tomentosa</i>	IH	IH	IH	IH	IH
	<i>M.scaber</i>	IH	IH	IH	IH	IH
Chloroform	<i>P. tomentosa</i>	IH	IH	IH	IH	IH
	<i>M. scaber</i>	IH	IH	IH	IH	IH
Griseofulvin		IH				IH

Key: IH = Infection healed; GRP1 = Rabbits group one infected and treated; Rab 1 to 5 = rabbit one to five.

**Table 7.** Effect of *P. tomentosa* and *M. scaber* extracts on rabbits infected with *T. mentagrophyte*.

Plant extracts	Plants	Extract conc. (mg/ml)				
		GRP 2 (Rab1)	GRP 2 (Rab 2)	GRP 2 (Rab 3)	GRP2 (Rab 4)	GRP2 (Rab 5)
		10	20	40	80	160
Hexane	<i>P. tomentosa</i>	IH	IH	IH	IH	IH
	<i>M. scaber</i>	IH	IH	IH	IH	IH
Chloroform	<i>P. tomentosa</i>	IH	IH	IH	IH	IH
	<i>M. scaber</i>	IH	IH	IH	IH	IH
Griseofulvin		NR				NR

Key: IH = Infection healed; NR = No response to treatment; GRP2 = Rabbits group two infected and treated; Rab 1 to 5 = rabbit one to five.

hexane extract of *M. scaber* which showed positive result of very slight erythema.

## DISCUSSION

The hexane and chloroform extracts of *P. tomentosa* and *M. scaber*, that exhibited significant antidermatophytic

activities, 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 antidermatophytic activities of the fractions against the test organisms was tested and the results obtained showed that the chloroform fraction 4 (CHL4) and chloroform fraction (CHL1) of *P. tomentosa* and *M. scaber*

**Table 8.** Effect of *P. tomentosa* and *M. scaber* extracts on rabbits infected with *M. gypseum*.

Plant extracts	Plants	Extract conc. (mg/ml)				
		GRP 3 (Rab1)	GRP 3 (Rab 2)	GRP 3 (Rab 3)	GRP3 (Rab 4)	GRP3 (Rab 5)
		10	20	40	80	160
Hexane	<i>P. tomentosa</i>	IH	IH	IH	IH	IH
	<i>M. scaber</i>	IH	IH	IH	IH	IH
Chloroform	<i>P. tomentosa</i>	IH	IH	IH	IH	IH
	<i>M. scaber</i>	IH	IH	IH	IH	IH
Griseofulvin		NR				NR

Key: IH = Infection healed; NR = No response to treatment; GRP2 = Rabbits group two infected and treated; Rab 1 to 5 = rabbit one to five.

**Table 9.** Grading of skin reactions due to oedema, erythema and eschar formations on infected experimental rabbits treated with different extracts of *P. tomentosa* and *M. Scaber*.

Treatment samples	Skin reactions	1	2	3	4	5
Extracts of <i>P. tomentosa</i>	Erythema and eschar formations	-	-	-	-	-
	Very slight erythema (barely perceptible)	-	-	+	-	-
	Well defined erythema (Moderate to severe)	-	-	-	-	-
	Severe erythema (beet redness)	-	-	-	-	-
Extracts of <i>M. scaber</i>	Oedema formations	-	-	-	-	-
	Very slight oedema (Barely perceptible)	-				
	Slight oedema (edges of area well defined by definite raising)	-	-	-	-	-
	Moderate oedema (edges raised) approximately 1 milliliter	-	-	-	-	-
	Severe oedema (raised more than milliliter and extending beyond the area of exposure)	-	-	-	-	-

Key:

- 1 - Grading of skin reaction in respect of rabbits treated with hexane extract of *P. tomentosa*
  - 2 - Grading of skin reaction in respect of rabbits treated with chloroform extract of *P. tomentosa*
  - 3 - Grading of skin reaction in respect of experimental rabbits treated with hexane extract of *M. scaber*
  - 4 - Grading of skin reaction in respect of experimental rabbits treated with chloroform extract of *M. scaber*
  - 5 - Grading of skin reaction in respect of infected rabbits treated with Griseofulvin (Positive control)
- + = presence  
- = Absence

exhibited significant antidermatophytic activities on the following organism, *T. rubrum*, *T. mentagrophyte* and *M. gypseum*. The two fractions of the column proved to be the most active fractions in the organic solvent extracts (leaves) of these plants. It was found out that the organic solvent extracts of *P. tomentosa* and *M. scaber* were more potent than Griseofulvin in controlling growth of dermatophytes. Similar observations were made by Wokoma et al. (2007) and Mukhtar and Huda (2005), who found garlic and lettuce (*Pistia stratiotes*) extracts though different plant species to be more potent than Griseofulvin.

Antidermatophytic activities of isolated flavonoids and saponins compound of the column fractions (CHL4) and (CHL1) of *P. tomentosa* and *M. scaber* (leaves) exhibited significant high antidermatophytic activities against *T.*

*rubrum*, *T. mentagrophyte* and *M. gypseum*. The isolated compounds showed activity more than the convention antifungal drug Griseofulvin which served as the positive control.

Determination of skin irritation and sensitization of the active extracts of plants indicated that the infected rabbits treated with both hexane and chloroform extracts of the active plants showed positive results on treatment. Both the extracts of the plants proved more active than the conventional antifungal drug Griseofulvin, which served as the positive control. Findings from this aspect indicated that the flavonoids and saponins compounds of these plants present in large quantity may lead to the significant effect exhibited by the plants on the species of dermatophytes. F.A.O. (1989) reported that the styptic and astringent properties of saponins are useful in the

treatment of inflammation and skin eruption conditions. Grading of skin reactions in respect to the different groups of rabbits treated with the two extracts of the plants at different concentrations indicated the skin reactions and responses to treatments obtained from different groups of rabbits. Erythema, eschar and oedema formations were observed and responses recorded. Only rabbits treated with hexane extract of *M. scaber* showed positive results of very slight erythema on the infected area, this could not be necessarily because this plant is irritant but it could be due to other environmental factors that the animals were exposed to during investigations. And also couple with the fact that some of the animals died during period investigation, all these might not be necessary because of the effects of the extracts, but this may be because of other factors like change of environment, feeding method and handling. *M. scaber* as reported by Van-wyk et al. (1997) and Gill (1992) is an effective antifungal agent, and also revitalizes areas of hypopigmentation and hyperpigmentations, the crushed leaves of the plant are use in dressing fresh cuts, wounds and ulcers. Findings from this work also corresponded with the reports of Abere et al. (2007); the juice from the crushed plant *M. scaber* is known to be applied topically for the treatment of skin diseases such as ring worm, lice itching, scabies and other fungal diseases or applied to dressings for fresh cuts, wounds and ulcers.

## Conclusion

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. The two fractions when tested were active against most of the dermatophytes, which showed that the bulk of the active components were in the two fractions of the column. The toxicity studies (Animal testing) conducted indicated that all the two plants leaves extracts were safe for topical application on the skin which proved the claim made by traditional healers. The results of this research reinforce the use of the two plants in Nigerian traditional medicine for treating skin infections and underline the importance of the ethnobotanical approach for the selection of these plants for the discovery of new bioactive substances Our experience with many patients attending dermatology clinics in Nigeria is the fact that there is the reoccurrence of dermatophytosis infections after treatment with some existing drugs (Cox and Balick, 1994). The fact that the extracts of these plants inhibited agents of these infections is particularly encouraging. Also the global problem of antibiotic resistance supports the use of the two plants as alternative treatment for mycotic infections caused by dermatophytes.

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