



## Isolation and Characterization of Active Fraction of *Pergularia tomentosa* against Dermatophyte

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### ABSTRACT

*Pergularia tomentosa* is reported to have antifungal (Hassan *et al.*, 2007), anti-bacterial, (Dangoggoet *et al.*, 2002) and anti-dermatophyte activity (Gill, 1992). This work was aimed at isolating the most active fraction against some dermatophyte isolate, phytochemical screening of crude, n-hexane, chloroform and ethanol extracts indicate the presence of alkaloid, flavonoid, tannin, glycoside, saponin, and phenols in all fractions but volatile oils are absent. The main work was to isolate the active fraction, So, fractionation of was done by suspending 100g of the dried plant sample in 500ml n-hexane, chloroform and then ethanol. The minimum percentage inhibition of the three fractions (n-hexane, chloroform and ethanol) were assayed by Agar well dilution method, which shows that, chloroform fraction has highest percentage inhibition of 58.15, and 55.42% against *Malassezia* and *Trichophyton* than n-hexane 7.40 and 15.11% and ethanol extract 20.32 and 25.13% respectively. The chloroform extract was then fractionated using column chromatography, dichloromethane, ethyl acetate and ethanol were used as eluent, and five fractions were obtained. All the column chromatographic fractions were also tested for its antidermatophytic activity using the same method above, dichloromethane fraction shows highest percentage inhibition against the dermatophyte (*Malassezia* and *Trichophyton*) with the percentage inhibition 82.22 and 92.32% respectively than other five fractions of ethyl acetate: (12.22 and 34.36), (20.56 and 30.13) and ethanol fractions (17.78% and 27.03%), and (10.00% and 19.22%) respectively. The dichloromethane fraction were then subjected to: physical examination (color, physical state, TLC analysis and melting point examination), and spectroscopic techniques (IR, GC-MS) were utilized.

**Keywords:** Isolate, Chloroform, n-hexane, *Malassezia*, *Trichophyton*

### INTRODUCTION

Knowledge of chemical constituents of plant is important for the discovery of therapeutic agent (Ubani 2002). Plants have the ability to synthesize wide variety of chemical compounds that are used to perform important biological functions, and to defend the plant against attack from predators such as insect, fungi and herbivorous mammals, (Abdullah M.A. 2006). Many of these phytochemicals have beneficial effects on long term health when consumed by humans, and can be used to effectively cure human disease, (Allison Onyechere and *et al.*, 2008). In developing countries, infectious diseases remain the main cause of the high mortality rates, majority of rural dwellers have limited access to formal and adequate health services and thus heavily resort to traditional healers (WHO 1996). Indigenous herbal medicines are widely used against many infectious diseases, but only few of them have been studied chemically and biologically in order to identify their bioactive constituent (Longanga 2000, in Ubani 2012). The chemistry of plant is very important as it involves both plant biochemistry and natural product chemistry. It is concerned with many varieties of organic compounds that are accumulated, their metabolism and biological function. To fully understand the mode of action of a particular plant, one needs to study its chemical constituents that lead to phytochemical study of plant which is very essential, (Sumner and Judith, 2000).



Phytochemicals are non-nutritive plant chemicals that have protective or diseases preventive properties. It is well known that plant produces these chemicals to protect itself, but recent research demonstrate that they protect humans against diseases, (Brian FH, Thomas-Bigger J, Goodman G, 2001). Phytochemistry is a scientific discipline used to describe medicinal plant consisting on the study of how to extract, concentrate, analyse, and standardize and preserve herbal plant (Evans, W C, 2009). Plants are rich in wide variety of secondary metabolites, the most important of these metabolites include: alkaloid, tannins, steroids, terpenoids, saponins, flavonoids and glycosides which have been found in-vitro to have anti-microbial property, (Banso2001 and Cowam 1999) and (Irobi O N and et al. 1993)

*Pergularia tomentosa* (milk weed) belongs to the family *Asclepiadecea*, it is a perennial plant, and is found mostly in the Sahara and sub-Sahara Desert (Gill, 1992). The local name of the plant is "fatakko" or "malaiduwa" in Hausa, and it is mostly found in northern part of Nigeria, (Husain, H.I, and et al. 1999). It is a perennial shrub about 50-60cm high, reaching 1m in good conditions, with older woody stems around which the younger once wind. The stems are covered with erect green hairs. The leaves are opposite, entire, 1-2 cm long, heart-shaped, with long stalks that are also covered with green hairs. There are small flowers with 5 yellow-whitish free petals. The fruits are oblong, globulous follicles, covered in fleshy bristles. At the slightest touch the plant secretes a white sticky fluid from the leaves and fruits. It flowers in spring in the northern Sahara and any time of year in the central Sahara. Ecologically, *Pergularia tomentosa* is a hardy chamaephyte that shows rather wide amplitude for soil from sandy, clayey to gravelly stony sites. It is found on wadi beds as well as on gravelly plateau (regs). The plant thrives in hot deserts where the rainfall does not exceed 100 mm. (Salima and *et el* 2005).

### Traditional uses of plant

The parts of the plant used include latex leaves and roots. These are collected in spring in the northern Algerian Sahara and any time after a rainy period in the central Algerian Sahara. They are prepared as an infusion, decoction or powder and mixed with other plants, and taken by mouth or used externally, (Salima and *et el* 2005). The information gathered from traditional healers of the northern part of Nigeria has shown that for over hundred years the juice 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 the antifungal activity of this plant and results obtained shows that the plant has antifungal effect against *Aspergillus niger* (Gill, 1992). The plant was reported to have mulluscidal 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 sacorma cells. Also toxicity study shows that the plant has antidermatophytic activities against *T. rubrum* *T. mentagrophyte* *M. gpyseum*, (Shinkafi S.A 2014). (Hassan S.W and *at el* 2007), Revealed that the plant has significant nutritive value. Pharmacological studies have confirmed that the different parts of the plant have had a wide application in different folk medicine remedies, such as in case of diarrhea, bronchitis, constipation, skin diseases, tuberculosis and used as poultice, depilatory,



laxative, anthelmintic, and abortifacient. (Mossallam, and BaZaid, 2000), (Abou- Zeid et al., 2008; Clauzel, 2006). (Hammiche and Maiza. 2006). Crude alkaloids were extracted from aerial part of the plant. Newly emerged fifth instar larvae were treated by forced ingestion, results showed that the test compound has Insecticidal activity, exhibited a considerable larvicidal effect with a dose-dependent mortality (Fatma A. And et al. 2013). On the other hand, it also caused antifeeding effect, weight loss of larvae with a reduction in protein and carbohydrate contents. These results indicated that *P. Tomentosa* is a promising naturally occurring agent for locust larval control, (Fatma A. And et al. 2013) *Pergularia tomentosa* represent a source of antifungal (Hassan et al., 2007) antibacterial (Dangoggo et al., 2002), enzymes screening (Lahmar I. 2015).

## MATERIAL AND METHOD

### Sample Collections and preparation

The plant sample were obtained from Wamakko town in Wamakko Local Government, Sokoto state and was identified and confirmed at Usmanu Danfodiyo University, Sokoto herbarium (Botany Unit, department of Biological science). The sample was washed with tap water and air dried, and was pulverized in to fine powder using pestle and mortar and then sieved to removed coarse parts, the powder was stored in an air tight polythene bag, (Trease GE, Evans WC, 1978).

### Test Organism

The dermatophyte fungal strains used was clinical isolate obtained from the mycology laboratory of usmanu Danfodiyo university Sokoto; *Malassezia* (dandruff causing organism), *Trichophyton*.

### Sample extractions

The plant extracts were prepared using the solvents: n-Hexane, chloroform and ethanol. 100g of the sample were taken and homogenized with 500cm<sup>3</sup> of n-Hexane in 1000cm<sup>3</sup> conical flask, shaken for several minutes and allowed to stand overnight at room temperature, the resulting liquid were first decanted and then filtered using What man filter paper (no 1), the filtrate was evaporated on water bath. The residue was air dried in the laboratory and extracted again with the same procedure above with chloroform and then ethanol, the extract in each case was evaporated to dryness, and taken to mycology lab for anti-dermatophyte test.

### Determination of extraction yield (% yield)

The yield (% w/w) from the dried extracts was calculated as:

$$\text{Yield (\%)} = (W_1 / W_2) \times 100$$

Where  $W_1$  is the weight of the extract after evaporation of solvent, and  $W_2$  is the weight of the plant powder.

### Phytochemical analysis of Crude extracts.



Plant extract were tested for the presence of active principles such as Triterpenoids, Steroids, Glycosides, Saponins, Alkaloids, Flavonoids, Tannins, and phenol compound carbohydrate. (Harbone ).B 2005), in (Kumar S.B 2012), Following standard procedures.

### Antifungal Assays

The effect of various plant extracts on the fungal strains were assayed by Agar well dilution method. The minimum concentrations of the plant extracts to inhibit the microorganisms were also determined by micro dilution method using plant fractions serially diluted in sterile nutrient broth.

### Reagents

Savoured Dextrose Agar medium: The commercially available (HiMedia) Savoured dextrose agar medium (39g) was suspended in 100ml of distilled water. The medium were dissolved completely by boiling and was then autoclaved at 15 lbs pressure (121°C) for 15 minutes.

### Procedure

The fungal species was maintained in savored dextrose agar (SDA) medium in 90mm Petri dishes. 10, 20 and 30mg of ethanol extract was reconstructed using water and was mixed with 15cm<sup>3</sup> of SDA. The Hexane, Chloroform and ethanol extract were also prepared using the same procedure above. The 90mm Petri-dishes was filled with 20cm<sup>3</sup> final volume of SDA containing the requisite amount of diluted extract solution, the SDA was allowed to solidify in the Petri-dishes at room temperature and were then inoculated at the centre with (2x2mm) cut from the periphery of a 14 days dermatophyte fungal colonies. 15cm<sup>3</sup> of water instead of extract and 15cm<sup>3</sup> of agar was mixed together as a negative control. The treated and control Petri-dishes were incubated at room temperature for 14 days for superficial mycosis. Growth was observed each day to the last day. From these, the percentage inhibition were calculated using the relation, (Shinkafi S A. 2014);

$$I(\%) = \frac{dc - dt}{dc} \times 100$$

Where;

I; Inhibition

DC; diameter of colony of control culture

DT; diameter of colony of treated culture

The result were presented as mean  $\pm$  standard deviation of the percentage inhibitions obtained.

### Chromatography

The Column chromatography was done to isolate and purify the constituents present in the active (chloroform) extracts.

### Packing of the Column:

Column preparation: The lower end of the clean dried glass column were plugged with glass wool. The columns were packed with slurry of silica gel in dichloromethane by



pouring the silica gel slurry into the column in a stepwise manner. The side of the column were taped gently. As the silica gel settles, the column outlet was adjusted. 0.5g of sample was dissolved in a small quantity of ethanol and mixed with small amount of silica gel to adsorb the sample, the mixture were then loaded on to the silica gel in the column, followed by small amount of glass wool and then eluted with Dichloromethane, Ethyl acetate and then Ethanol. Five fractions were obtained, antifungal activity were carried out on each fraction.

### Thin Layer Chromatography

TLC is a method for analyzing mixtures by separating compounds in the mixture. It is used to determine the number of components. Small amount of the dichloromethane chromatographic fraction was dissolved in ethanol; using micro pipette, a small amount of dissolved substance was transferred to one end of the plate coated with silica gel, and allowed to evaporate leaving behind a small spot of dry substance. The bottom of the plate was placed into a shallow pool (TLC tank) of a development solvent (dichloromethane/ethanol) in the ratio 60:40, the lid was covered, the solvent then travels up the plate by capillary action to about  $\frac{3}{4}$  of the plate. The plate was removed and lines were drawn with pencil at front of the solvent. The visualization of the movement of spot was done directly. The R<sub>f</sub> value was calculated by dividing the distance travelled by the substance by the distance travelled by the solvent.

## RESULTS

**Table 1:** Result of phytochemical content of crude and organic solvent extract of *pergulariatomentosa*.

Fraction	Crude extract	n-Hexane fraction	Chloroform fraction	Ethanol Fraction
<b>Alkaloid</b>				
Dragendorff's test	++	++	+++	++
Mayer's test	++	++	++	++
Hager's test	++	++	++	-
Wagner's	++	++	++	++
<b>Flavonoid</b>				
Shinoda's test	++	++	+++	++
<b>Tannin</b>				
Alkaline test	++	-	+	+
<b>Glycoside</b>				
Fehling solution test	++	-	+	+
<b>Saponin</b>				
Foam Test	++	++	++	+
<b>Phenol</b>				
Iron III Chloride test	++	-	++	++
<b>Volatile Oil</b>				
Dilute HCl Test	-	-	-	-

(+) Presence, (-) absence of the respective phytochemicals

**Table 2:** Result of percentage mass of extract obtained.

Solvents used	% extract(w/w) obtained
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n-Hexane	4.33
Chloroform	7.67
Ethanol	5.61

**Table 3:** Result of percentage inhibition of fungal isolate by organic solvent extract of *Pergulariatomentosa*

Solvent	concentration	malassezia	<i>Trichoptyton</i>
N-Hexane	10mg 0.00±0.00%	8.00±0.60%	20mg 7.00±0.00%
	10.21±13.23%	30mg 7.40±2.31%	15.11±72.51%
Chloroform	10mg 38.08±4.49%	40.34±11.00%	20mg 40.05±13.8%
	43.08±20.15%	30mg 58.15±0.64%	55.42±33.11%
Ethanol	10mg 0.00±0.00%	11.01±22.10%	20mg 14.60±30.22%
	18.3051.10%	30mg 20.32±30.06%	25.13±33.0%

**Table 4:** Result of percentage inhibition of fungal isolate by the chromatographic fractions of Chloroform extract of *Pergulariatomentosa*

Solvent	Fractions	<i>Malassezia</i>	<i>Trichoptyton</i>
Dichloromethane	Fraction I	82.22±0.22%	92.23±0.11%
Ethyl acetate	Fraction II	12.22±0.38%	34.36±0.10%
// // //	Fraction III	20.56±0.81%	30.13±0.15%
Ethanol	Fraction IV	17.78±0.42%	27.03±0.44%
	Fraction V	10.00±0.32%	19.22±0.33%

**Table 5:** Physical properties of the isolated active fraction

Solvent Property	
Melting point	137°C
Colour	Yellow
State	Paste form

**Table 6:** TLC Result of the active fraction of *Pergularia tomentosa*

Solvent system	Components	R <sub>f</sub> Value
Dichloromethane: Ethanol	(70:30)	2
		0.83
		0.47

**Table 7:** GC-MS Result for isolated fraction

Peak Number	Retention time	% area	Base peak	Molecular ion peak
1	28.71	3.48	140	270
2	33.27	44.29	157	294



3	33.38	43.37	135	292
4	33.59	8.52	148	298
5	36.14	0.06	519	
6	36.56	0.11	179	326
7	38.08	0.06105	207	
8	38.41	0.12		519

**Table 8:** IR Result for the isolated compound

Frequency	Assignment	Intensity	Functional group
3420	N-Hstr.	Medium	Amines
2920	C-Hstr.	Strong	Alkanes
2850	CH <sub>3</sub>	Medium	Alkanes
2370	C=N	Medium	Nitriles
1725	C=O	Strong	Carboxylic Acid
1650	C=C	Medium/Weak	Alkenes
1430	N-Hbend	Medium/strong	Amines
680	C-X	Strong	Chlorides

## DISCUSSION

### Phytochemical screening.

Table 1 shows the result of phytochemical analysis of *Pergularia tomentosa* leaves crude ethanol extract and organic fractions obtained. The result revealed that the crude ethanol extract contains Alkaloid, Flavonoid, Saponin, Tannin, glycoside and phenolic compounds, while volatile oil is absent. In the organic fraction extract, it shows that the n-Hexane contains alkaloid, saponin, flavonoid and tannin. While glycoside phenol and volatile oil are absent. In chloroform extract, alkaloid, flavonoid, saponin and phenols are present while tannin, glycoside and volatile oil are absent. In the last ethanol fraction extract, Alkaloid, Flavonoid, Saponin, Tannin, glycoside and phenolic compounds are present, while volatile oil is absent. Phytochemicals found to inhibit bacteria, fungi and pest. The present of Phytochemicals in the leaf of *Pergularia tomentosa* for example, saponins have hypotensive and cardio depressant properties (Olayele MT, 2007). Glycosides are naturally cardio active drugs used in the treatment of congestive heart failure and (1999) cardiac arrhythmia. Flavonoids possess antifungal, antiviral and antibacterial activity, (Neeti 2004).

### Result of Percentage Mass of Extract

Table 2 give the result of percentage mass of extract obtained by different solvents, 100g of the plant sample extracted with n-Hexane give 4.33% of the extract, the resulting residue extracted with Chloroform gives 7.61%, lastly the chloroform residue extracted with Ethanol gives 5.61%. The percentage extract of n-hexane may be due to the low polar nature of hexane compare to chloroform.

### Result of Percentage inhibition of fungal isolate by organic solvent extract of *Pergularia tomentosa*.

The result of antifungal activity of *Pergularia tomentosa* against tested organisms were presented in table 3. The result revealed that, the chloroform extract has high activity



against the tested organism, with the percentage inhibition of  $58.15 \pm 0.64$  percent against malassesia and  $55.42 \pm 33.11$  percent against trichophyton at 30mg concentration. Hexane extract shows lowest activity against the tested organisms with the percentage inhibition of  $0.00 \pm 0.00$  percent and  $8.00 \pm 0.60$  percent at 10mg concentration, and  $0.00 \pm 0.00$  percent and  $8.00 \pm 0.60$  percent at 20mg, and  $7.40 \pm 2.31$  percent and  $15.11 \pm 72.51$  percent at 30mg against malassesia and trichophyton respectively.

#### Percentage inhibition of fungal isolate by the Chromatographic fractions of Chloroform extract of *Pergularia tomentosa*

The Chloroform extract were separated using column chromatography, each fractions obtained were tested for its antifungal activity, the result were given in table 4. Fraction 1 has nearly total inhibition against fungal isolate with total inhibition of  $82.22 \pm 0.22$  percent and  $92.23 \pm 0.11$  percent against *Malassezia* and *Trichoptyton* respectively. All other fractions show little inhibition of  $10.00 \pm 0.32$  to  $34.36 \pm 0.10$  percent against the fungal isolate.

#### GC-MS Result.

Table 5 give the summary of GC-MS analysis of the active compound isolated from the leaf of *Pergularia tomentosa*. The give 8 peaks of varying intensities, first four with high intensity and another four with low intensity. Peak 1 of GC chromatogram of retention time 28.71 give mass spectra of unknown compound with molecular ion peak at  $m/z$  270, with base peak at  $m/z$  140, and another fragment at  $m/z$  125, 110 and 95. When compare with NISTO<sub>2</sub> Reference spectral library in the MS spectra, it suggested a compound with molecular formula  $C_{17}H_{37}O_2$  Hexadecanoic acid, methyl ester. Peak 2 of GC chromatogram of retention time 33.27, give spectra of unknown compound with molecular ion peak at  $m/z$  294.47, with base peak at  $m/z$  137, with another fragments at  $m/z$  124, 109 and 95. When compare with NISTO<sub>2</sub> Reference spectral library in the MS spectra, it suggests a compound with molecular formula  $C_{19}H_{34}O_2$  9, 12-Octadecadienoic acid, ethyl ester. Peak 3 of GC chromatogram of retention time 33.28, give spectra of unknown compound with molecular ion peak at  $m/z$  292 with base peak at  $m/z$  135 and another fragments at 140, 125 and 110. When compare with NISTO<sub>2</sub> Reference spectral library in the MS spectra, it suggests a compound with molecular formula  $C_{19}H_{32}O_2$  9, 12, 15-Octadecatrienoic acid, methyl ester. Peak 4 of GC chromatogram of retention time 33.59, give spectra of unknown compound with molecular ion peak at  $m/z$  298.72, with base peak at  $m/z$  139, and another fragments at  $m/z$  124 and 109. When compare with NISTO<sub>2</sub> Reference spectral library in the MS spectra, it suggests a compound with molecular formula  $C_{19}H_{38}O_2$  Octadecanoic acid. Peak 7 show very low intensity, with GC chromatogram of retention time 38.08, give spectra of unknown compound with molecular ion peak at  $m/z$  326.43, and base peak at  $m/z$  179.5. When compare with NISTO<sub>2</sub> Reference spectral library in the MS spectra, it suggests a compound with molecular formula  $C_{12}H_5Cl_5$  2, 2, 3, 4, 5-pentachloro 1, 1-biphenyl.

#### IR Result.





Table 6 give a summary of the IR result of the active chromatographic fraction isolated from the chloroform extract of *Pergularia tomentosa*. The spectrum shows absorption at  $3420\text{cm}^{-1}$ , and  $2370\text{cm}^{-1}$  which represent N-H vibration for amine, C=N vibration for Nitrile respectively. Absorption at  $1725\text{cm}^{-1}$  represent C=O for carboxylic acid. Absorption at  $2850\text{cm}^{-1}$  and  $2920\text{cm}^{-1}$  represent  $\text{CH}_3$  vibration and C-H vibration for alkane. While  $1650\text{cm}^{-1}$  and  $680\text{cm}^{-1}$  represent C=C vibration for alkenes and chloride respectively.

## REFERENCES

- Abdullah M.A. (2006) *Medicinal and economic plant of Nupe land*. First Edition, Evans book and publication 221.
- Allison Onyechere L.N and Opara F.N (2008) Phytochemical and antimicrobial activity of extract of *Carica papaya*. Proceeding of the 21<sup>st</sup> Annual compherence of the bio technology society of Nigeria 16<sup>th</sup>- 19<sup>th</sup> july 2008, University of Abekuta, Nigeria.
- Banso A. and Olutimayin, T (2001) *Phytochemical and antimicrobial evaluation of aqueous extract of Danielle Oliver and Neuclea latifolia*, Nigerian Journal of Biotechnology 12(1) 114-118.
- Dangoggo S. M., Faruq U. Z. and Hassan L. G. 2002. *Preliminary phytochemical analysis and antibacterial activity of Pergularia tomentosa*. Sokoto Journal of Veterinary Sciences.
- Evans, W C (2009) Trease and Evans Pharmacology, 14 ed. WB sounders Company Ltd
- Gill LS, 1992. *Ethnomedicinal Uses of Plants in Nigeria*. Uniben Press. pp: 78-124.
- Harbone, J.B (1973) *Phytochemical Methods, A Guide of Mordan Techniques of Plant Analysis*, 3<sup>rd</sup> Chapman and Hall pp7-11.
- H. W Hassan and etel (2007). *Nutritive value. Phytochemical and antifungal property of pergularia tomentosa*. International journal of phamacology. 3(4) 334-340.
- Hassan S.W., Umar R.A., Ladan M.), Nyemike P., Wasagu R.S.U., Lawal M. and Ebbo A.A. 2007. *Nutritive Value, Phytochemical and Antifungal Properties of Pergularia tomentosa L. (Asclepiadaceae)*. International Journal of Pharmacology 3 (4); 334-340.
- Hamliche H. and Maiza K. 2006. Traditional Medicine in Central Sahara: Pharmacopoeia of Tassili N'ajjer. *Journal Of Ethnopharmacology*, Vol 105, P. 358-367
- Irobi O N and Daramola, S O (1993), *Antifungal activity of crude extract of mitracarpus villosus*, Journal of Ethnopharmacology, Vol. 40 p137-140
- Kalsi, p s (2004). *Spectroscopy of organic compounds*, New age international Ltd, 4835/24. Ansari road, Daryaganj, New delhi, India.
- Longanga, O A, Verduyck, A. and Fariers. A (2000) In Ubani 2012. *Contribution to the ethno botanical, phytochemical and pharmacological studies of traditional used plants in the treatment of dysentery and diarrhea in Lomela area, Democratic Republic of Congo (DRC)*. Journal of Ethnopharmacology 1(3): 411-423
- Mossallam H.A. and Bazaid S.A. 2000. An Illustrated Guide to The Wild Plants of Taif. Kingdom of Saudi Arabia. Publication Committee for Tourism Activation-Taif, Saudi Arabia.

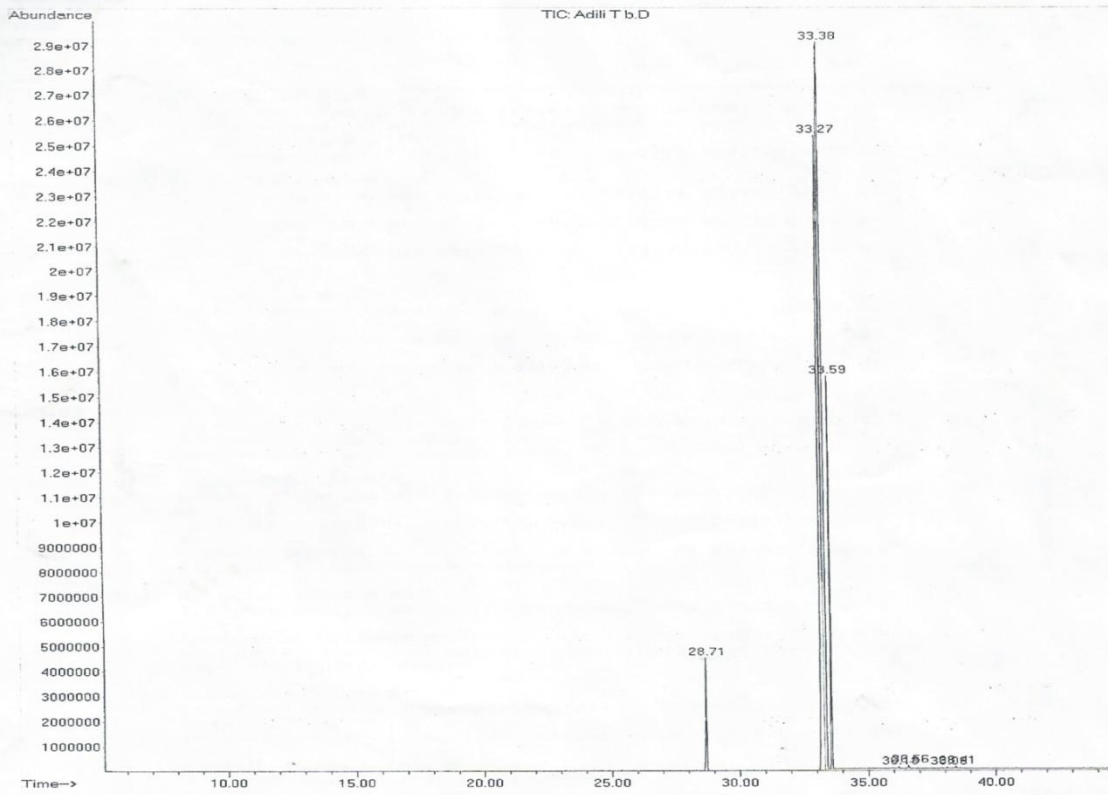


- Olayele MT, Cytotoxicity and antibacterial activity of methanolic extract of *Hibiscus sabdariffa*. *Journal of Medicinal Plants Research*.2007; 1: 9–13.
- Salima Benhaou, and Richchmdi Zainab Ghrabi 2005. *A guide to medicinal plant in Africa*. IUCN, pp268.
- Shabana MM, Muhom YW, Genenah AA, Aboutabi EA, Amker HA 1990. *Study into wild Egyptian plants of potential medicinal activity*. Ninth communication. Hypoglycaemic activity of some selected plants in normal fasting and alloxanised rats. *Arch Exp Vet. Med*, 44:389-394.
- Shinkafi S A. (2014) *Antidermatophytic activities of column chromatographic fractions and toxicity studies of Pergularia tomentosa L. and Metacarpus scaber Zucc used in the treatment of dermatophyte*. *Advancement in Medicinal Plant Research*, Vol 2(1) pp 7-15
- Trease GE, Evans WC, 1978. *Pharmacognosy*. 11th Ed. Baclliere Tindall London p 333.
- Ubani. C S, Oje. OA, Ihekogwo, Fin. P., Eze. E.A and Joshua. P E. (2002) *Effect of Varying soil minerals and phytochemical parameters on Antimicrobial susceptibility of Metacarpus villosus ethanol extract, using samples from south east and south southern region of Nigeria*. *Electronic Journal of Environmental, Agricultural and food chemistry*, 11(6) 630-637.
- WHO. SWG (1996). *Resistance to antimicrobial agent; A Bulletin of the World Health Organization*.
- WHO (1978) *The promotion and development of traditional medicine* Technical Report No 622 Geneva
- Hussaini, H.I, Alrajy D. and Hashem, S.M (1999). Molluscidal activity of *Pergularia Tomentosa* (L). Methyl and Methplacarb, against Land Snails. *International Journal of Pest Management*
- Abou-Zeid A.M., Altalhi A.D. and Abdu El-Fattah R.I. 2008. Fungal Control Of Pathogenic Fungi Isolated from Some Wild Plants in Taif Governorate, Saudi Arabia, *Mal. J. Microbial*. Vol 4(1), 30-39.
- Fatma Acheuk, Bahia Doumandji-Mitiche(2013) Insecticidal activity of alkaloids extract of *Pergularia tomentosa* (Asclepiadaceae) against fifth instar larvae of *Locustae migratoria cinerascens* (Fabricius 1781) (Orthoptera: Acrididae). *International Journal of Science and Advanced Technology (ISSN 2221-8386)*
- Harbone JB, 1973. *Phytochemical methods. A guide to modern techniques of Plant Analysis*. 3rd Chapman and Hall, London pp 7-14, 85, 200 and 280.
- Cowan, M.M (1999). *Plant Product as an antimicrobial agent*. *Clinical Microbial, Rev*;12:564-582

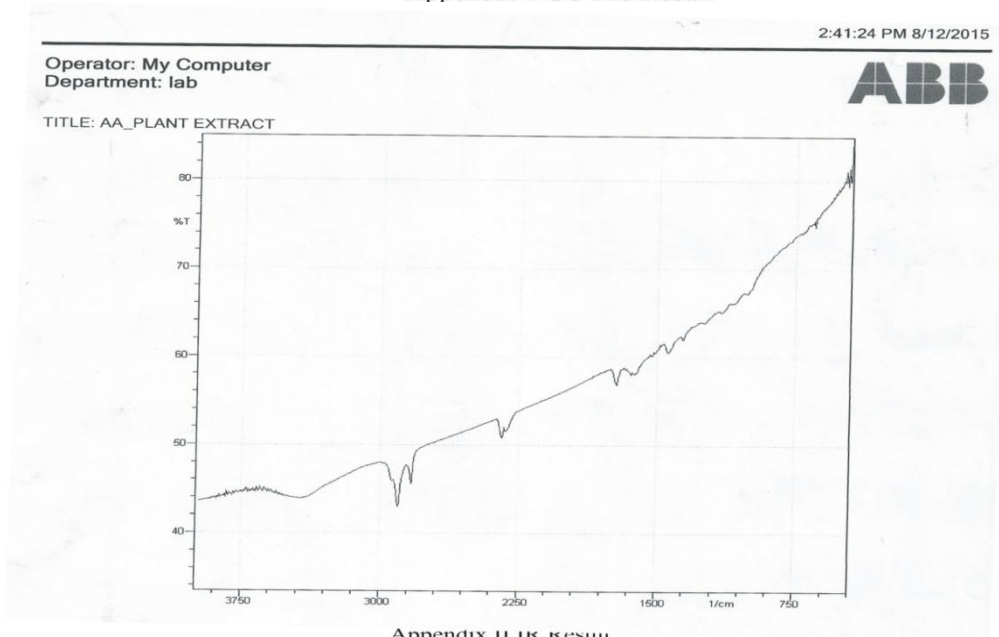


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Appendix 1 GC-MS Result



Appendix 11 IR Result