IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736.Volume 9, Issue 9 Ver. II (September. 2016), PP 118-131
www.iosrjournals.org

Activity-Guided Isolation and Antimicrobial Assay of A Flavonol From Mitracarpus Verticillatus (Schumach. & Thonn.) Vatke

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Abstract:*The antimicrobial activity of a methanol extract of Mitracarpus verticillatus was evaluated against some bacteria and dermatophytes. The Mitracarpus verticillatus methanol extract possesses both antibacterial and antifungal activities. This extract was subsequently fractioned and the three different fractions were screened for antibacterial activity and secondary metabolites. Methanol extract residue displayed a higher degree of antibacterial activity, than the other two fractions (dichloromethane soluble fraction and ethyl acetate soluble fraction). The fractions were all positive for alkaloids, glycosides, steroids, and cardiac glycosides. Methanol extract residue showed the presence of tannins, saponins, flavonoids and phlobatannins; while anthraquinones and terpenes were detected in the other two fractions. A compound was isolated via thin layer chromatographic method from the methanol extract residue. The ultraviolet and visible spectrophotometric and infrared spectroscopic studies of this isolated compound suggest it to be flavonol.*

Keywords -*Antibacterial Activity, Antifungal, Mitracarpus verticillatus, Mitracarpus verticillatum, Flavonol*

I. Introduction

*Mitracarpus verticillatus*is an annual herb which grows as a weed. It is half-woody at the base, erect, with terete branches, from a few inches to 2ft high. The flowers are white and densely crowded at the nodes. It has lanceolate leaves and reproduces by seeds. *M. verticilatus* is a common weed in upland areas from the forest to the savanna zones. It is wide spread in tropical Asia and Africa. The plant has other names such as *Mitracarpum verticillatum* VATKE, *Mitracarpus scaber* ZUCC,*Staurospermum verticillatum* SCHUM AND THONN,*Mitracarpus villosus* (SW)DC,*Mitracarpus scabrum*,*Mitracarpum scabrum* and *Spermacoce hirta* L. [1-3].The plant is employed in traditional medicine in Senegal for the treatment of sore throat and leprosy. In Nigeria, Hausas and Yorubas use it for parasitic conditions: for itch, ringworm and craw-craw. It is also used as an antidote to arrow poison. It is widely employed in traditional medicine in west Africa for headaches, toothaches, amenorrhea, dysperpsia, hepatic diseases, venereal diseases and leprosy. The juice of the plant is also applied topically for the treatment of skin diseases – infectious dermatitis, eczema and scabies [3-5]. The National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria, has formulated an extract of the plant into a cream, Niprifan®, which is a highly effective topical antifungal agent. Likewise, in Mali, a lotion and a skin ointment made with the aerial part of the plant are used for skin diseases and infections [5]. The use of *M. verticillatus*in traditional medical care for treatment of infections is well documented. There are also quite a number of literature reports on its chemistry and pharmacology [6-11]. However, this work was aimed at investigating the most potent fraction of the aerial part of the plant with focus on isolation and determination of chemical structures of the organic metabolites in the active fraction.

II. Materials and Method

2.1 Plant Material

Fresh aerial part of the plant was collected from Bauchi Bassa, Nigeria, in April 2006. The plant was authenticated by Dr Ibrahim Abdulkareem of Savana Research Centre, Forestry Research Institute of Nigeria, Zaria. The plant material was air dried for about four weeks and pulverized.

2.2 Extraction

Extraction was carried out for 24hours using a Soxhlet extractor. Methanol was used as the solvent. The extract was concentrated to dryness using a rotary evaporator.

2.3 Fractionation of Crude Extract

50g of the crude Methanol Extract was dissolved in 40ml of dichloromethane and filtered after decanting.This process was repeated 12 times. The 12 portions were combined and concentrated to dryness using a rotary evaporator. This concentrate was labelled Dichloromethane Soluble Fraction (DSF).The insoluble

fraction from the above process was dissolved in 40ml ethylacetate and filtered after decanting. This was repeated 15times. The 15 portions were combined and concentrated to dryness using a rotary evaporator. This concentrate was labelled Ethylacetate Soluble Fraction (ESF). The remaining insoluble fraction from above was then labelled Methanol Extract Residue (MER).Each of the above fractions including the residue was tested for antimicrobial property.

2.4 Phytochemical Screening of Fractions

The Dichloromethane Soluble Fraction (DSF), Ethylacetate Soluble Fraction (ESF) and Methanol Extract Residue (MER) were separately tested for the presence of Tannins, Saponins, Cardiac glycosides, Steroidal ring, Alkaloids, Flavonoids, Anthraquinones, Terpenoids, Carbohydrates, Glycosides, Phlobatannins and Resins using standard procedures [12-16].

2.5.1 Detanning of the Methanol Extract Residue (MER)

MER was completely detanned by soxhlet extraction for 12 hours using a thimble and acetone (as solvent). The Detanned Extract (DE) was concentrated to dryness using a rotary evaporator and tested for the presence of flavonoid. DE was positive for lead acetate test, sodium hydroxide test, ferric chloride test and Shinoda test.

2.5.2 Chromatographic Analysis of the Detanned Extract (DE)

The Detanned Extract was subjected to further purification. Analytical thin Layer Chromatographic (TLC) analysis was carried out using Chloroform-Methanol (90:10v/v), Chloroform-Methanol (80:20v/v), Chloroform-Methanol (70:30v/v), Chloroform-Methanol (60:40v/v) and Chloroform-Methanol (95:5v/v) as developing solvents. A small quantity of the Detanned Extract was dissolved in acetone to make a dilute solution. The solution was spotted using a capillary tube. The spotted plates were allowed to air-dry. The plates were developed in different solvent systems and observed in daylight and in Ultraviolet light. The plates were then treated with ammonia solution vapour and 5% methanolic aluminum chloride solution separately to locate the components resolved by the various solvent systems.

TLC plates of 0.5mm thickness were prepared and used for analytical thin layer chromatography. Run time was noted for each solvent mix. Developed plates were viewed under the UV light to detect spots, after which they were sprayed with conc. ammonia solution and 5% methanolic aluminium chloride (on separate plates) respectively. The various colours and R_f values of the spots were noted.

Solvent system $CHCl_3$-MeOH (90:10 v/v) gave the best resolution (six spots). Therefore, preparative TLC was then carried out using silica gel plates of 1mm thickness and $CHCl_3$-MeOH (90:10 v/v) solvent mix. They were each run for a period of 1hr 17mins and with the exact condition and format used to develop the analytical (reference) plate. The preparative TLC plates were matched with the analytical (reference) plates so as to identify the various bands. The preparative plates showed six bands which were scraped into respective beakers, extracted with methanol, filtered and then concentrated to dryness giving six fractions.Flavonoid tests were carried out on each fraction. Only one fraction tested positive. The fraction that tested positive for flavonoid was further subjected to analytical TLC, and it gave only one component.

2.6 Spectroscopic Analysis

A very dilute solution of the isolated flavonoid fraction in methanol was used to run the UV-Visible spectrum;after which, shift-reagents were employed. Thus, sample with 3 drops of 5% methanolic aluminum chloride and sample with 5% methanolic aluminium chloride and 3 drops of 2M HCL were used.Powdered sodium acetate and boric acid were also used as shift reagents.Flavonoid fraction solution in methanol was used to run the IR.1mg and 400mg of fraction and KBr respectively were mixed to make KBr disc which was used to repeat the IR analysis.

2.7 Biological Screening of the Extract

The methanol extract, MER, ESF and DSF were tested for antimicrobial activity against some disease causing microorganisms using standard procedures [17,18]. All glassware used(beakers, test tubes, universal bottles, macarney bottles and bijour bottles), cork-borer and wire loop were all sterilized.

2.7.1 The Test Organisms Used

The bacteria used in this research are *Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa, Proteus mirabilis* and *Salmonella parathyphi* A. They are clinical isolates obtained from School of Medical Laboratory Technology, NVRL, Vom. The fungi used are clinical isolates collected from the Dermatophilosis Research Centre, NVRL, Vom. They are: *Microsporum audouinii, Trichophyton rubrum, Trichophyton violaceum, Aspergillus fumigatus, Aspergillus flavus and Trichophyton mentagrophytes.* All the fungi used are dermatophytes.

2.7.2 Preparation of Test Materials

Extracts

Stock solutions of 200mg/ml of the various extracts were prepared by dissolving 2g of each extract in 10ml methanol sterilized universal bottles. From these stock solutions, further dilutions were made (doubling dilutions) to give lower concentrations as follows: 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml 3.125mg/ml, 1.56mg/ml and 0.78mg/ml. Other concentrations were also prepared: 600mg/ml, 500mg/ml, 400mg/ml and 300mg/ml were prepared by dissolving 6g, 5g, 4g, and 3g of the extract in 10ml methanol respectively. These solutions of the extracts were kept in the refrigerator for further use.

Nutrient Agar (NA)

This was prepared by dissolving 28g of the agar in 1litre of distilled water. The solution was sterilized in an autoclave at 121°C for 15minutes and allowed to cool to about 45°C before pouring into the sterilized Petri-dishes. 20ml of the solution was poured into each Petri-dish. Prepared plates were kept in the incubator overnight to check for contamination.

Sabouraud Dextrose Agar (SDA)

This was prepared by dissolving 65g of the SDA in 1litre of distilled water. The solution was then sterilized by autoclaving at 121°C for 15minutes. It was allowed to cool to about 45°C. 20ml of the cool solution and extracts were poured into sterilized Petri-dishes.

2.7.3 Antimicrobial Assay

Inoculation of Bacteria

The uncontaminated nutrient agar plates were inoculated with the bacteria by streaking the surface of the agar with a loop-full of solution of the microorganisms. A platinum wire loop was used for this purpose. A loop-full usually contain about 10^6 bacteria.

Application of Extract

Six wells were bored on the inoculated nutrient agar plates using a cork borer which was constantly sterilized. 50µl of different concentrations of the extract were introduced into the wells using a micro pipette and sterile sharps. 50µl of methanol which was the solvent used to prepare the solution of the extract, was introduced into one of the wells, this served as a control. 50µl of 2.5mg/ml of streptomycin was also introduced into one of the wells. This served as the standard chemotherapy. The plates were incubated at 37°C overnight and observed for zones of inhibition around the wells.

Inoculation of Dermatophytes

From the stock solution of extract already prepared 1ml each of 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml were transferred into sterile plates containing 20ml of SDA to prepared 9.5mg/ml, 4.8mg/ml, 2.4mg/ml, 1.2mg/ml and 0.6mg/ml respectively. The plates with mixtures (SDA + extract) were incubated overnight to check for contamination. Plates for control had no extract mixed into it. After incubation and drying in the incubator, the dermatophytes were introduced into the centre of the plates by seeding (planting the fungi in the middle of the plate). The inoculated plates were incubated at room temperature. The results were measured after 2,5,7, and 9 days.

III. Results and Discussion

3.1 Antimicrobial Result

The results of the antibacterial activity of the crude methanol extract of *Mitracarpusverticillatus* (Tables 1 and 2) show that the plant significantly inhibited the growth of all the test organisms from a concentration of 100mg/ml and above. The most inhibited of all the bacteria tested is *Staphyloccus aureus* with zone of inhibition of up to 36mm.

Table 1: Result of the Antibacterial Activity of the Crude Methanol Extracts of*Mitracarpus verticillatus*

Bacteria	Zone of Inhibition * (mm)						
	Extract Dilution (mg/ml)				STD	Solvent	Control
	200	100	50	25			
Proteus mirabilis	13.0	12.5	10.0	NI	11.0	NI	TG - NI
Bacillus cereus	10.0	8.0	NI	NI	14.0	NI	TG - NI
Staphylococcus aureus	17.5	16.0	13.0	9.5	29.0	NI	TG - NI
Salmonella parathyphi A.	10.0	9.0	NI	NI	34.0	NI	TG - NI
Pseudomonas aeruginosa	11.0	8.0	8.0	NI	18.0	NI	TG - NI

KEY:NI-No Inhibition TG-Total Growth STD-Streptomycin (2.5mg/ml)

*The values in the tables are average values of four readings

Table 2: Results of Antibacterial Activity of the Crude Methanol Extract on *Mitracarpus verticillatus*

Bacteria	Zone of Inhibition * (mm)						
	Extract Dilution (mg/ml)				STD	Solvent	Control
	600	500	400	300			
Proteus mirabilis	15.0	15.0	14.0	13.5	14.0	NI	TG - NI
Bacillus cereus	14.0	12.0	12.0	12.0	20.0	NI	TG - NI
Staphylococcus aureus	36.0	36.0	28.0	24.0	30.0	NI	TG - NI
Salmonella parathyphi A.	13.0	12.0	10.0	10.0	26.0	NI	TG - NI
Pseudomonas aeruginosa	13.0	13.0	12.0	12.0	25.0	NI	TG - NI

Key:NI-No Inhibition TG-Total Growth STD-Streptomycin (2.5mg/ml)
*The values in the tables are average values of four readings

From the results of the antifungal activity of the crude methanol extract (Tables 3, 4, 5 and 6), it can be observed that the plant greatly inhibited the growth of the test organisms even at concentration as low as 0.6mg/ml. comparing the growth diameters of the control with those containing the crude extract, it can be deduced that at higher concentrations, the growth of the organisms may be totally inhibited.

Table 3: Results of Antifungal Activity of the Crude Methanol Extract of *Mitracarpus verticillatus* Day 2

Fungi	Diameter of Growth (mm)					
	Extract Dilution (mg/ml)					Control
	9.5	4.8	2.4	1.2	0.6	
Microsporum audouinii	MG	4.0	8.0	11.0	12.0	16.0
Trichophyton rubrum	9.0	-	-	-	-	17.0
Trichophyton violaceum	NG	NG	NG	NG	NG	NG
Aspergillus fumigatus	NG	NG	NG	NG	NG	NG
Aspergillus flavus	NG	NG	NG	NG	NG	NG
Trichophyton mentagrophytes	NG	NG	NG	NG	NG	NG

Key: NG-No Growth MG-Minimal Growth

Table 4: Results of Antifungal Activity of the Crude Methanol Extract of *Mitracarpus verticillatus* Day 5

Fungi	Diameter of Growth (mm)					
	Extract Dilution (mg/ml)					Control
	9.5	4.8	2.4	1.2	0.6	
Microsporum audouinii	14	15	20	22	27	39
Trichophyton rubrum	29	-	-	-	-	51
Trichophyton violaceum	NG	NG	MG	MG	5	10
Aspergillus fumigatus	MG	NG	12	13	11	25
Aspergillus flavus	14	14	14	15	17	38
Trichophyton mentagrophytes	NG	NG	14	15	19	19

Key:NG-No Growth MG-Minimal Growth

Table 5: Results of Antifungal Activity of the Crude Methanol Extract of *Mitracarpus verticillatus* Day 7

Fungi	Diameter of Growth (mm)					
	Extract Dilution (mg/ml)					Control
	9.5	4.8	2.4	1.2	0.6	
Microsporum audouinii	19	18	22	25	35	42
Trichophyton rubrum	32	-	-	-	-	60
Trichophyton violaceum	MG	13	9	14	14	19
Aspergillus fumigatus	15	10	16	17	15	32
Aspergillus flavus	21	19	20	20	26	60
Trichophyton mentagrophytes	NG	10	23	17	27	33

Key:NG-No Growth MG-Minimal Growth

Table 6: Results of Antifungal Activity of the Crude Methanol Extract of *Mitracarpus verticillatus* Day 9

Fungi	Diameter of Growth (mm)					
	Extract Dilution (mg/ml)					Control
	9.5	4.8	2.4	1.2	0.6	
Microsporum audouinii	20	19	25	25	34	45
Trichophyton rubrum	36	-	-	-	-	64
Trichophyton violaceum	12	18	11	19	19	22
Aspergillus fumigatus	19	13	17	20	18	37
Aspergillus flavus	27	25	24	21	29	81
Trichophyton mentagrophytes	NG	16	29	20	33	40

Key:NG-No Growth MG-Minimal Growth

After fractionating the crude methanol extract to three fractions and fractions tested for their antibacterial properties, it was observed that all the fractions have antibacterial activities but in varying degrees. The methanol extract residue was the most potent with zones of inhibition between 9-27mm. It also showed inhibition for all the test organisms including *Pseudomonas aeruginosa*, which is known to be a very recalcitrant gram-negative organism (Table 7). The antibacterial activity of the dichloromethane soluble fraction is next to methanol extract residue with zones of inhibition in the range between 8 – 24mm and like Methanol Extract Residue, it showed inhibition for all the test organisms (Table 8). Ethylacetate soluble fraction (Table 9) showed weak inhibition against *Bacillus cereus* even at concentration of 200mg/ml (11mm zone of inhibition). The range of the zones of inhibition for all the organisms is 9-19mm.

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Table 7: Result of Antibacterial Activity of Methanol Extract Residue (MER)

Bacteria	Zone of Inhibition * (mm)						
	Extract Dilution (mg/ml)				STD	Solvent	Control
	200	100	50	25			
Proteus mirabilis	16.0	13.5	11.5	9.0	12.5	NI	TG – NI
Bacillus cereus	18.0	16.5	10.5	10.0	22.0	NI	TG – NI
Staphylococcus aureus	27.0	15.5	14.5	11.0	20.0	NI	TG – NI
Salmonella Parathyphi A.	21.0	17.5	10.5	10.5	20.0	NI	TG – NI
Pseudomonas aeruginosa	15.0	15.0	12.5	10.5	21.5	NI	TG – NI

Key: NI-No Inhibition TG-Total Growth STD-Streptomycin (2.5mg/ml)
*The values in the tables are average values of three readings

Table 8: Result of Antibacterial Activity of Dichloromethane Soluble Fraction (DSF)

Bacteria	Zone of Inhibition * (mm)						
	Extract Dilution (mg/ml)				STD	Solvent	Control
	200	100	50	25			
Proteus mirabilis	14.0	12.0	10.0	10.0	14.0	NI	TG – NI
Bacillus cereus	19.0	18.5	9.0	NI	21.0	NI	TG – NI
Staphylococcus aureus	24.0	19.0	15.0	14.0	30.0	NI	TG – NI
Salmonella Parathyphi A.	16.0	11.0	11.0	8.0	28.0	NI	TG – NI
Pseudomonas aeruginosa	15.0	12.0	10.0	NI	23.0	NI	TG – NI

Key: NI-No Inhibition TG-Total Growth STD-Streptomycin (2.5mg/ml)
*The values in the tables are average values of three readings

Table 9: Result of Antibacterial Activity of Ethylacetate Soluble Fraction (ESF)

Bacteria	Zone of Inhibition * (mm)						
	Extract Dilution (mg/ml)				STD	Solvent	Control
	200	100	50	25			
Proteus mirabilis	13.0	10.5	9.0	9.0	14.0	NI	TG – NI
Bacillus cereus	11.0	NI	NI	NI	20.0	NI	TG – NI
Staphylococcus aureus	19.0	15.5	12.5	10.5	22.5	NI	TG – NI
Salmonella Parathyphi A.	19.0	18.5	18.5	16.0	19.5	NI	TG – NI
Pseudomonas aeruginosa	14.0	11.5	11.0	10.0	24.0	NI	TG – NI

Key: NI-No Inhibition TG-Total Growth STD-Streptomycin (2.5mg/ml)
*The values in the tables are average values of three readings

3.2 Phytochemicals

It can be deduced from the phytochemical analysis that the methanol extract residue contains more metabolites than ethylacetate and dichloromethane soluble fractions (Table 10). The methanol extract residue showed presence of tannins, saponins, cardiac glycosides, steroids, alkaloids, flavonoids, phlobatannins, while anthraquinones, terpenes and resins were absent. It is evident that the organic metabolites present in the methanol extract residue are very polar compounds which are more soluble in water [19,20]. The presence of the phenolic compounds in methanol extract residue could account for its high antibacterial activity. Ethylacetate and dichloromethane showed presence of anthraquinones and terpenes which were absent in methanol extract residue.The three fractions showed abundance of alkaloids. The presence of alkaloids may have been partly responsible for the weak antimicrobial activity exhibited by the ethyl acetate and dichloromethane soluble fractions.

Table 10: Results of Phytochemical Screening Tests

Test	Methanol extract residue	Ethylacetate soluble fraction	Dichloromethane soluble fraction
Test for Tannins: Ferric Chloride test	+++	-	-
Test for Saponins: Froth test	+	-	-
Test for Cardiac Glycosides: Keller – Kelliani test	+++	++	++
Test for Steroidal ring: Salkowski's test	+++	++	++
Test for Alkaloids: Wangner's reagent	++	+++	+++
Mayer's reagent	+++	+++	+++
Test for Flavonoids: Shinoda Test Lead Acetate Test Sodium Hydroxide Test Ferric Chloride Test	 +++ +++ +++ +++	 - - - -	 - - - -
Test for Anthraquinones Borntrager's Test	-	++	+++
Test for Terpenes Liebermann – Burchard Test	-	++	+++
Test for Carbohydrates: Molisch reagent	+++	+	-
Conc. H_2SO_4	+++	+	+
Test for Glycosides: Feliling's reagent	+++	+	+
Test for Phlobatannins: 1% Aqueous HCL Test	+++	-	-
Test for Resins: Acetic anhydride+Conc.H_2SO_4	-	-	-

Key: +++ Abundant ++ Present + Trace - Absent

3.3 TLC Result

Thin layer chromatographic analysis of the detanned methanol extract residue on silica gel plates with $CHCl_3$: MeOH (90:10v/v) solvent system showed the presence of 6 major components. Treatment of the plates with detecting reagents (5% methanolic aluminium chloride solution) confirmed one of the components as a flavonoid (Table 11)

Table 11: Thin Layer Chromatographic Analysis of the Detanned Extract (DE)

Mobile Phase	R_f Values of Components	Colours in UV Light		
		Alone	With ammonia	With 5% methanolic aluminium chloride
$CHCl_3$-MeOH 90:10v/v	6 components:		2 components	4 components
	0.6	Dark brown	Bright yellow-green	Yellow
	0.17	Pink		
	0.37	Pink		Pink
	0.59	Pink		
	0.88	Blue	Sky blue	Blue
	0.96	Red		Red
$CHCl_3$-MeOH 80:20v/v	5 components:		2 components	3 components
	0.18	Dark brown	Bright yellow-green	Yellow
	0.35	Pink		
	0.60	Pink		
	0.91	Blue	Sky blue	Blue
	0.97	Red		Red
$CHCl_3$-MeOH 70:30v/v	4 components:		2 components	3 components
	0.21	Dark brown	Bright Yellow-green	Yellow
	0.815	Pink		

	0.91	Blue	Sky blue	Blue
	0.99	Red		Red
$CHCl_3$-MeOH 60:40v/v	4 components:		2 components	3 components
	0.22	Dark brown	Bright yellow-green	Yellow
	0.80	Pink		
	0.87	Blue	Sky blue	Blue
	0.97	Red		Red
$CHCl_3$-MeOH 95:50v/v	5 components:		2 components	3 components
	0.00	Dark brown	Bright yellow-green	Yellow
	0.10	Pink		
	0.24	Pink		
	0.77	Blue	Sky blue	Blue
	0.97	Red		Red

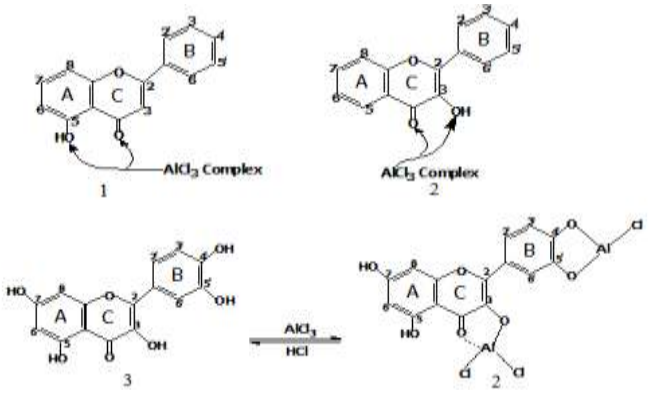
3.4 Result of Spectroscopic Analysis

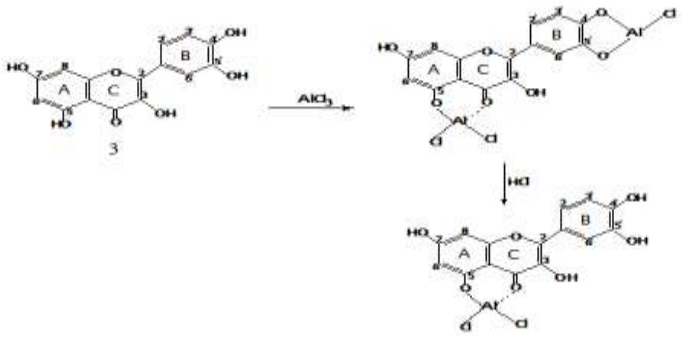
The flavonoid fraction isolated using preparative thin layer chromatographic analysis showed two bands in the ultraviolet and visible regions of the spectrum (Table 12). The two bands are Band I(355nm) and Band II (255nm). This further suggests that the isolated compound is indeed a flavonoid, since it exhibits the characteristic two major bands [19,21]. The wavelengths of the absorption maxima are within the region for flavonols (350 – 390nm and 250 – 270nm) [19,22]. Therefore, the compound maybe a flavonol.

Table 12: UV and Visible Spectral Data of the Isolated Flavonoid Fraction

Methanol solution with	Absorption maxima (nm)		Remarks
	Band I	Band II	
Sample	355	255	Within flavonol range
Sample + 3 drops of 5% $AlCl_3$	420	270	65nm Bathochromic shift (Band I) and 15nm Bathochromic shift (Band II)
Sample + 3 drops of 5% $AlCl_3$ and 3 drops of 2M HCl	355, 400	270	65nm and 20nm hypsochromic shift (Split of Band I)
Sample + 3mg powdered NaOAc	Weak band	270	15nm Bathochromic shift (Band II)
Sample + 3mg powdered NaOAc and H_3BO_3	375	260	20nm bathochromic shift (Band I) and 5nm Bathochromic shift (Band II)
Sample + 3mg powdered NaOAc and H_3BO_3 and 3 drops of 2M HCl	355	Weak band	20nm hypsochromic shift (Band I)

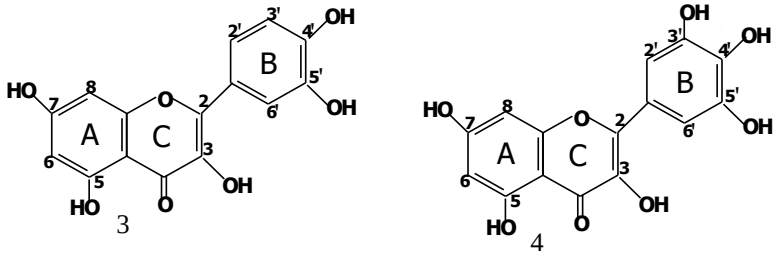
Pronounced bathochromic shifts of the spectral bands of the isolated compound were observed on addition of 5% methanolic aluminum chloride to the sample. The 355 and 255nm absorption maxima were shifted to 420 and 270nm respectively, which suggests the presence of a free hydroxyl group in position 5 of ring A (Table 12). On treatment with 2M HCl, band I was shifted to 355 – 400nm, a hypsochromic shift indicating that some of the complexes formed with $AlCl_3$ had decomposed on addition of HCl (Table 12). The split in band I showing two peaks at 355nm and 400nm, suggests that complexes other than the one formed with carbonyl group and hydroxyl group at position 5 of ring A may have been formed i.e. complexes formed may be as shown in structures **1** and **2**. [19,22]





Complex **2** is unstable in acidic medium, therefore, easily broken down by the addition of 2M HCl producing a hypsochromic shift (65nm) resulting to the peak at 355nm. While complex **1** is stable giving rise to the second peak at 400nm.The above possibility implies that there is also a free hydroxyl group in position 3 of ring C. The presence of this hydroxyl group (in position 3) suggests that the compound may be a flavonol. [19,22]

Interaction of sodium acetate with the compound produced bathochromic shift in band II of 15nm, while band I became weakened. This shift in band II indicates the presence of a free hydroxyl group in position 7 of ring A and further suggests that the compound is a flavonoid. (Table 12) Band I and band II of the spectrum demonstrated 20nm and 5nm bathochromic shifts respectively in the presence of powdered sodium acetate and boric acid. The shift in band I implies that there is an ortho dihydroxyl group also called a catechol structure (which is absent from kaempferol nucleus) present. This catechol structure complexes with boric acid giving rise to the bathochromic shift in band I (Table 12). On addition of 2M HCL, band I showed a hypsochromic shift (20nm) returning to its original position at 355nm (Table 12). This suggests that the isolated compound may likely be a derivative of quercetin (**3**) or myricetin (**4**). [19,22].



Infrared Spectral Data of the Isolated Compound

Compound (Fraction 1) in methanol: Absorption peaks appear at 3400cm^{-1}, 2930cm^{-1},1715cm^{-1}, 1650cm^{-1}, 1600cm^{-1}, 1400cm^{-1}, 1350cm^{-1}, 1200cm^{-1}, 1070cm^{-1}, 915cm^{-1}, 900cm^{-1}, 820cm^{-1} and 780cm^{-1}.

Compound (Fraction 1) in KBr: Absorption peaks appear at 3400cm^{-1}, 2940 cm^{-1}, 1720 cm^{-1}, 1650 cm^{-1}, 1600 cm^{-1}, 1500 cm^{-1}, 1385 cm^{-1}, 1280 cm^{-1}, 1200 cm^{-1}, 1070 cm^{-1}, 950 cm^{-1}, 820 cm^{-1} and 780 cm^{-1}.

The infrared spectrum of the isolated flavonoid compound (in methanol) and the spectrum in KBr gave some similar peaks. The spectra showed strong absorption in the area associated with O–H (hydroxyl) stretching at Vmax 3400cm^{-1}. The presence of the band at Vmax 2940 which shows C-H stretching for alkyl groups. The presence of bands at 1650cm^{-1}, 1600cm^{-1} and 1500cm^{-1} suggest that the compound is unsaturated, contains phenyl nucleus and is aromatic. The peaks at 1200cm^{-1} and 1070cm^{-1} is associated with C-O stretching for alcohols, phenols and aromatic ether. Other peaks in the spectrum are 780cm^{-1}, 820cm^{-1}, 915cm^{-1}, 900cm^{-1} and 950cm^{-1} which indicates the presence of substituted benzene rings [23-25].

IV. Conclusion

Reports have shown that the extract of *Mitracarpus verticillatus* has antibacterial and antifungal activities [4,26]. This work also reports that the antimicrobial activity is highest in the methanol extract residue. The potency of the extract can be attributed to the class of the major natural product components present. The methanol extract residue is the fraction that gave the highest antimicrobial activity. The flavonoid component of this fraction may be responsible for its broad antimicrobial activity against the test organisms since flavonoids have been reported to have antibacterial, antifungal and antiviral activities [10,27]. In the light of the various results obtained from phytochemical screening, thin layer chromatographic analysis, ultraviolet/visible, and

infrared spectroscopic studies, it can be inferred that the compound isolated from the methanol extract residue is a flavonol.

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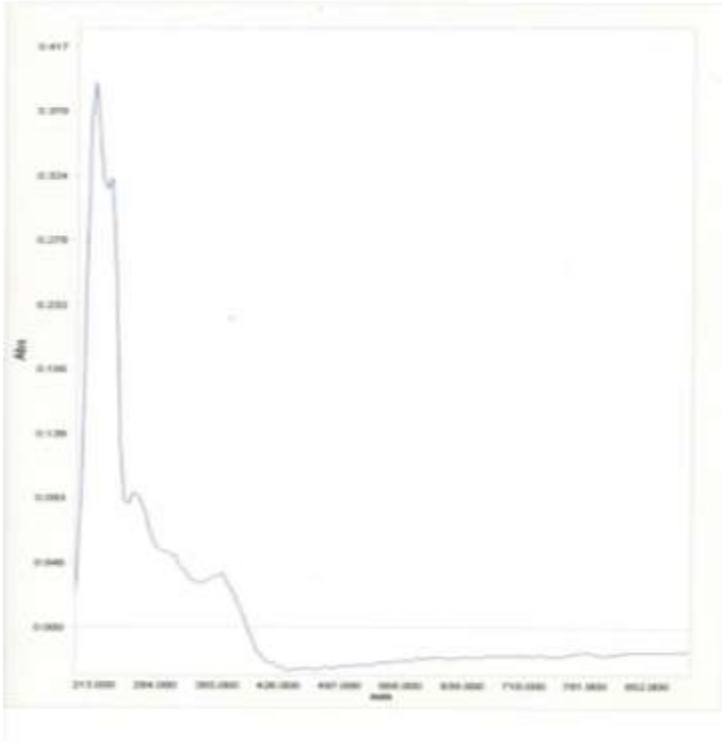
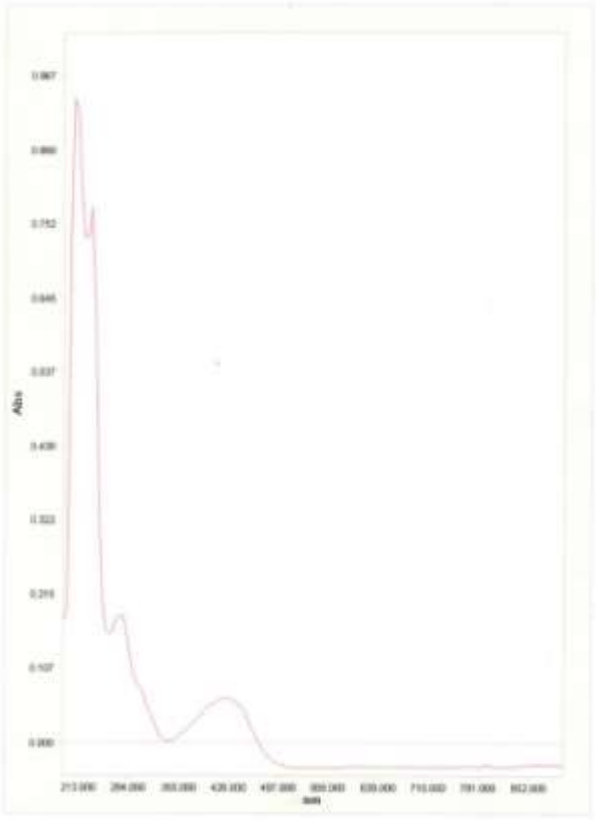
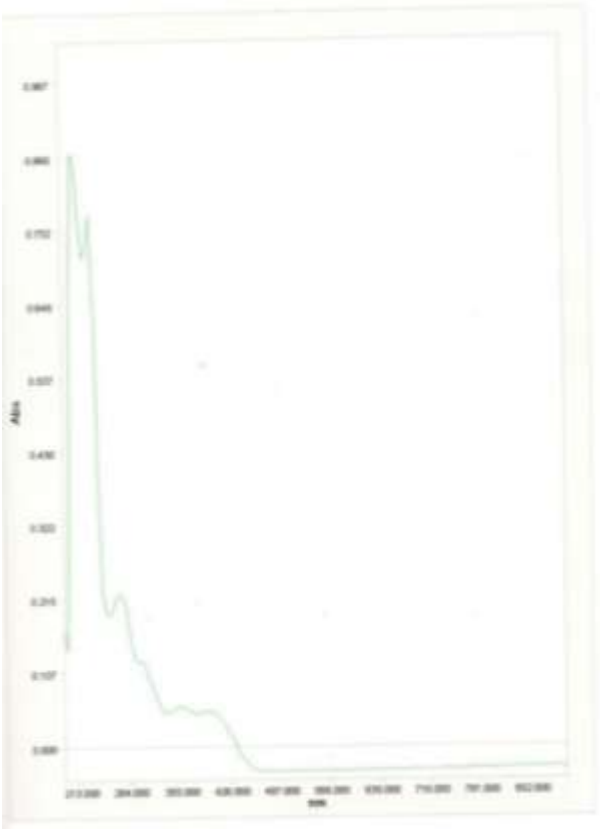
Supporting Document



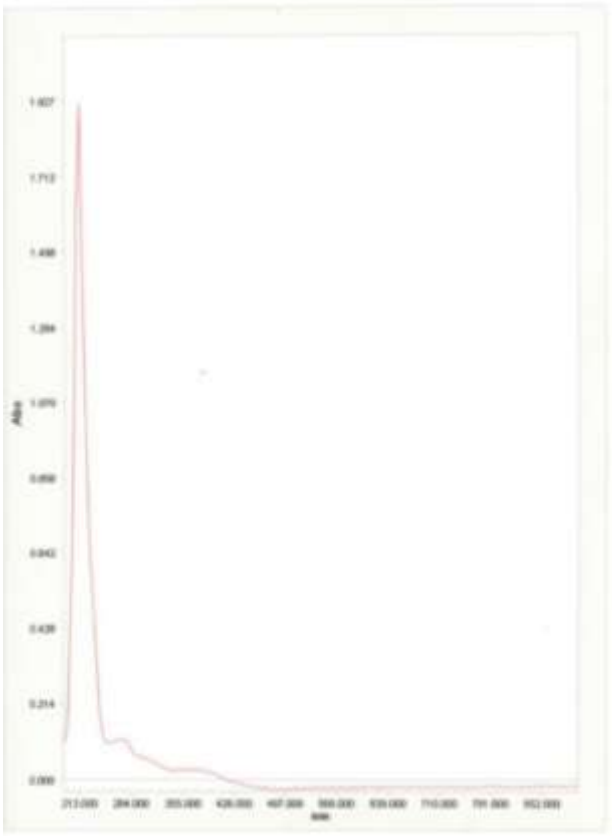
Appendix I: UV/Vis Spectrum of Isolated Compound



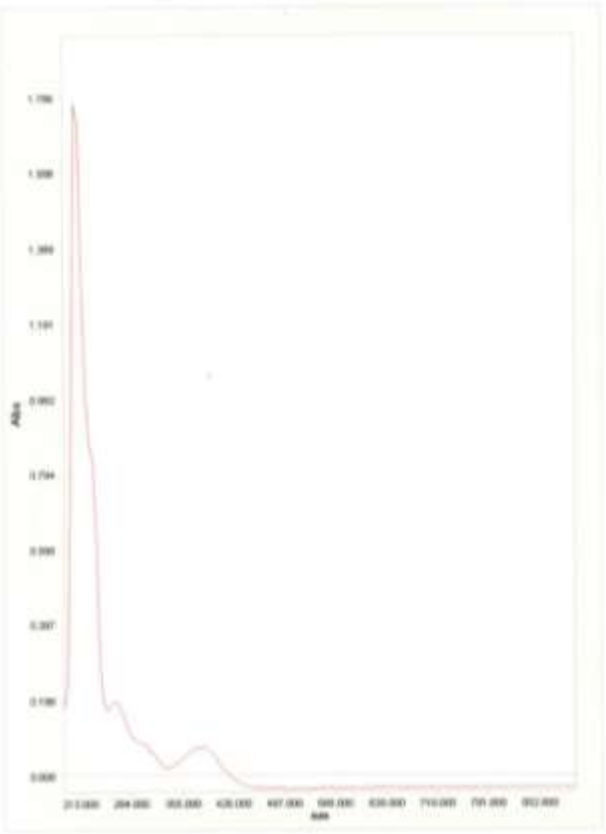
Appendix II: UV/Vis Spectrum of Isolated Compound + $AlCl_3$



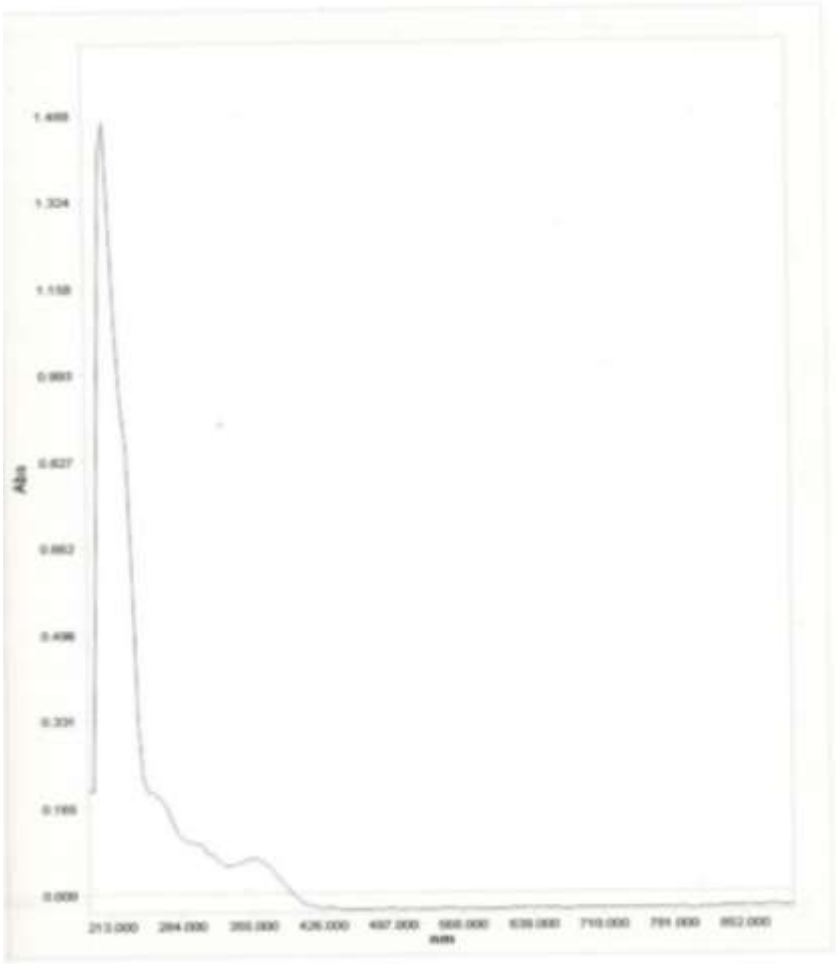
Appendix III: UV/Vis Spectrum of Isolated Compound + $AlCl_3$ + HCl



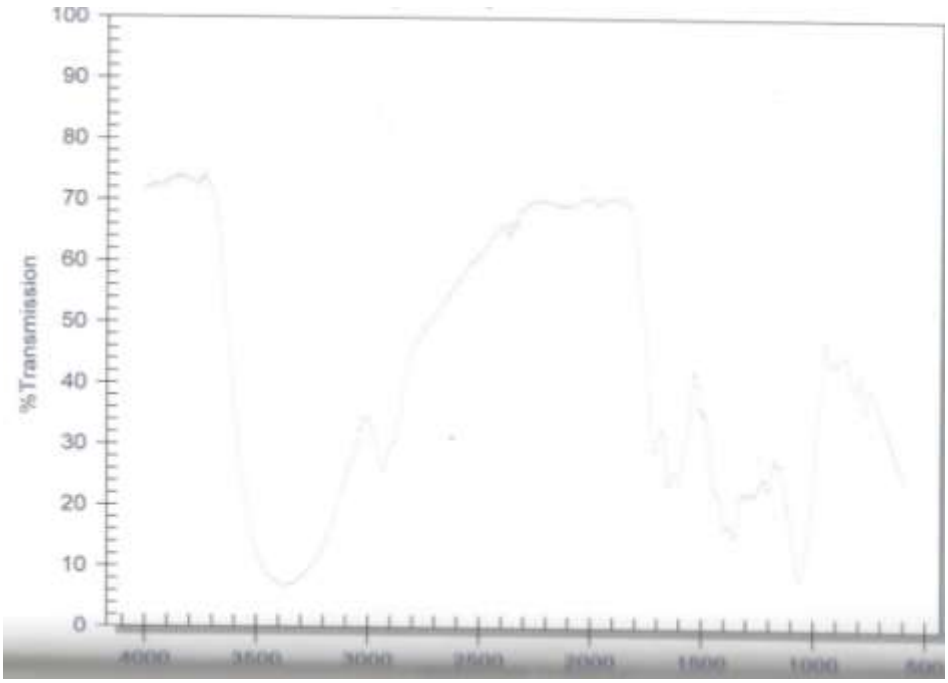
Appendix IV: UV/Vis Spectrum of Isolated Compound + NaOAc



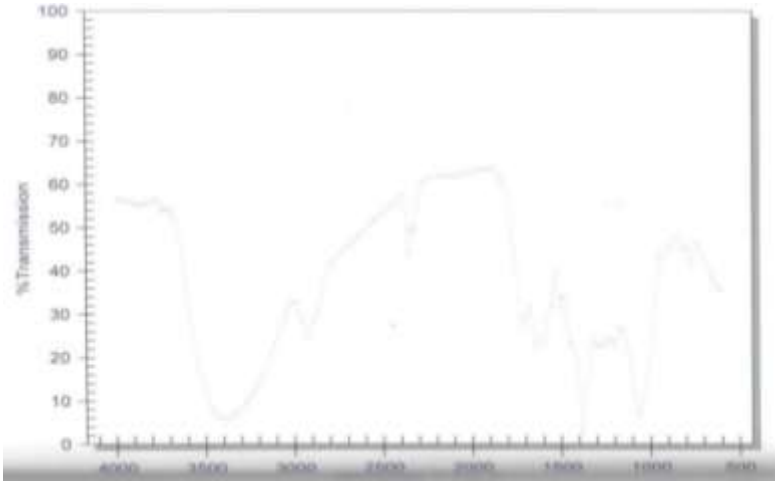
Appendix V: UV/Vis Spectrum of Isolated Compound + NaOAc + H_3BO_3



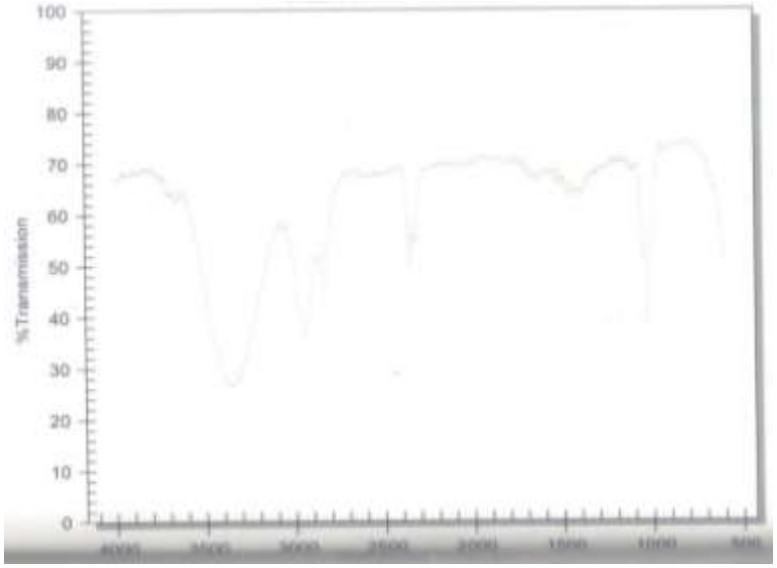
Appendix VI: UV/Vis Spectrum of Isolated Compound + NaOAc + H_3BO_3 + HCl



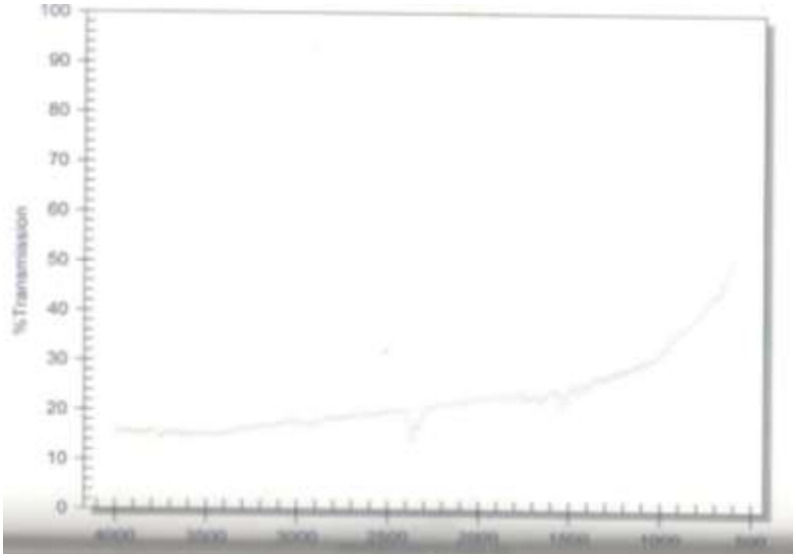
Appendix VII: FT-IR Spectrum of Isolated Compound in MeOH



Appendix VIII: FT-IR Spectrum of Isolated Compound in KBr



Appendix IX: FT-IR Spectrum of Methanol



Appendix X: FT-IR Spectrum of KBr