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# Anti-urease and antioxidant activities of the leaf extracts from *Murraya paniculata* (L.) Jack (Rutaceae)

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## Abstract

This report is on the urease inhibitory and free radical scavenging activities of the leaf extracts of *Murraya paniculata*. The pulverized plant sample (314 g) was extracted by successive cold maceration using n-hexane (non-polar), acetone (moderately polar) and absolute ethanol (polar) for 72 hrs. Phytochemical screening done using standard phytochemical methods, urease inhibition using modifed Berthelot method and free radical scavenging using 2, 2-diphenyl-1-picryl hydrazyl (DPPH) spectrophotometric assays. The bioactive extract was fractionated on chromatography column packed with normal phase silica gel (200-400 mesh size) and eluted using gradient mixtures of n-hexane, dichloromethane and methanol. The pooled fractions from the column were assessed for bioactivity. The functional group investigation was done using infra-red (IR) spectroscopy. Alkaloids, flavonoids, triterpenoids, phenols, steroids, and carbohydrates were present in the extract. The acetone extract displayed the highest free radical scavenging activity (54.186±0.0004) with IC 50 value: 0.84 mg/ml, and urease inhibition. Its dichloromethane-methanol column chromatography fraction had the highest free radical scavenging activity at 0.8 mg/ml (60.65±0.062) with an IC50 value: 0.49mg/ml. The activities were quite significant compared to the standard (Ascorbic acid) at $P<0.05$. The bioactive dichloromethane-methanol column chromatography fraction evidenced vibration stretching bands [3440 $cm^{-1}$ (O-H), 2975 $cm^{-1}$ (C-H), 1745 $cm^{-1}$ (C=O), 1634 $cm^{-1}$ (C=C), 1290 $cm^{-1}$(C-N), and 1134/1007 $cm^{-1}$ (C-O)] from the IR spectroscopy thus corroborating the detected phytochemicals. These current results validate the use of the *M. paniculata* in managing oxidative stress and other reported traditional uses in addition to the potentials for its use in the discovery and development of eco-friendly urease inhibitors for sustainable agriculture.

**Keywords:** *Murraya paniculata*; Antioxidants; Urease Inhibitors; Sustainable Agriculture

## 1. Introduction

Traditional medicinal systems such as Ayurvedic, Homeopathic, Unami, Naturopathy, Tibetan, and even folklore medicines have served an essential role in rural health care, and this usually involves the use of plant extracts [1]. Many commercially used drugs have come from the information of indigenous knowledge of plants and their folk uses. The Medicinal properties of plants are attributed to the active secondary metabolites, most of which probably evolved for chemical defenses against predation or infections. More often, natural products provide lead structures, starting point for chemical modification to derive an optimal drug. Natural products have always contributed extensively towards modern medicine by playing a substantial role in drug discovery. The quest for novel therapeutic leads from natural resources has been going on for ages and has resulted in several significant discoveries, including antibiotics, anticancer

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agents, anti-inflammatory compounds, and analgesics. The vast genetic diversity available in plants, animals, and microorganisms presents a wealth of possibilities for the betterment of mankind in the manufacture of food, materials, and medicine. Free radicals are molecules or fragments of molecules that has an unpaired electron in their atomic or molecular orbitals. The body produces them naturally as they perform vital roles in various cellular functions. However, excessive production of these free radicals induces molecular and cellular damage, resulting in diseases such as cancer [2,3] and respiratory diseases [4]. Antioxidants are molecules that inhibit the oxidation of other molecules. They reduce and prevent damage that result from free-radical reactions by donating electrons to neutralize free radicals. Urease of *Helicobacter pylori* produce abundant amount of ammonia (10–15% of total proteins by weight) and makes the development and survival of bacteria possible by increasing the pH of the environment [5]. Therefore, it plays a necessary role in the treatment of gastric diseases. *Helicobacter pylori* infection is the main cause of gastrointestinal disorders, particularly gastritis, peptic ulcer, duodenal, and gastric ulcer. The enzyme makes the stomach medium more tolerable by neutralizing gastric acid through hydrolysis of urea to form carbon dioxide ($CO_2$) and ammonia ($NH_3$). The innate and adaptive immune system utilizes inflammation as a general response against pathogenic invaders, such as free radicals and urease. However, when these inflammatory responses are unregulated, they lead to several disorders, such as cancer, allergies, cardiovascular dysfunctions, and autoimmune diseases. With the current mutation of these diseases, orthodox medicine are often times less reliable resulting in treatment failures. Thus, the need for a search into plant kingdom for new potent drug candidates becomes imperative. Plant kingdom contains naturally occurring free radical scavengers, such as polyphenols, flavonoids, and carotenoids, which have anti-inflammatory properties [6,7,8]. *Murraya paniculata* generally known as orange Jessamine, is a tropical evergreen plant that has tiny, white fragrant flowers. It belongs to the Rutaceae family. The leaves are taken orally to ease pain [9]. Because of the stimulant and astringent activities of the leaves, it is also used to treat diarrhea and dysentery [10]. The bark of *M. paniculata* is used in treating cough, rheumatism, and mental disorder [ [11]. The cooked leaves are also reportedly used to set joints while the boiled twigs for stomachache [12]. Locals in Southern Asia manage gastrointestinal diseases with *M. paniculata* [13], such as ulcers linked to the presence of urease. Also, this plant is used locally to treat cardiovascular diseases [13], such as myocardial ischemia, which has been linked to the presence of free radicals. In order to validate its ethnomedicinal uses in the treatment of ailments whose pathophysiology are assocaited with ureolytic organisms and oxidative stress, this study is aimed at evaluating the urease inhibitory and free radical scavenging activities of the leaf n-hexane (non-polar), acetone (moderatley polar) and ethanol (polar) extracts from the leaves of *Murraya paniculata*.

**Figure 1** *Murraya paniculata* showing the leaf and flower taken from premises of the Choba campus of University of Port Harcourt, Nigeria

## 2. Material and methods

### 2.1. Plant material

Fresh leaves of *Murraya paniculata* were collected from the University of Port-Harcourt botanical garden. Identification of the plant was done by Dr Chimiezie of the Department of Plant Science and Biotechnology. It was further authenticated with voucher number UPH/V/1347 at the Herbarium of the Department of Plant Science and Biotechnology, University of Port-Harcourt, Choba, Rivers State, Nigeria. The leaves were collected, air dried and pulverized using table blender. The powdered material was kept in an airtight container for analysis.

### 2.2. Extraction and column chromatography

The dried and powdered leaves of *Murraya paniculata* (314 g) was macerated successively with n-hexane, acetone and ethanol respectively, for a period of 72 hours for each solvent. The combined filtrate for each was evaporated using a rotary evaporator at 40°C. The weight of the extracts and their percentage yield were noted after concentration. Extracts were then stored in a glass container inside a desiccator until when needed. The bioactive acetone extract was fractionated on column chromatography packed with silica gel G (200-400 mesh size) eluting with gradient mixture of N-hexane, dichloromethane and methanol in the proportion of n-hexane (100%), n-hexane: DCM (50:50), DCM (100%), DCM: methanol (50:50), methanol (100%). The eluates were collected in whole at 500ml intervals. The fractions were recovered using a rotary evaporator at reduced pressure and temperature. Fractions were monitored on analytical thin layer chromatography (TLC) silica gel $GF_{254}$

### 2.3. Phytochemical study

Phytochemical screening to detect the presence secondary metabolites was conducted on the dried powder and the extracts obtained using standard described methods [14,15].

### 2.4. *In vitro* Urease Inhibitory assay of the extracts from M. paniculata

The modified Berthelot method described [16] was adopted. Briefly, the solutions of the test extracts (n-hexane, acetone and absolute ethanol) were prepared separately by dissolving 0.01g of the extracts in 10ml of ethanol to obtain a 1mg/ml test solution. Thiourea reference solution used for the comparison was made by dissolving 0.1g of thiourea in 10ml of ethanol to obtain the reference stock solution (10mg/ml). A 1ml solution of the thiourea stock solution was further diluted to 10ml with ethanol to obtain the 1mg/ml thiourea working solution for comparison. The content of the Blood Urea Nitrogen (BUN) enzyme vial was reconstituted in 10ml of deionised water as specified in the manufacturer's test kit. The assay began by transferring a 1ml aliquot of the various test extracts, reference thiourea solution, ethanol (negative control) into separately labeled test tubes. To each of these test tubes, 1ml of the reconstituted BUN enzyme solution was added, mixed and followed by the addition of 10µl of urea. The mixture was then incubated for 10minutes at room temperature. The percentage inhibition of urease activity was then calculated using the formula:

$$\text{Percentage urease inhibitory activity} = 100\frac{(\text{Acontrol} - \text{Asample})}{\text{Acontrol}}$$

Where $A_{control}$ = Absorbance of the control solution (containing all reagents except the extracts), $A_{sample}$ = Absorbance of the plant extracts or thiourea reference solution

### 2.5. Quantitative Antioxidant Assay

This method was done following the standard method [17,18] with a few modifications. The assay was carried out on the n-hexane, acetone and ethanol extracts. Using a beaker thoroughly wrapped with aluminum foil, 0.0001 DPPH solution was prepared by dissolving 0.01g of DPPH powder in 100ml of ethanol. The wrapped beaker was kept in a dark chamber to prevent stray light. A 1mg/ml concentration of the three extracts was prepared by dissolving 0.03g of extract in 30ml of ethanol. The standard (ascorbic acid, 1mg/ml) was also prepared followed by a tenfold serial dilution. This was repeated on the acetone extract.

### 2.6. Spectroscopy

#### *2.6.1. Fourier-Transform Infrared spectroscopy*

Buck scientific M530 USA FTIR was employed for the analysis. The instrument was furnished with a potassium bromide beam splitter and deuterated triglycine sulphate detector. A film of the sample was properly mixed with potassium bromide and put on the salt pellet. The FTIR spectra were shown as transmitter values, scanned at frequency range of 4000 - 600 $cm^{-1}$.

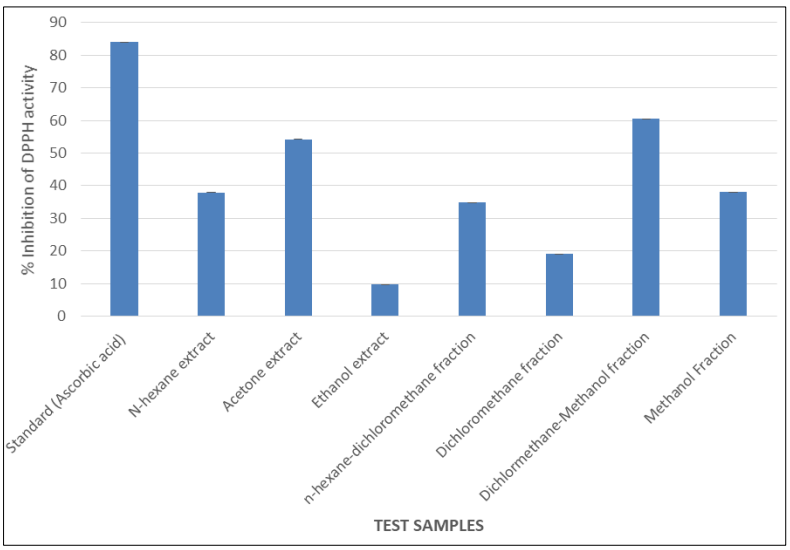
### 2.7. Statistical Analysis

Data were expressed as Percentage Mean Inhibition ± Standard Deviation. The significant difference between the mean percentage inhibition of the different concentrations and the standard drug were established using a one-sample t-test at 95% confidence intervals.

## 3. Results and discussion

The yields of the extract: N-hexane (0.89 %w/w) < ethanol (2.52 % w/w) < acetone (2.58 % w/w) was observed. It is an indisputable fact that plants are the banks of chemical compounds. The beneficial effect of these compounds cannot be over-emphasized. Figure 2 represents the free radical scavenging activity of the extracts and column fractions from *M. paniculata* using on DPPH. At 1mg/ml, the acetone extract of the plant had the most activity (54.186%) compared with the reference standard (ascorbic acid). The trend in percentage inhibition of DPPH activity was acetone extract (54.186%)> n-hexane extract (37.903%)> ethanol extract (9.690%) in comparison with ascorbic acid, which exhibited a percentage inhibition of 84.047%. The urease inhibitory activity of the extracts using the Phenol hypochlorite method are shown in Figure 3. The trend in percentage inhibition at 1mg/ml of urease activity was acetone extract (6.74%)> ethanol extract (0.99%) > n-hexane extract (-47.8%) which were all significantly different ($p<0.05$). These values were compared with the percentage inhibition of Thiourea (96.8%), which was used as the reference standard. None of the extracts showed significant urease inhibitory activity. Further dilution of the acetone extract was made and assessed on the DPPH at a concentration range of 0.05mg/ml - 1.0mg/ml (Fig 4). The $IC_{50}$ (0.75mg/ml) and $IC_{90}$ (1.35mg/ml) values were extrapolated from the dose-response curve. Separation of the active acetone extract was achieved using gradient mixtures of n-hexane, dichloromethane, and methanol on column chromatography. Four fractions were attained from the separation and further assayed for DPPH reduction as seen in Fig 2. Fraction F3 showed the highest activity (60.46%), in comparison with F1, F2, and F4 and although it showed a lower percentage inhibition in comparison with the standard drug (84.047%). Average percentage inhibition of fractions F1, F2, F3 and F4 was 34.82%, 19%, 60.46%, and 38.01% respectively at 1mg/ml. The values were all significantly different in comparison with the standard drug at $P<0.05$. The bioactive fraction F3 was further assayed using different concentrations to determine the $IC_{50}$ and $IC_{90}$ values. Figure 5 shows that the percentage inhibition of DPPH by bioactive column fraction F3 was also dose-dependent. Average inhibition increased as the concentrations increased. The highest percentage inhibition was at 0.8mg/ml (60.65%) in comparison with the other concentrations, although it was lower than the percentage inhibition of the standard drug (85.60%). The $IC_{50}$ and $IC_{90}$ values as shown in Figure 6 were 0.488mg/ml and 1.468mg/ml, respectively; at 0.488mg/ml, F3 will yield a 50% percentage inhibition of DPPH activity, and at 1.468mg/ml, there would be a 90% inhibition of DPPH activity.

Table 1 shows the class of phytochemical compounds contained in the crude *M. paniculata* leaves and its Acetone extract. The result shows the presence of alkaloids, cardiac glycosides, triterpenoids, phenolics, flavonoids and, cardiac glycosides in both the crude plant and the acetone extract. In this current study, the presence of secondary metabolites such as phenols, flavonoids, alkaloids, steroids, and triterpenoids correlated with earlier studies [19]. Several antibacterial alkaloids have serve as skeletons for for the design and development of essential antibacterial drugs such as metronidazole, quinolones, linezolid, and trimethoprim [20, 21]. Flavonoids scavenge free radicals by donating a Hydrogen atom or a single electron to the radical stabilizing it or by their metal chelating activity. The flavonoids possess hepatoprotective, anticancer, antibacterial, anti-inflammatory, anti-diabetic, and anti-viral properties [22-26].




values represent the percentage mean ± SD from n=3. Where a sample is indicated as a fraction, it is the pooled fraction obtained after chromatography separation of the bioactive acetone extract

**Figure 2** Free radical scavenging activity of the extracts and column chromatography fractions from *M. paniculata*

The mild anti-urease activity of the acetone extract could also be exploited in future anti-urease drug and agrochemicals discovery. Urease is an amid hydrolase. It has a $Ni_2^+$ which contains three complex subunits responsible for the catalysis of the disintegration of urea into ammonia and carbonic acid [27]. Urease is a virulent factor in urinary tract infection (UTI) caused by gram-positive bacteria *Staphylococcus saprophyticusi* [28], in *H. pylori* associated ulcer [29], *Streptococcus salivarius* associated dental plaque and calculus deposit [30] and several other bacterial diseased cases [31]. In case of UTIs, these pathogenic microbes can cause swelling of the urethra, bladder, and kidneys, leading to the growth of urinary stones. Urease plays a fundamental part in nitrogen metabolism by plants [31]. Its activity is mostly distributed in soil and aquatic environment, as reported [31]. An increase in urease concentration leads to environmental problems by discharging a large quantity of ammonia into the air during urea fertilization. It instigates plant damage by impoverishing their essential nutrients through ammonia toxicity and the release of carbon(vi)oxide. To solve the problems encountered in the use of urea such as fertilizer, it is necessary to inhibit the hydrolysis of urea using environmentally or eco-friendly urease inhibitors. Thus, the mild anti-urease action of the acetone extract observed in this study (see Figure 3), could be exploited for eco-friendly agrochemicals discovery and development as alternatives for the environmentally less friendly synthetic derivatives.

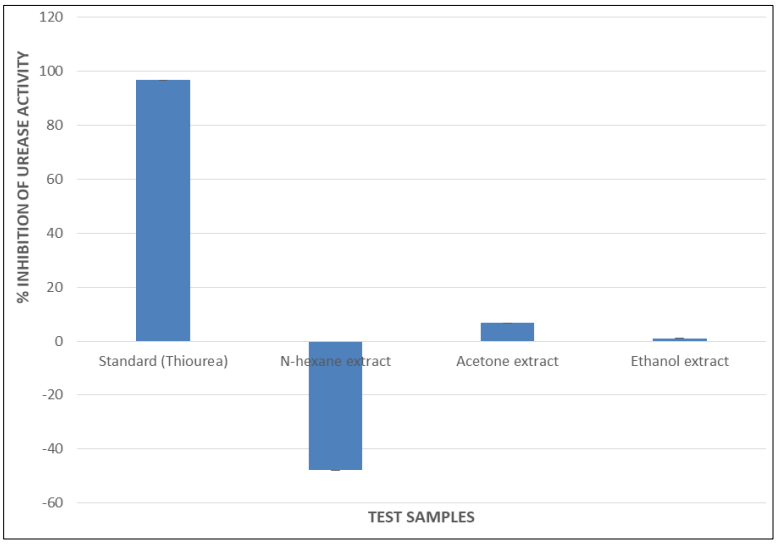



**Figure 3** Urease inhibitory activity of extracts from *M. paniculata*

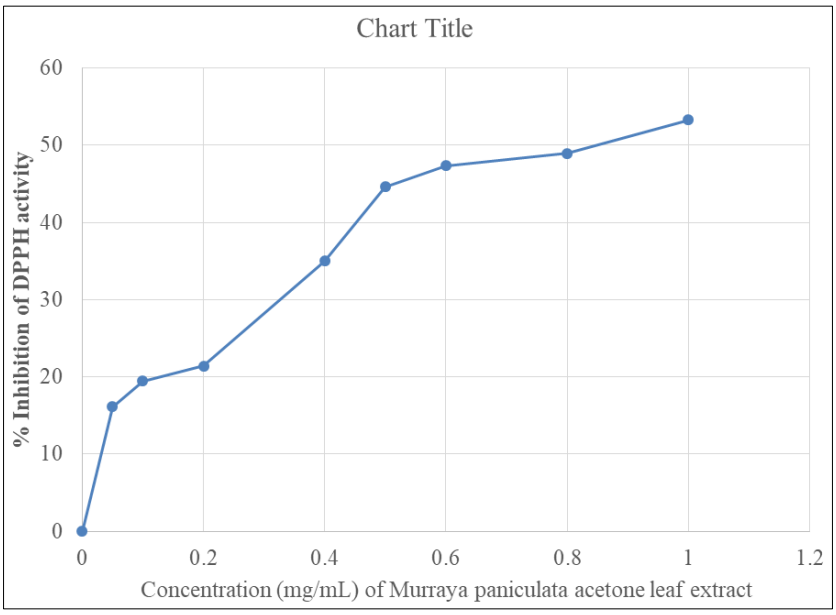



**Figure 4** Concentration - dependent Free radical scavenging activity of the acetone extract from *M. paniculata* leaf

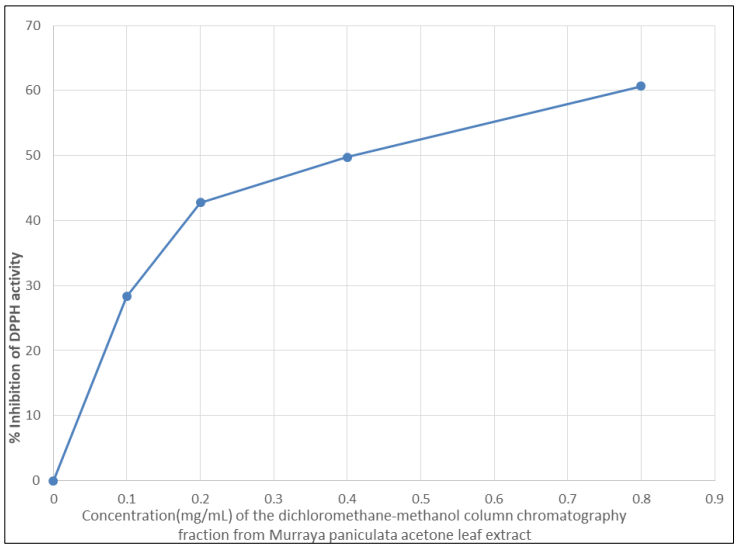



**Figure 5** Concentration-dependent free radical scavenging activity of the bioactive dichloromethane-methanol column chromatography fraction from the bioactive acetone extract of *M. paniculata*

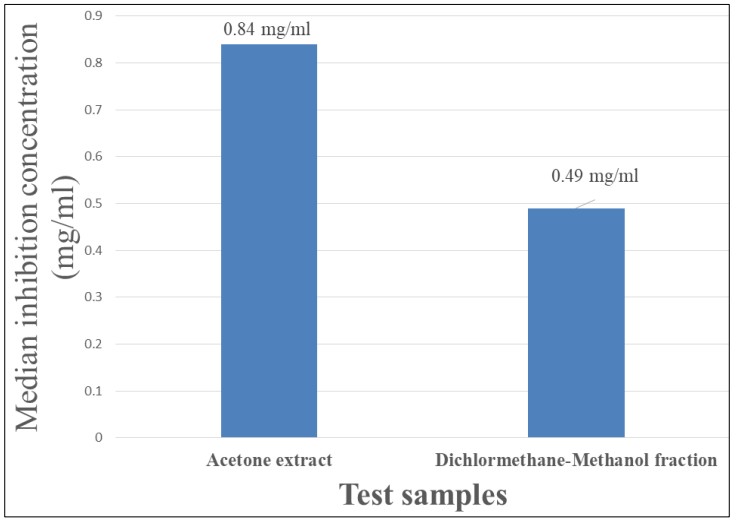



**Figure 6** Median inhibitiopn concentration ($IC_{50}$ in mg/mL) of the acetone leaf extract and its bioactive dichloromethane-methanol column chromatography fraction as obtained from their respective concentration response Curve

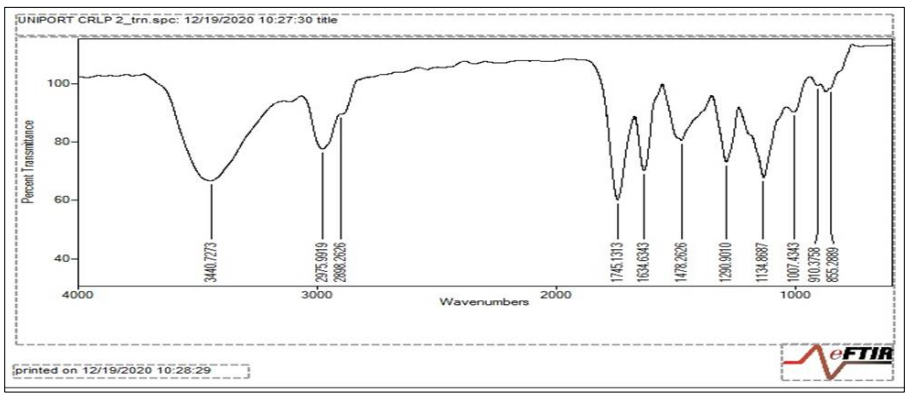



**Figure 7** The FTIR spectrum of the bioactive dichloromethane-methanol column chromatography fraction from the bioactive acetone extract of *M. paniculata*

**Table 1** Result of phytochemical screening of *M. paniculata* leaves

| Secondary metabolites | Dried *M. paniculata* | Acetone extract |
|---|---|---|
| Alkaloids | + | + |
| Phenolics | + | + |
| Phlobatannin | - | - |
| Flavonoids | + | + |
| Anthraquinones | - | - |
| Triterpenoids | + | + |
| Cardiac glycosides | + | + |
| De-oxysugars | + | + |
| Saponin | - | - |

Key: += positive, -=negative

**Table 2** Infrared Spectrum of the bioactive dichloromethane-methanol column chromatography fraction from the bioactive acetone extract of *M. paniculata*

| S/No. | Observed absorption band ($Vcm^{-1}$) | Description of bands |
|---|---|---|
| 1 | 3440 | O-H stretching of Phenol or alcohol |
| 2 | 2975/2898 | C-H stretching (aymmetric and symmetric) |
| 3 | 1745 | C=O stretching evidence of Carbonyl group |
| 4 | 1634 | C=C stretching Evidence of aromatic or olefinic compounds |
| 5 | 1478 | C-H bending |
| 6 | 1290 | C-N stretching Evidence of tertiary amine |
| 7 | 1134/1007 | C-O stretching Evidence of ether bond of phenol or alcohol |
| 8 | 910/825 | Aromatic C-H out-of-plane bands |

The FTIR spectroscopy analysis of the bioactive dichloromethane-methanol column chromatography fraction from the bioactive acetone extract of *M. paniculata* is evident of the O-H functional groups associated with phenolic ccompounds which is seen at the strong broad band stretching vibration at 3440cm-1 for -OH (see Figure 7 and as rationalised in Table 2). The strong band at 1745$cm^{-1}$ indicates a C=O stretching which could be a carbonyl group of a ketone, aldehyde, or esters. The sharp band at 1634cm-1 suggests the presence of C=C stretching of aromatic or olefinic groups. At 2975cm-1 and 2898cm-1, there are assymmetric and symmetric C-H stretching vibrations.The medium to weak band at 1290$cm^{-1}$ indicates the presence of C-N stretching vibrations due to tertiary amine as seen in naturally occuring alkaloids which are detected from the phytochemical screening results in Table 1.

## 4. Conclusion

The free radical scavenging and the mild anti-urease activities of the acetone extract from the leaf extract of *Murraya paniculata* confirms the usefulness of this plant in ethnomedicine in the management of oxidative stress as well as other reported traditional uses such as in the treatment of mental disorders and ailments associated ureolytic pathogens. Also, the mild anti-urease activities of the acetone extract could be explored for the discovery and development of eco-friendly urease inhibitors as additives to urea-based fertilizers for sustainable agriculture.

## Compliance with ethical standards

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*Disclosure of conflict of interest*

The authors declare no conflict of interest.

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