



Research Article

Phytochemical and in-vitro antimicrobial evaluations of solvents partitioned portions of methanol crude extract of the root of *Raphanus sativus* Linn

Sule Ibrahim<sup>1\*</sup>, Olufunke A. Sodipo<sup>2</sup>, Fanna I. Abdulrahman<sup>1</sup>, Yakubu James<sup>1</sup>, Mamza U. Tanko<sup>1</sup>,

<sup>1</sup>Department of Pure and Applied Chemistry, Faculty of Physical Sciences, University of Maiduguri, P.M.B. 1069, Maiduguri, Nigeria

<sup>2</sup>Department of Clinical Pharmacology and Therapeutics, University of Maiduguri, P.M.B 1069, Maiduguri, Nigeria

\*Corresponding author's Email: [suleibrahim@umaid.edu.ng](mailto:suleibrahim@umaid.edu.ng), [doi.org/10.55639/607.080706](https://doi.org/10.55639/607.080706)

ARTICLE INFO:

ABSTRACT

**Keywords:**

Antimicrobial,  
Partitioned portions,  
Phytochemical,  
*Raphanus sativus*

Radish can be found as a cultigen throughout the world however, the original gene center lies between the eastern Mediterranean and the Caspian Seas. *Raphanus sativus* L. is a root vegetable and an annual herb consumed all over the world as food. The plant is considered medicinal and taken by many to control urinary tract infection. The *Shuwa* people slice it raw on fried meat and consume it to control stomach upset. This work was carried out to evaluate antimicrobial efficacy of solvents partitioned portions of methanol extract of the root of *Raphanus sativus* L. The antimicrobial activity was determined against four Gram +ve, -ve and fungal strain each at three different concentrations in mg/mL. Chloroform, n-butanol and aqueous partitioned portions had inhibited growth of *S. aureus*, *S. pyogenes*, *B. subtilis*, *C. spp.*, *E. coli*, *S. typhi*, *K. pneumoniae*, *P. aeruginosa* and *C. albicans* but *S. pyogenes*, *B. subtilis* and *P. aeruginosa* were resistant against aqueous portion meanwhile ethyl acetate portion had only inhibited growth of *S. pyogenes* and *B. subtilis*. Notably, chloroform portion had significantly ( $P \leq 0.05$ ) inhibited the growth of *C. spp.* at all concentrations. In addition, MIC, MBC/MFC, activity, percent activity, bacterial/fungal susceptibility and spectral intensity indexes of portions were all calculated. The qualitative phytochemical tests of partitioned portions of the root of *R. sativus* revealed the presence of biologically active secondary metabolites and carbohydrates.

**Corresponding author:** Sule Ibrahim, Email: [suleibrahim@umaid.edu.ng](mailto:suleibrahim@umaid.edu.ng)

Department of Pure and Applied Chemistry, Faculty of Physical Sciences, University of Maiduguri, P.M.B. 1069, Maiduguri, Nigeria

## INTRODUCTION

Plant and man co-existed since the beginning of life however; man has been exploiting plants for his benefits which include food and therapy among others. Radish can be found as a cultigen throughout the world. In summary, radish occurs scattered throughout Africa; it has been recorded in over a dozen countries (Selvakumar, 2023). In Nigeria, *R. sativus* is grown mostly in Plateau state. *Raphanus sativus* L. (Brassicaceae) is a root vegetable and is eaten in different forms to control piles, urinary tract infection, diarrhoea, sugar level and rheumatism. The Shuwa people slice it on fried meat and eat to prevent stomach upset. Majority of people use plants as their foremost alternative source of treatment of diseases (Susanna *et al.*, 2019). Medicinal plants and their extracts have been used in treatment since the time immemorial (Kebede *et al.*, 2021). Medicinal properties (antimicrobial among others) of plants are widely exploited for the benefit of mankind regarding healthcare. Plants are the storehouse of unlimited therapeutic agents (Nasim *et al.*, 2022). It has been reported that 100,000 plant species have been tested for their medicinal uses (Fitzgerald *et al.*, 2020). Today, 85-90 % of the modern society still focuses on plant-based remedies, because of their better adaptability, less side effects and economic affordability (Wangchuk, 2018). Antibiotics have been used successfully to treat infections for many years and have made the management of infectious diseases easier (Hutchings *et al.*, 2019). However, recent evidence indicates that the gains achieved through antibiotics are threatened by the development of antimicrobial resistance (AMR) (ACDC, 2018). This recent trend has rendered standard treatment ineffective leading to high morbidity and mortality rate (Emelda *et al.*, 2020). AMR is being worsened by unprofessional use of antibiotics (Sean *et al.*, 2023). According to Khalid *et al.* (2019), 92.2 % of Nigerian youths engage in self-medication. In that regard, high rate of antibiotic resistance among common Gram-positive and Gram-negative bacteria has been reported (Dada *et al.*, 2023). It has been stated that the abuse of antibiotics by the public is an important risk factor for antibiotic resistance (Khalid *et al.*, 2019). However, plants remain major players in combating the growing antimicrobial resistance (Dar *et al.*, 2016).

Hence, this study was designed to conduct phytochemical and in-vitro antimicrobial susceptibility study of solvents partitioned portions of methanol extract of the root of *R. sativus* Linn.

## MATERIAL AND METHOD

### Sample Collection and Identification

Fresh roots of *R. sativus* were collected from Zabbolorfarms, Farms Nigeria Limited at Bassa local government Area of Plateau state, Nigeria. The plant materials were transported in a wool sacked-bag to University of Maiduguri, identified and authenticated by a Plant Taxonomist in the Department of Biological Science, University of Maiduguri, Borno State, Nigeria to be from the *Raphanus sativus* L., given a voucher specimen number UM/DC/03/342 and deposited at the Herbarium of the Postgraduate Research Laboratory of Pure and Applied Chemistry Department, University of Maiduguri, Maiduguri, Borno State.

### Plant Material

This study used the root of *R. sativus* L. The roots of *R. sativus* L. were obtained fresh from Zabbolorfarms, Farms Nigeria Limited at Bassa Local Government Area of Plateau state, Nigeria.

### The Extraction of Plant Material

The grated fresh root of *R. sativus* L., 5 kg was extracted with 20 liters of 85 % methanol using cool maceration method (Ibrahim *et al.*, 2022). The plant material was soaked in solvent for 72 hrs with periodic shaking and allowed to stand at room temperature for a proper extraction of soluble phytochemicals. The liquid mixture of the extract was filtered using a clean muslin cloth followed by filtration with Whatmann No. 1 filter paper. This process was repeated on same plant material for 24 hrs. until the required plant material was exhausted. The crude extract was concentrated to dryness at room temperature. The crude methanol extract weighed, % yield recorded, and coded the extract as CME (crude methanol extract), and was kept in a desiccator until needed for further analyses. Two hundred and fifteen grams (215 g) of CME was partitioned using chloroform and ethyl acetate as solvents in graded polarities. The insoluble portion was then suspended in distilled water and further partitioned with *n*-butanol and aqueous soluble portion was considered. Each portion was concentrated to dryness under reduced pressure. The portions

were weighed, percentage yield determined and coded as CFP (chloroform partitioned portion root extract), EAP (ethyl acetate partitioned portion), NBP (*n*-butanol partitioned portion) and AQP (aqueous partitioned portion).

#### **Chemicals, reagents and solvents**

All chemicals, solvents and reagents were of Analar grade (Merck, Germany, JHD China Sigma-Aldrich, Germany, BDH England). Standard susceptibility antibiotic discs of ciprofloxacin and ketoconazole were obtained from the Department of Microbiology, University of Maiduguri Teaching Hospital, Borno state, Nigeria.

#### **Preliminary qualitative phytochemical evaluation**

The chloroform, *n*-butanol, aqueous and ethyl acetate were qualitatively evaluated for the presence of sugar, phlobatannins, tannins, alkaloids, cardiac glycosides, glycosides, terpenoids, saponins, flavonoids using standard procedure (Junaid and Patil, 2020; Elvino *et al.*, 2022).

#### **Preparation of media**

The powdered nutrient agar and nutrient broth media (Biolife, England: Oxoid, England) were obtained from a chemical store in Maiduguri, Nigeria and prepared according to manufacturing guidelines. Cultures were maintained on agar slants and kept in fridge at 4 °C until needed.

#### **Experimental organism**

A total of twelve (12) microorganisms were used for microbial test in this study: four Gram negative bacteria (*Salmonella typhi*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) and four Gram positive bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes* *Corynaebactrium spp* and *Bacillus subtilis*) and four fungal strains (*Candida albicans*, *Aspergillus flavus*, *Aspergillus niger* and *Rhizopus stolonifera*). These microorganisms were obtained from the Department of Microbiology, University of Maiduguri Teaching Hospital, Borno state.

#### **Antimicrobial susceptibility studies**

The preliminary antimicrobial activity of the crude extract and solvent partitioned portions of the root of *R. sativus* on test organisms were evaluated using disc diffusion as described by Heatley and adopted by Sodipo *et al.* (2016). Three different concentrations of 200 mg/mL, 400 mg/mL and 800 mg/mL of the crude and partitioned portions of *R. sativus* extract were

prepared by dissolving 200 mg, 400 mg and 800 mg respectively into 10 mL each of distilled water as vehicular. The same concentrations were maintained for column pooled fractions. Fraction E' only 200 and 400 mg/mL concentrations were used. However, the column pooled fractions were only tested against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi* and *Candida albicans* being the most susceptible of *n*-butanol partitioned portion. The crude methanol root extract, partitioned portions, column fractions and pure isolate(s) were subjected to bioassay-guided antimicrobial studies against four each of Gram-positive and Gram-negative bacteria and four fungal strains. Preliminary antimicrobial susceptibility was done as described by Magaldi *et al.* (2004), Valgas *et al.*, (2007) and Schwalbe *et al.* (2007) using disk diffusion technique. For antimicrobial susceptibility, the bacterial suspensions were prepared by suspending 3-5 well-isolated colonies from appropriate agar plates into 3 ml cation adjusted Mueller Hinton broth (CAMHB; BD Diagnostic Systems, adjusted to pH 5.9) (No *et al.*, 2002) and the turbidity was adjusted to a 0.5 McFarland standard. Also minimum inhibitory concentration (MIC), minimum fungicidal concentration (MFC) and minimum bactericidal concentration (MBC) of *R. sativus* extract solutions were determined as described by Vollekova and Sochorova (2001).

Some indices which include Activity Index (AI), Percentage Activity (PA), Bacterial / Fungal Susceptibility Index (BFSI), Average Percent of Bacterial / Fungal Susceptibility (APB/FS) and Spectral Intensity Index (SII) were calculated as described by Shahidi (2004), to establish relationship of activity (efficacy and potency) of the root extract of *R. sativus* compared with the standard drugs (Ciprofloxacin and Ketoconazole).

#### **Statistical analysis**

Data generated from the antimicrobial activity were analysed using GraphPad InStat 2000 Model, Version 3.10 for windows (Graphpad Software, 2000). One-way Analysis of Variance (ANOVA) test followed by Tukey-Kramer's Multiple Comparison test was used to analyze and compare the results at 95 % confidence level. Results were presented as the mean  $\pm$  standard error of mean (SEM) and results which differ with  $p \leq 0.5$  was considered significant.

## RESULTS AND DISCUSSION

### Partitioning Profile of Methanol root extract of *R. sativus*

The result of the methanol cool maceration yielded 244.00 g (4.88 %) and 215 g was exhaustively and successively partitioned with chloroform, ethyl acetate, *n*-butanol and aqueous and gave 1.34g (0.62 %), 2.00g (% .93), 34.00 g (15.81 %) and 150.00 g (69.76 %) respectively. The low percentage yield recorded could attribute to the cool maceration method used for extraction of bioactive compounds.

### *In-vitro* Microbial Susceptibility Against solvents partitioned portions of *R. sativus* (Disc Diffusion Assay)

The microbial growth inhibition of the solvents partitioned extracts of chloroform, ethyl acetate; *n*-butanol and water were measured by their diameters of inhibition zones against the selected microbial pathogens. The test concentrations were 800, 400 and 200 mg/mL and the standard drugs were ciprofloxacin and ketoconazole at 10 µg/mL and 200 mg/mL respectively. The diameter zone of inhibitions (DIZs) of the four portions at each test concentration was statistically compared against DIZs of the standard drugs. Also, MIC, MBC/MFC, activity, percent activity, bacterial/fungal susceptibility and spectral intensity indexes were all calculated and the results were presented in Tables 1- 8.

The antimicrobial effects of the partitioned portions at 200 mg/ml were recorded in Table 1. At the lowest concentration, only chloroform portion inhibited the growth of *S. aureus*, *S. pyogenes*, *B. subtilis*, *Corynaebacterium spp*, *S. typhi*, *K. pneumoniae*, *P. aeruginosa* and *C. albicans* while *n*-butanol inhibited growth of *S. aureus*, *B. subtilis*, *Corynaebacterium spp*, *E. coli*, *S. typhi*, *K. pneumoniae*, and *C. albicans* and water portion had activity only against *S. pyogenes*. Ethyl acetate portion was totally ineffective against all microbes at 200 mg/mL concentration. The ineffectiveness of the ethyl acetate portion suggested that the fraction does not contain antimicrobial phyto-compound(s) against test microbes. This observation was in total agreement to earlier finding of Sun *et al.* (2001), Shaalan *et al.* (2005) who pointed out those fractions from same extract always have different phytochemicals and perhaps different biological activity.

The growth inhibition zones of the partitioned portions against the test organisms at 400 mg/mL were presented in Table 2. At this concentration, all the portions had certain degrees of growth inhibitions against some microbes; however, ethyl acetate portion yet had the least inhibition effect against two Gram (+) *S. pyogenes* and *B. subtilis* while aqueous portion inhibited the growth of *S. aureus*, *S. pyogenes*, *E. coli*, *K. pneumoniae* and *C. albicans*. Nonetheless, higher activity was observed against two Gram (+) *S. aureus* and *S. pyogenes*. At 400 mg/mL concentration, maximum growth inhibitions were recorded against *S. aureus*, *S. pyogenes*, *B. subtilis*, *Coryneabacterium, spp.* *S. typhi*, *K. pneumoniae*, *P. aeruginosa* and *C. albicans* by chloroform portion while *n*-butanol portion had maximum growth inhibition against *E. coli*, *S. aureus*, *S. pyogenes*, *B. subtilis*, *C. bacterium*, *S. typhi*, *K. pneumoniae*, *P. aeruginosa* and *C. albicans*.

The growth inhibition effects of the partitioned portions at 800 mg/mL are presented in Table 3. As observed earlier above in the previous Tables, the activity order was chloroform portion > *n*-Butanol portion > water soluble portion > ethyl acetate portion. Chloroform had the highest growth inhibition against *S. aureus*, *S. pyugene*, *B. subtilis*, *Coryneabacterium spp.* *E. coli*, *S. typhi*, *K. pneumoniae*, *P. aeruginosa* and *C. albicans* while *n*-butanol followed second on the same microbes except on *S. pyogenes* water portion took the second lead while acted on *S. aureus*, *S. pyogenes*, *E. coli*, *S. typhi*, *K. pneumoniae* and *C. albicans*. The least being ethyl acetate only acted on two Gram (+) *S. pyogenes* and *B. subtilis*. However, as observed in all cases standard drugs lead over the extracts. The staggered antimicrobial effects observed could support by the fact that microbial microorganisms behave differently (susceptibility, resistance, tolerance, and persistence) under different antibiotic exposure (Brauner *et al.*, 2016). This observation further confirms that the solvent partitioned portions had different phyto-compounds

The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of solvent partitioned portions against test organisms were determined and results presented in Table 4. Among the partitioned extracts, chloroform portion had same strength

by 50 and 100 mg/mL as minimum inhibitory and minimum bactericidal concentrations against *S. aureus* and *S. pyogenes* however; water soluble portion had 200 mg/mL for both MIC and MBC. *B. subtilis* had 50 mg/mL for both MIC and MBC by chloroform portion, 200 mg/mL for both MIC and MBC by *n*-butanol portion. Chloroform portion was strongest with 50 mg/ml for both MIC and MBC while *n*-butanol portion had 200 mg/mL for both MIC and MBC. The strength of chloroform portion was 200 mg/ml against *E. coli* for both MIC and MBC similarly; *n*-butanol portion had 100 mg/ml against *E. coli* for both MIC and MBC. Meanwhile, *S. typhi* was more susceptible to *n*-butanol portion with 50 and 100 mg/mL for minimum inhibitory and minimum bacterial concentrations respectively followed by chloroform portion with 100 mg/mL for both for minimum inhibitory and minimum bacterial concentrations respectively. The *n*-Butanol portion had 200 mg/ml for both minimum inhibitory and bactericidal concentrations against *K. pneumoniae*, chloroform portion had 100 mg/mL for both minimum inhibitory and bactericidal concentrations. For *P. aeruginosa*, chloroform portion had 50 and 100 mg/mL for minimum inhibitory and bactericidal concentrations respectively. *C. albicans* was more susceptible to chloroform portion with 50 mg/ml for both minimum inhibitory and bactericidal concentrations followed by *n*-butanol portion with 200 mg/mL for minimum inhibitory and bactericidal concentrations respectively. From the findings of this study, based on the values of the antimicrobial activity of solvent portioned portions of *R. sativus* root could be biocidal because the highest MBC: MIC ratio observed was 2:1. Cushnie *et al.* (2020) reported that if MBC value of an antimicrobial agent is not four times higher than its MIC value, provisionally the substance can be considered biocidal.

The activity indexes of the solvent partitioned portions at test concentrations and bacterial and fungal susceptibility indexes were calculated and presented in Table 5. From the result, it is apparent that chloroform portion had the highest activity indexes against *S. aureus* with 37, 48 and 65 percent at 200, 400 and 800 mg/mL respectively. Zero percent (0%) activity index was recorded against *S. aureus* at all concentrations by ethyl acetate,

30, 38 and 55, percent were by *n*-butanol portion and 0, 27 and 35, were by water portion at 200, 400 and 800 mg/mL respectively. *S. aureus* was 100% susceptible to chloroform and *n*-butanol portions, while was 66.66% susceptible to water soluble portion and had 0% susceptibility to ethyl acetate portion. The susceptibility indexes of *S. pyogenes* to solvent partitioned portions were 100 percent for chloroform and *n*-Butanol portions and 66.66 percent for ethyl acetate and water portions. Meanwhile, the activity indexes of these portions against *S. pyogenes* were 26, 35 and 48% by chloroform portion, 0, 18, 26 percent by ethyl acetate portion, 0, 20 and 37% by *n*-butanol portion and 21, 28 and 39 percent by water portion at 200, 400 and 800 mg/mL respectively. *B. subtilis* was 100 percent susceptible to chloroform and *n*-Butanol portions while was 66.66% to ethyl acetate portion and was 0 percent susceptible to water portion. Activity indexes of these portions against *B. subtilis* were 34, 42 and 58% by chloroform portion, 0, 22 and 32%t by ethyl acetate portion, 22, 29 and 36% by *n*-Butanol and 0% by water portion at 200, 400 and 800 mg/mL respectively. *Corynebacterium spp.* was resistant to ethyl acetate and water portions however, was 100% susceptible to chloroform and *n*-butanol portions. Meanwhile, 28, 35, and 51% activity indexes were recorded against *Corynebacterium spp.* by chloroform portion, 0 percent activity was recorded by ethyl acetate and water portions while 17, 23 and 29% activity indexes were recorded by *n*-butanol portion. *E. coli*, on the other hand had against it 23, 28 and 39% activity indexes by chloroform, 28, 37 and 48% activity indexes by *n*-Butanol, 0, 22 and 37% activity indexes by water portion and 0 percent activity index by ethyl acetate portion at 200, 400 and 800 mg/mL respectively. By activity indexes, *E. coli* was 100 percent susceptible to *n*-butanol and chloroform portions and 66.66% susceptible to water portion while 0% susceptible to ethyl acetate portion. Going by susceptibility indexes, *S. typhi* was 100% subdued by *n*-butanol and chloroform portions and 33.33% susceptible to water portion while 0% susceptible to ethyl acetate portion. The following activities were 31, 44, 60% recorded against *S. typhi* by chloroform portion, 30, 40, 48% by *n*-butanol portion and 0 percent by ethyl acetate portion at 200, 400 and 800 mg/mL respectively while

only 27% by water portion at 800 mg/mL. Meanwhile, *K. pneumoniae* had 28, 38 and 55% activities by chloroform portion, 24, 32 and 42 percent by *n*-butanol portion, 0, 22 and 33% by water portion and 0% by ethyl acetate portion. *K. pneumoniae* was 100, 0, 100 and 66.66% susceptible to chloroform, ethyl acetate, *n*-butanol and water portions respectively. Interestingly, *P. aeruginosa* had against it 51, 68 and 82% activities by chloroform portion, 0, 46 and 57 percent by *n*-butanol portion however, 0% by ethyl acetate and water portions at 200, 400 and 800 mg/mL respectively. Meanwhile, it was 100 and 66.66% susceptible to chloroform and *n*-butanol portions while 0 percent susceptibility was recorded for ethyl acetate and water portions. For *Candida albicans* had against it 35, 44 and 59% activities by chloroform portion, 22, 28 and 39% activities by *n*-butanol portion, 0, 22, and 32% activities by water portion while 0% activity by ethyl acetate portion at 200, 400 and 800 mg/mL respectively when ciprofloxacin was the standard. However, when standard was ketoconazole, 32, 53 and 71 percent activities for chloroform portion, 27, 33, and 46% activities for *n*-Butanol portion, 0, 27, 39% activities for water portion but 0 percent activity for ethyl acetate at 200, 400 and 800 mg/mL respectively. Meanwhile, *Candida albicans* was 100% susceptible to *n*-butanol and chloroform portions and 66.66% susceptible to water portion while it was resistant to ethyl acetate portion.

The spectral intensity index and percent total activity of solvent partitioned portions against bacterial and fungal strains at 200, 400 and 800 mg/mL were calculated and presented in Table 6. Chloroform portion had 100% activity each and 100% total activity each against Gram (+) and Gram (-) bacteria while had 25 percent activity each and 25% total activity each at 200, 400 and 800 mg/mL against fungal. The intensity of activity of chloroform portion against bacterial and fungal strains was increased by increased in concentration as evidently observed by spectral intensities at different concentrations 9.70, 12.49 and 17.20 mm for bacterial strain and 2.83, 3.58 and 4.75 for fungal strain at 200, 400 and 800 mg/ml respectively. Similarly, ethyl acetate portion had only 50% activity each against Gram (+) bacteria at 400 and 800 mg/mL respectively while 0% activity

each against Gram (-) and fungal strains at all concentrations. The percent activity of ethyl acetate against bacterial strain was 25 at 400 and 800 mg/mL and 0% each for fungal strain at all concentrations. Meanwhile, the spectral intensity was similar to that of chloroform portion however, for ethyl acetate were 1.79 and 2.53 mm for bacterial strains at 400 and 800 mg/mL and 0 mm each at all concentrations for fungal strains. Subsequently, *n*-butanol had 75% activity each for Gram (+) bacteria at 200, 400 and 800 mg/mL respectively and 100 percent activity each for Gram (-) bacteria at all concentrations. There was 25% activity each at 200, 400 and 800 mg/mL for fungal strain. Meanwhile, there was 75% total at 200 mg/mL and 100 percent total activity each at 400 and 800 mg/mL for bacterial strain and 25% total activity each at all concentrations for fungal strain. The spectral intensity of *n*-butanol 6.0, 8.67 and 9.56 mm at 200, 400 and 800 milligrams per mills for bacterial strain and 1.83, 2.25 and 3.08 mm at 200, 400 and 800 milligrams per mills for fungal strain. Lastly, water portion had 25 % activity at 200 mg/ml and 50 % activity each at 400 and 800 mg/mL for Gram (+) bacteria and 0, 50 and 75 % activities at 200, 400 and 800 mg/mL respectively for Gram (-) bacteria while for fungal strain there was 0 % activity at 200 mg/mL and 25 % activity each at 400 and 800 mg/mL. The percent total activity for water portion were 12.5, 50 and 75 at 200, 400 and 800 mg/mL respectively for bacterial strain and 0 at 200 mg/ml and 25 each at 400 and 800 mg/mL respectively for fungal strain. As usual, the spectral intensity increased by increased in concentration therefore, 1.0, 1.02 and 6.84 mm for bacterial strain and 0, 1.83 and 2.58 mm for fungal strain.

Averagely, from Table 7, bacterial strain was 100 % susceptible to chloroform portion, 91.66 % susceptible to *n*-butanol portion, 41.83 % susceptible to water portion and 16.66 % susceptible to ethyl acetate portion while fungal strain was 25 % susceptible each to chloroform and *n*-butanol portions and 16.66 % susceptible to water portion however, 0 % susceptible to ethyl acetate portion.

The qualitative phytochemical tests of partitioned portions of the root of *R. sativus* revealed the presence of alkaloids, flavonoids, cardiac glycosides, terpenoids, phyosterols and carbohydrates while saponins,

anthraquinones, and tannins were not detected. Cardiac glycosides were not detected in residual aqueous. These phytochemicals could be most probably responsible for the observed antimicrobial activity of the root extract of *R. sativus*. This idea was earlier suggested by Makhafola *et al.* (2014); Njeru and Muema (2020) who reported that bioactivity of plant extracts is the effect of secondary metabolites

produced for purposes of normal plant defenses; to deter, stun, poison or kill threatening species, but inadvertently inhibiting various physiological targets/processes required for growth, biosynthesis of macromolecules, metabolism and virulence of microbial systems. Details of phytochemicals present from the partitioned portions are shown in Table 8.

**Table 1:** Antimicrobial effects of solvents partitioned portions of *R. sativus* at 200 mg/mL against standard drugs

Test organism	Mean $\pm$ SEM Diameters of Inhibition Zone (mm) of Extracts and standard drugs					
	Chloroform	Ethyl acetate	<i>n</i> -Butanol	Aqueous	Cip 10 $\mu$ g/mL	Ktz 200 mg/mL
<i>S. aureus</i>	10.33 $\pm$ 0.33 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	8.66 $\pm$ 0.33 <sup>c</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	27.66 $\pm$ 0.33 <sup>d</sup>	NA
<i>S. pyogene</i>	10.33 $\pm$ 0.33 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	8.00 $\pm$ 0.00 <sup>c</sup>	38.00 $\pm$ 0.00 <sup>d</sup>	NA
<i>B. subtilis</i>	11.33 $\pm$ 0.33 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	7.00 $\pm$ 0.00 <sup>c</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	33.00 $\pm$ 0.00 <sup>d</sup>	NA
<i>C. spp</i>	11.33 $\pm$ 0.33 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	7.00 $\pm$ 0.00 <sup>c</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	40.00 $\pm$ 0.00 <sup>d</sup>	NA
<i>E. coli</i>	7.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	8.66 $\pm$ 0.33 <sup>c</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	31.33 $\pm$ 0.33 <sup>d</sup>	NA
<i>S. typhi</i>	9.33 $\pm$ 0.33 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	9.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	30.00 $\pm$ 0.00 <sup>c</sup>	NA
<i>K. pneumoniae</i>	8.66 $\pm$ 0.33 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	7.00 $\pm$ 0.00 <sup>c</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	30.66 $\pm$ 0.33 <sup>d</sup>	NA
<i>P. aeruginosa</i>	9.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	18.66 $\pm$ 0.33 <sup>c</sup>	NA
<i>C. albicans</i>	11.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	7.00 $\pm$ 0.00 <sup>c</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	31.66 $\pm$ 0.33 <sup>d</sup>	27.00 $\pm$ 0.00 <sup>e</sup>
<i>A. niger</i>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	NA	20.33 $\pm$ 0.33 <sup>b</sup>
<i>R. stolonifera</i>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	NA	24.66 $\pm$ 0.33 <sup>b</sup>
<i>A. flavus</i>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	NA	26.66 $\pm$ 0.33 <sup>b</sup>

**Key:** mean values with different superscript letters across the row are significantly ( $P \leq 0.05$ ) different,  $n=3$ , SEM = standard error of mean, Cip = ciprofloxacin, Ktz = ketoconazole, NA = not applicable

**Table 2:** Antimicrobial effects of solvents partitioned portions of *R. sativus* at 400 mg/mL against standard drugs

Test organism	Mean $\pm$ SEM Diameters of Inhibition Zone (mm) of Extracts and standard drugs					
	Chloroform	Ethyl acetate	<i>n</i> -Butanol	Aqueous	Cip 10 $\mu$ g/mL	Ktz 200 mg/mL
<i>S. aureus</i>	13.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	11.00 $\pm$ 0.00 <sup>c</sup>	7.66 $\pm$ 0.33 <sup>d</sup>	28.00 $\pm$ 0.00 <sup>e</sup>	NA
<i>S. pyogenes</i>	13.33 $\pm$ 0.33 <sup>a</sup>	7.00 $\pm$ 0.00 <sup>b</sup>	7.66 $\pm$ 0.33 <sup>b</sup>	10.66 $\pm$ 0.33 <sup>c</sup>	38.00 $\pm$ 0.00 <sup>d</sup>	NA
<i>B. subtilis</i>	14.00 $\pm$ 0.00 <sup>a</sup>	7.33 $\pm$ 0.33 <sup>b</sup>	9.33 $\pm$ 0.33 <sup>c</sup>	0.00 $\pm$ 0.00 <sup>d</sup>	33.00 $\pm$ 0.00 <sup>e</sup>	NA
<i>C. spp</i>	14.33 $\pm$ 0.33 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	9.00 $\pm$ 0.00 <sup>c</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	40.00 $\pm$ 0.00 <sup>d</sup>	NA
<i>E. coli</i>	9.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	11.66 $\pm$ 0.33 <sup>c</sup>	8.00 $\pm$ 0.00 <sup>d</sup>	31.33 $\pm$ 0.33 <sup>e</sup>	NA
<i>S. typhi</i>	13.33 $\pm$ 0.33 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	12.00 $\pm$ 0.00 <sup>c</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	30.33 $\pm$ 0.33 <sup>d</sup>	NA
<i>K. pneumoniae</i>	11.66 $\pm$ 0.33 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	10.00 $\pm$ 0.00 <sup>c</sup>	7.00 $\pm$ 0.00 <sup>d</sup>	30.66 $\pm$ 0.33 <sup>e</sup>	NA
<i>P. aeruginosa</i>	11.66 $\pm$ 0.33 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	8.00 $\pm$ 0.00 <sup>c</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	18.00 $\pm$ 0.00 <sup>d</sup>	NA
<i>C. albicans</i>	14.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	9.00 $\pm$ 0.00 <sup>c</sup>	7.00 $\pm$ 0.00 <sup>d</sup>	31.66 $\pm$ 0.33 <sup>e</sup>	27.00 $\pm$ 0.00 <sup>f</sup>
<i>A. niger</i>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	NA	20.33 $\pm$ 0.33 <sup>b</sup>
<i>R. stolonifera</i>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	NA	24.66 $\pm$ 0.33 <sup>b</sup>
<i>A. flavus</i>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	NA	26.66 $\pm$ 0.33 <sup>b</sup>

**Key:** mean values with different superscript letters across the row are significantly ( $P \leq 0.05$ ) different,  $n=3$ , SEM = standard error of mean, Cip = ciprofloxacin, Ktz = ketoconazole, NA = not applicable

**Table 3:** Antimicrobial effects of solvents partitioned portions of *R. sativus* at 800 mg/mL against standard drugs

Test organism	Mean $\pm$ SEM Diameters of Inhibition Zone (mm) of Extracts and standard drugs					
	Chloroform	Ethyl acetate	<i>n</i> -Butanol	Aqueous	Cip 10 $\mu$ g/mL	Ktz 200 mg/mL
<i>S. aureus</i>	18.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	15.00 $\pm$ 0.00 <sup>c</sup>	9.66 $\pm$ 0.33 <sup>d</sup>	28.00 $\pm$ 0.00 <sup>e</sup>	NA
<i>S. pyogenes</i>	18.33 $\pm$ 0.33 <sup>a</sup>	10.00 $\pm$ 0.00 <sup>b</sup>	10.00 $\pm$ 0.00 <sup>b</sup>	15.00 $\pm$ 0.00 <sup>c</sup>	38.00 $\pm$ 0.00 <sup>d</sup>	NA
<i>B. subtilis</i>	19.00 $\pm$ 0.00 <sup>a</sup>	10.00 $\pm$ 0.00 <sup>b</sup>	12.00 $\pm$ 0.00 <sup>c</sup>	0.00 $\pm$ 0.00 <sup>d</sup>	32.66 $\pm$ 0.33 <sup>e</sup>	NA
<i>C. spp</i>	20.33 $\pm$ 0.33 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	11.66 $\pm$ 0.33 <sup>c</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	40.00 $\pm$ 0.00 <sup>d</sup>	NA
<i>E. coli</i>	12.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	15.00 $\pm$ 0.00 <sup>c</sup>	12.00 $\pm$ 0.00 <sup>a</sup>	31.33 $\pm$ 0.33 <sup>d</sup>	NA
<i>S. typhi</i>	18.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	14.66 $\pm$ 0.33 <sup>c</sup>	8.00 $\pm$ 0.00 <sup>d</sup>	30.00 $\pm$ 0.00 <sup>e</sup>	NA
<i>K. pneumoniae</i>	17.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	13.00 $\pm$ 0.00 <sup>c</sup>	10.00 $\pm$ 0.00 <sup>d</sup>	30.66 $\pm$ 0.33 <sup>e</sup>	NA
<i>P. aeruginosa</i>	15.33 $\pm$ 0.33 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	10.00 $\pm$ 0.00 <sup>c</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	18.00 $\pm$ 0.00 <sup>d</sup>	NA
<i>C. albicans</i>	19.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	12.00 $\pm$ 0.00 <sup>c</sup>	10.00 $\pm$ 0.00 <sup>d</sup>	32.33 $\pm$ 0.33 <sup>e</sup>	27.00 $\pm$ 0.00 <sup>f</sup>
<i>A. niger</i>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	NA	20.33 $\pm$ 0.33 <sup>b</sup>
<i>R. stolonifera</i>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	NA	24.66 $\pm$ 0.33 <sup>b</sup>
<i>A. flavus</i>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	NA	26.66 $\pm$ 0.33 <sup>b</sup>

**Key:** mean values with different superscript letters across the row are significantly ( $P \leq 0.05$ ) different,  $n=3$ , SEM = standard error of mean, Cip = ciprofloxacin, Ktz = ketoconazole, NA = not applicable

**Table 4:** Minimum inhibitory and minimum bactericidal/fungicidal concentrations of solvent partitioned portions

Test organism	Concentration of the extracts (mg/mL)		
	Chloroform	<i>n</i> -Butanol	Aqueous
<i>S. aureus</i>	50 <sup>a</sup> 100 <sup>b</sup>	100 <sup>a</sup> 100 <sup>b</sup>	ND
<i>S. pyogenes</i>	50 <sup>a</sup> 100 <sup>b</sup>	ND	200 <sup>a</sup> 200 <sup>b</sup>
<i>B. subtilis</i>	50 <sup>a</sup> 50 <sup>b</sup>	200 <sup>a</sup> 200 <sup>b</sup>	ND
<i>C. bacteria</i>	50 <sup>a</sup> 50 <sup>b</sup>	200 <sup>a</sup> 200 <sup>b</sup>	ND
<i>E. coli</i>	200 <sup>a</sup> 200 <sup>b</sup>	100 <sup>a</sup> 100 <sup>b</sup>	ND
<i>S. typhi</i>	100 <sup>a</sup> 100 <sup>b</sup>	50 <sup>a</sup> 100 <sup>b</sup>	ND
<i>K. pneumoniae</i>	100 <sup>a</sup> 100 <sup>b</sup>	200 <sup>a</sup> 200 <sup>b</sup>	ND
<i>P. aeruginosa</i>	50 <sup>a</sup> 100 <sup>b</sup>	ND	ND
<i>C. albicans</i>	50 <sup>a</sup> 50 <sup>c</sup>	200 <sup>a</sup> 200 <sup>c</sup>	ND

**Key:** values with super script <sup>a</sup> = MIC, <sup>b</sup> = MBC, <sup>c</sup> = MFC, ND =not determined



**Table 5:** Activity, bacterial and fungal susceptibility indexes of partitioned portions at concentrations

Test organisms	Activity index (%) of partitioned portions at test concentrations												Bacterial /fungal susceptibility index (%)			
	200 mg/mL				400 mg/mL				800 mg/mL				CFP	EAP	NBP	AQP
	CFP	EAP	NBP	AQP	CFP	EAP	NBP	AQP	CFP	EAP	NBP	AQP				
<i>S. aureus</i>	37	0	30	0	48	0	38	27	65	0	55	35	100	0	100	66.66
<i>S. pyugene</i>	26	0	0	21	35	18	20	28	48	26	27	39	100	66.66	66.66	100
<i>B. subtilis</i>	34	0	22	0	42	22	29	0	58	32	36	0	100	66.66	100	0
<i>C. spp</i>	28	0	17	0	35	0	23	0	51	0	29	0	100	0.00	100	0
<i>E. coli</i>	23	0	28	0	28	0	37	22	39	0	48	37	100	0.00	100	66.66
<i>S. typhi</i>	31	0	30	0	44	0	40	0	60	0	48	27	100	0.00	100	33.33
<i>K. pneumoniae</i>	28	0	24	0	38	0	32	22	55	0	42	33	100	0.00	100	66.66
<i>P. aeruginosa</i>	51	0	0	0	68	0	46	0	82	0	57	0	100	0.00	66.66	0
<i>C. albican</i>	35	0	22	0	44	0	28	22	59	0	39	32	100	0.00	100	66.66
	32*		27*		53*		33*	27*	71*		46*	39*				
<i>A. niger</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0	0
<i>R. stolonifera</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0	0
<i>A. flavus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0	0

**Key:** CFP= chloroform, EAP = ethyl acetate, NBP = *n*-Butanol and AQP = aqueous, plain value = percent activity using ciprofloxacin as standard while value with asterisk = percent activity using ketoconazole

**Table 6:** Percent total activity and spectral intensity index of partitioned portions against bacterial and fungal strains at 200, 400 and 800 mg/mL

Portions	Percent activity (%) at test concentrations									Percent total (%) at test concentrations						Spectral intensity index (mm) at test concentrations					
	Gram (+)			Gram (-)			Fungal strain			Bacterial strain			Fungal strain			Bacterial strain			Fungal strain		
	200	400	800	200	400	800	200	400	800	200	400	800	200	400	800	200	400	800	200	400	800
CFP	100	100	100	100	100	100	25	25	25	100	100	100	25	25	25	9.70	12.49	17.20	2.83	3.58	4.75
EAP	0	50	50	0	0	0	0	0	0	0	25	25	0	0	0	0	1.79	2.53	0	0	0
NBP	75	75	75	100	100	100	25	25	25	75	100	100	25	25	25	6.0	8.67	9.56	1.83	2.25	3.08
AQP	25	50	50	0	50	75	0	25	25	12.5	50	75	0	25	25	1.0	1.02	6.84	0	1.83	2.58

**Table 7:** Average percentage bacterial/fungal susceptibility to partitioned portions

Portions	Average percentage bacterial/fungal susceptibility (%)	
	Bacterial strain	Fungal strain
CFP	100	25
EAP	16.66	0
NBP	91.66	25
AQP	41.83	16.66

**Table 8:** Results of Qualitative Phytochemical Tests of Partitioned Portions of *R. sativus* Root

SN	TESTS	CFP	EAP	NBP	AQP
1	Alkaloids				
	Dragendroff's reagent	+	+	+	+
2	Mayer's reagent	-	-	-	-
	Flavonoids				
3	Shinoda's test	+	+	+	+
	Ferric chloride	-	-	-	-
	Lead acetate	-	-	-	-
	Sodium hydroxide	-	-	-	-
4	Cardiac glycosides				
	Keller-Killiani	+	+	+	+
5	Saponins				
	Frothing	-	-	+	-
6	Terpenoids	+	+	+	+
7	Triterpenoides				
	Salkowski's	+	+	+	-
8	Phytosterols				
	Salkowski's test	+	+	+	-
	Liebermann-Burchard's test	-	+	+	-
9	Carbohydrates				
	Molisch's	-	-	+	+
	Borfoed's	-	-	+	+
	Fehling's	+	+	+	+
	Combined reducing sugar	+	+	+	+
10	Ketoses	+	+	+	+
	Anthraquinones				
10	Borntrager's test	-	-	-	-
	Tannins				
	Lead acetate	-	-	-	-
	Sodium hydroxide	-	-	-	-

Key: + = presence, - = absence, CFP= chloroform partitioned portion, EAP= Ethyl acetate partitioned portion; n-BP=n-butanol partitioned portion and AQP=Aqueous Partitioned Portion

## CONCLUSION

Conclusively, chloroform, ethyl acetate, n-butanol and aqueous portions had demonstrated a concentration dependent activity, however, of the four portions, chloroform had the best activity, and likely, the active antimicrobial agent is from chloroform portion. Phytochemicals responsible for the observed activities might be alkaloids, flavonoids, cardiac glycosides or terpenoids. To optimize antimicrobial properties of *R. sativus* root, further research should be carried out using chloroform as solvent of extraction.

## REFERENCES

- ACDC, (2018). Africa Center for Disease Control, Africa CDC framework for antimicrobial resistance, 2018-2023
- Brauner, A., Fridman, O., Gefen, O., and Balaban, N. Q. (2016). Distinguishing between resistance, tolerance and persistence to antibiotic treatment. *Nat. Rev. Microbiol.* 14: 320–330.
- Cushnie, T. P. T., Cushnie, B., Echeverría, J., Fowsantear, W., Sutthiwan, T. S., Dodgson, J. L. A., Law, S. and Clow, S. M. (2020). Bioprospecting for antibacterial drugs: A multidisciplinary perspective on natural products source material, bioassay-guided selection and avoidable pitfalls. *Pharm. Res.* 37 (7): 125.
- Dada, S., Odetoyn, B., Adeyemo, S and Olaniran, O. (2023). High prevalence of multidrug-resistant bacteria in fomites in a tertiary institution in Southwestern Nigeria. *Journal of Contemporary Studies in Epidemiology and Public Health* 4(2):ep23006. <https://doi.org/10.29333/jconseph/13132>
- Dar, K. B., Bhat, A. H., Amin, S., Anees, S., Masood, A., Zargar, M. I. and Ganie, S. A. (2016). Efficacy of aqueous and methanolic extracts of *Rheum Spiciformis* against pathogenic

- bacterial and fungal strains. *J. Clin. Diagn. Res.*, 10: 18-22. <https://doi.org/10.7860/JCDR/2016/18036.8486>.
- Elvino N, Moses B, Doice M, and Pardon N, (2022). Extraction Methods, Quantitative and Qualitative Phytochemical Screening of Medicinal Plants for Antimicrobial Textiles. *A Review Plants (Basel)*. 11(15): 1-26.
- Emelda, E. C., David, A. O., Oluwatoyin, B. A., Ebelechukwu, E. A., Rahman, G. L., Ismail, A., Folasade, T. O., and Rosemary, A. A. (2020). A national survey of public awareness of antimicrobial resistance in Nigeria. *Antimicrob Resist Infect Control*. 9 (72): 1-11 <https://doi.org/10.1186/s13756-020-00739-0>.
- Fitzgerald M, Heinrich M and Booker A (2020) Medicinal Plant Analysis: A Historical and Regional Discussion of Emergent Complex Techniques. *Front. Pharmacol.* 10:1480.
- Graphad Software (2000): Graphad Software Instat Guide to choosing and interpreting statistical tests, Graphad Software, Inc, San Diego, California USA Version 3.10 32 bit for windows: [www.graphad.com](http://www.graphad.com).
- Hutchings MI, Truman AW and Wilkinson B (2019). Antibiotics: past, present and future. *Current Opinion in Microbiology*, 51: 72'80. <https://doi.org/10.1016/j.mib.2019.10.008>.
- Ibrahim, S., Sodipo, O. A. and Abdulrahman F. I. (2022). Proximate content, elemental, phytochemical and antimicrobial evaluations of crude methanol root extract of raphanus sativus linn. *JSR*, 1(1): 33-50.
- Junaid, R. S. and Patil, M. K., (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies* 8 (2): 603-608.
- Kebede, T., Gadisa, E. and Tufa, A. (2021). Antimicrobial activities evaluation and phytochemical screening of some selected medicinal plants: A possible alternative in the treatment of multidrug-resistant microbes. *PLoS one* 16 (3): e0249253.
- Khalid, G. M., Jatau A., I., Ibrahim. U. I., Dungus, F. M., Shitu, Z., Sha'aban, A. and Burji, SIL. (2019). Antibiotics self-medication among undergraduate pharmacy students in Northern Nigeria. *Med. Access@ Point of Care*, 3: 1-8.
- Magaldi, S., Mata-Essayag, S., Hartung de Capriles, C., Perez, C., Colella, M.T., Olaizola, C. and Ontiveros, Y. (2004). Well diffusion for antifungal susceptibility testing, *Int. J. Infect. Dis.*, 8: 39-45.
- Makhafola, T. Y., McGaw, L. J. and. Eloff, J. N. (2014). In vitro cytotoxicity and genotoxicity of five Ochna species (Ochnaceae) with excellent antibacterial activity, *South Afr. J. Bot.* 91: 9-13. <https://doi.org/10.1016/j.sajb.2013.11.010>.
- Nasim, N, Sandeep IS, and Mohanty S, (2022). Plant-derived natural products for drug discovery: current approaches and prospects. *Nucleus (Calcutta)* 65 (3): 399-411.
- Neş, B. Ç., Emine, Y., Kültiğın, C., and Selin, S. K. (2022). Qualitative and quantitative phytochemical screening of Nerium oleander L. extracts associated with toxicity profile. *Scientific Reports* 12: 1-16.
- Njeru, S. N. and Muema, J. M. (2020). Antimicrobial activity, phytochemical characterization and gas chromatography-mass spectrometry analysis of *Aspilia pluriseta* Schweinf. Extracts. *Heliyon* 6: e05195.
- Nur, F. S., Qamar, U. A., Syed, M., Syed, A. A. S., Alfi, K., Sayeed, M., Meshari, A. A., Humaira, P. and Zainul, A. Z. (2022). Antibacterial Effects of Flavonoids and Their Structure-Activity Relationship Study: A Comparative Interpretation. *Molecules*; 27(4): 1-59 <https://doi.org/10.3390/molecules27041149>.
- No, H. K., Park, N. Y., Lee, S. H. and Meyers, S. P. (2002). Antibacterial Activity of

- Chitosans and Chitosan Oligomers with Different Molecular Weights. *Int. J. Food Microbiol.*, 74 (1-2): 65-72.
- Sausana, O. M., Tonny. A., Mary. A., Peter, A. J., Daniel, B., Doris, K., Alfred, A., Augustine, O., Yaw, D. B. and Christian, A. (2019). Medicinal Plants for Treatment of Prevalent Diseases. IntechOpen. <https://doi.org/10.5772/intechopen.82049>.
- Schwalbe, R., Steele-moore, L. and Goodwin, A. (2007). *Antimicrobial Susceptibility Testing protocols*. 430 p.
- Sean, C., Stella, N., Cathrin, C. L., Joel. T., Benn, S., Christiane, D., Céline, C., Paul, N. N. and Ben, S. C. (2023). The uncertain role of substandard and falsified medicines in the emergence and spread of antimicrobial resistance. *Nature Communications* 14: 1-9 <https://doi.org/10.1038/s41467-023-41542-w>.
- Selvakumar, R. (2023). An update on radish reeding strategies: an overview. Intecopen.doi:10.5772/intecopen.108725.
- Shaalán, E. A-S., Canyon, D., Younes, M. W. F., Abdel-Wahaba, H. and Mansour, A-H. (2005). A review of botanical phytochemicals with mosquitocidal potential. *Environment Intl* (31): 1149-1166.
- Shahidi, B. G. H. (2004). New approaches in Screening for Antimicrobials in Plants. *Asian J. Plt Sci.* 3 (1): 55-60.
- Sodipo, O. A., Akanmu, A. O., Mamza, U. T., Oke, E. O. and Gulani, I. (2016). In-vitro antimicrobial studies of the aqueous and methanol extracts of Watheria. Americana Linn (Sterculiaceae) in Maiduguri. *Nig. J. pharmaceut. Biomed. Res.* 1(1): 43-49.
- Sun, R, Sacalis, J. N., Chin, C. and Still, C. C. (2001). Bioactive aromatic compounds from leaves and stems of *Vanilla fragrans*. *J. Agric Food Chem.* (49):5161-5164.
- Valgas, C., De Souza, S. M., Smânia, E.F.A. and Smânia, J. A. (2007). Screening Methods to determine antibacterial activity of natural products. *Braz. J. Microbiol.*, 38: 369-380.
- Volleková, A., Kòst'álovà, D. and Sochorová, R. (2001). Isoquinoline alkaloids from *Mahonia aquifolium* stem bark is active against *Malassezia spp.* *Folia Microbiol*46: 107-111.
- Wangchuk, P. (2018). Therapeutic applications of natural products in herbal medicines, biodiscovery programs, and biomedicine. *J Biol Act Prod Nat.*, 8(1):1-20.
- Wrink, M. (2010). Functions and biotechnology of Plant Secondary Metabolites, Annual Plant. Reviewes, vol. 39, Wiley-Blackwell Pub. London, UK, p. 24- 39.