

## Evaluation of the Antibacterial Activity and Phytochemical Screening of *Nuxia congesta* Leaf in Gondar, Northern Ethiopia

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### Abstract

This study aims to evaluate antibacterial properties of *Nuxia congesta* species and identify the biologically active compounds present in the extracts of *Nuxia congesta*. Organic solvents (methanol, and ethanol, and inorganic solvents (chloroform) were used for extraction of leaf of *Nuxia congesta*. Some bioactive compound found in this plant was screened in the Gondar microbiology laboratory and Identified bacterial strains were purchased from Ethiopia public health institute, Addis ababa Ethiopia and from national animal health diagnostic center Sebeta, Ethiopia (NAHDIC.). The antibacterial activity of the solvent extract was tested on seven bacterial species using agar disk diffusion method at the same concentrations (500 mg/ml) three times in the presence of positive control (tetracycline) and negative control (dimethyl sulphoxide). Test strains were *E. coli*, *E. faecalis*, *S. aureus*, *P. aeruginosa*, *K. pneumonia*, *P. mirabilis* and *H. influenza*. The antibacterial properties were quantitatively evaluated by the zone of inhibition and minimum inhibitory concentration. Ethanol and methanol extract showed positive response for alkaloid, flavonoid, tannin, terpenoid and polyphenol while chloroform extract showed positive respond for flavonoid, tannin, and terpenoid. Ethanol and methanol extraction revealed antibacterial activities against the growth of test bacterial strains with varying antibacterial spectrum where most tested strains were less sensitive to chloroform extraction. The average minimum inhibitory concentration value of the ethanol and methanol extracts ranged from 31.25 mg/ml to 208.33 mg/ml and from 41.75 mg/ml to 208.33 mg/ml, respectively. *N. congesta* leaf demonstrated significant antibiotic activity against the tested bacteria.

**Keywords:** *Nuxia congesta*; Antibacterial activity; Ethanol; Methanol; Chloroform

### Introduction

Ethiopia has the huge livestock population in Africa and owns about 41.5 million heads of cattle, 28.2 million sheep and goats, 5.8 million equine, 1 million camels and over 42 million poultry [1]. The livestock production is a major economy among resource-poor small holder farmers by providing milk, meat, skin and manure. However their economic benefits have been reducing by the occurrence diseases [2].

Ethno veterinary medicine (EVM) is a holistic inter-disciplinary study of the local knowledge and the socio-cultural structures and environment associated with animal health care and husbandry [3]. Hence, to keep animals healthy, traditional healing practices have been applied for centuries and have been passed down orally from generation to generation [4].

Medicinal plants have been used since ancient time for the management of various humans and animals ailments. Those medicinal plants are used mainly in local, traditional medicine rather than exported to foreign markets. Demand is increasing and often exceeds supply which is estimated that about 80% of the world's population depends wholly or partially on the plant based traditional medicine for its primary healthcare needs [5].

In Ethiopia, about 800 species of plants are used in the traditional health care system to treat nearly 300 mental and physical disorders.

Traditional medicine still remains the main resource for a large majority of the people in Ethiopia for treating health problem [6].

Despite significant recent improvements in modern healthcare, many rural communities continue to have limited access to modern health care due to availability and affordability [7]. It is widely acknowledged that the wisdom of both professional and lay healers in applying traditional medicine to support health and manage illness [8-10].

A large number of farmers rely on a range of ethno veterinary knowledge to keep their livestock healthy and have been used for preventing and treating livestock ailments for several generations. Diversity of plant species which have pharmacological activities were identified so far and the active ingredients are extracted mainly from the root, stem, and leaf parts that processed to administer through appropriate routes and it is accessible and easy to prepare and administer, at little or no cost to the farmer [11].

Now days we are using few antibiotics to treat several infectious diseases and microorganisms. Especially bacteria have the tendency to adapt quickly to their immediate environment. Drugs for treating bacterial infections lose their effectiveness with time; because the targets of these drugs keep shifting their forms and drug lose its effectiveness. Infections caused by bacteria are responsible for considerable mortality and morbidity worldwide especially in developing countries due to poor sanitation, unhygienic and overcrowded living conditions also. The management of multi-drug resistant bacterial strains is difficult because treatment options are limited and if available are beyond the reach of the poor and this

increase risk to death and the cost of hospitalization and increase the cost on healthcare systems [12].

Drug use from medicinal plants has progressed from the formulation of crude drugs to the isolation of bioactive compounds like alkaloids, terpenoids, flavonoids, tannins and phenolic compounds, essential oils, lectins and polypeptides, polyacetylenes and proposed for use as antimicrobial activity and new drug discovery [13].

Currently, studies on herbal medicines appear under different names such as plant medicines, phytomedicines, and natural products. Under pharmacognosy usually referring to products processed from living organisms: plants, animals, insects, microorganisms and marine organisms. Atropine, morphine, quinine, ephedrine, warfarin, salicin, digoxin, vincristine, taxol, and hyosine are some examples of extracts from traditional plants currently used in modern medicines. Findings from ethnobotanical and ethnomedicinal studies have shown correlation between medicinal use and laboratory results. Natural sources are usually the starting points for most pharmacological agents [14].

Therefore, research is needed in the production of low cost, resistance free and locally available antimicrobials. Herbal scientists are also researching on alternative sources of resistance free drugs from medicinal plants. A few studies on the sensitivity of microorganisms to some plants have recently been carried out [15].

In view of this, scientific studies have to be conducted on the traditional medicinal plants to overcome the global problem of antimicrobial resistance and for the purpose of developing a new, effective and safe antimicrobial drug from *Nuxia congesta* plant. There is no preliminary antimicrobial screening for *N. congesta*; despite the plant is used to treat human and animal ailments. It was necessary to further study its antibacterial activity by using solvent fractions, which in turn could simplify the isolation and identification of active principle responsible for the antibacterial activity of the plant

*Nuxia congesta* is an ever green shrub or a tree it can grow from 3-25 m tall and its habitat is the dry montane forest and forest margins in the costal and mountain forest belts, occurring frequently along water courses at elevations up to 2400 meters. The genus *Nuxia* comprises of 40 species of shrubs and trees distributed over the southern region of the Arabian Peninsula, tropical Africa (Madagascar, South Africa, Uganda, Ethiopia, Comoro and the Mascarene Islands) and South Africa [16].

*Nuxia* is represented in Saudi Arabia by only two species, viz. *N. oppositifolia benth* and *N. congesta Fresen* [17]. In Ethiopia, *Nuxia congesta* is found in humid and semihumid highland evergreen forests in Moist and Wet Weyna Dega agro climatic zones and the tree is deciduous [18].

Several *Nuxia* species are plants of economic and medicinal interest with a rich diversity of ethnobotanical uses. Medicinal importance of *Nuxia* species was reported in different countries for treatment of several diseases. Some species are used for the treatment of urine albumin, venereal disease, and as purgatives [19].

Leaves of *N. sphaerocephala baker* are used in the traditional medicine of Madagascar to treat malarial splenomegaly and infantile hydrocephalus [20]. *Nuxia floribunda* leaves used to treat fever, cough, influenza, infantile convulsion and as anthelmintic [21]. In Bale Mountains, *Nuxia congesta* leaves and stem bark are used to treat rheumatism and scabies [22].

Antibiotic activity of this plant was not reported in the previous study. In few literatures, revealed traditional use of this plant as medicine was reported for treatment tonsillitis, malaria and other skin disease. In present study I tried to reveal phytochemical content of this plant, zone of inhibition (ZI) and minimum inhibitory concentration (MIC) by using alcohol (ethanol and methanol) and chloroform extract solvents.

This study was carried out with the aim to evaluate the antimicrobial properties of the extracts of *Nuxia congesta* and to identify the biologically active compounds present in the extracts of *Nuxia congesta*.

## Materials and Methods

### Plant collection site

The plant was collected in the general environment of Tara-gedam and Amba forests located in Libo Kemkem District (Wereda) in the South Gondar Zone of the Amhara Regional State, northwestern Ethiopia located at around 12°04.351'-12°10.926'N and 37°44.266'-37°50.057'E. Tara-gedam forest ranges from 2062-2496 m a.s.l. and Amba forest ranges from 2011-2541 m a.s.l. with the highest peak at Mt. Deboch. The climate data obtained from the National Meteorological Service Agency of Ethiopia shows that the mean annual maximum and minimum temperatures of the study area are 32.8°C and 8°C, respectively. The District receives rainfall of approximately 1300 mm per year and about 95.1% of the area is mid-highland while the rest is highland [23].

The vegetation of the area belongs to the dry evergreen montane forest type consisting of forests, bush lands, shrub lands and enrichment plantation interspersed with stands of natural vegetation [24]. Archival information shows that forested land is about 4,429.5 hectares, Libo Kemkem District in particular Tara-gedam has several recreational sites. Mt. Kualla, along with diverse geographical features of the forest, Tara-gedam Monastery, many caves and forested churches are very useful for archaeological studies and for the tourism industry [25].

The 2007 census report of the Central Statistical Agency of Ethiopia shows that Libo Kemkem District has an estimated population of 209,451 (106,564 males and 102,887 females). The inhabitants are mostly members of the Amhara ethnic community who speak the Amharic language with economies that are predominately based cultivation of crops mixed with livestock production [23]. Malaria, intestinal helminthiasis and pneumonia were the top three human diseases and the major livestock ailments were pasteurellosis, anthrax, internal and external parasites, blackleg, sheep pox, trypanosomiasis, respiratory tract infection, rabies and coccidiosis [26].

### Collection and preparation of plant materials

Leave of *Nuxia congesta* was collected from South Gondar special district Addis zemen, Amhara regional state January to February, 2017 and extracted in Gondar University. For the extraction organic solvents (methanol, ethanol and inorganic solvents (chloroform) were used for extraction of leaf. Some bioactive compound found in this plant was screened in the Gondar microbiology laboratory and Identified bacterial strains were purchased from Ethiopia public health institute, Addis ababa Ethiopia and from national animal health diagnostic center Sebeta, Ethiopia (NAHDIC.) sub cultured in the Gondar university college of veterinary medicine.

## Plant extraction

After collection and authentication, the leaf of *Nuxia congesta* thoroughly washed, gently with tap water to remove dirt. The leaf of the plant were dried under shade and then, grounded into a coarse size using electric mill (SPEC). Then, the coarse powder of the plant was subjected to crude extraction.

## Ethanol, methanol and chloroform extraction

Crude extractions were conducted [27]. Briefly, the powdered sample was macerated in extraction solvent i.e., 80% ethanol and methanol and chloroform for 3 days with a ratio of 100 gm:500 ml. The extracts were filtered using gauze and then with Whatman No 1 filter paper (SPEC). Then the filtrate was dried by rotary vaporizer (SPEC) under reduced pressure at 50°C to get the crude extracts. Then, the concentrated filtrate was frozen in a deep freezer and dried in lyophilizer (SPEC) to remove its aqueous content. Finally, the dried extracts were packed in a closed vessel and stored in deep freezer (SPEC) until required for the experiment.

## Soxhlet extraction

This method was utilized to extract volatile oily extracts from the leaves of *Nuxia congesta*. The extraction was conducted by using chloroform as a carrier chemical using a Soxhlet apparatus (SPEC). In this method, finely ground sample was placed in a porous bag or "thimble" made from a strong filter paper or cellulose was placed in thimble chamber of the Soxhlet apparatus. Extraction solvents was heated in the bottom flask, vaporizes into the sample thimble, condenses in the condenser and drip back. When the liquid content reaches the siphon arm, the liquid contents emptied into the bottom flask again and the process is continued [27].

## Phytochemical screening

The antimicrobial value of medicinal plants relies on some chemical substances produced by these plants: these chemicals called "secondary metabolites" and include alkaloids, terpenoids, flavonoids, tannins and phenol compounds, essential oils, lectin and polypeptides, polyacetylenes etc [28]. In the present study I tried to reveal some secondary metabolites such as alkaloid, tannins, phenol terpenoid and flavonoid of crude extract of *Nuxia congesta*. The qualitative phytochemical investigations of the ethanol extraction, methanol and chloroform extraction was carried out using standard tests as described below.

**Test for alkaloids:** 500 milligram of extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added to 2 ml of dilute ammonia. 5 ml of chloroform was add and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Dragendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Dragendorff's reagent) was regarded as positive for the presence of alkaloids [29].

**Test for tannins:** About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration [30].

**Test for phenols:** The alcohol and few drops of neutral ferric chloride solution were added to 2 ml of test solution. Then reddish blue color was appeared which indicates the presence of polyphenols [29].

**Test for terpenoids:** 0.5 g each of the extract was added 2 ml of chloroform. Concentrated H<sub>2</sub>SO<sub>4</sub> (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids [29].

**Test for flavonoids:** About 10 ml of ethyl acetate was added to 0.25 g of the crude extract and each fraction and heated on a water bath for 3 min. The mixture was cooled and filtered. Then, about 4 ml of the filtrate was taken and shaken with 1 ml of dilute ammonia solution. The layers was allow separating and the yellow color in the ammoniac layer indicated the presence of flavonoids [30].

## Inoculation preparation

Muller Hinton agar (MHA) and mannitol salt supplement for *S. aureus* was prepared following the manufacturer's protocol. After cooling the media to about 45°, it was poured to a pre-labeled sterile petridishes aseptically and allowed time for the congealing of the agar. Then, standard pathogenic bacteria was inoculated and spread on the respective prepared agar using inoculating wire loop and incubated for 24 hrs at 37°. This actively growing bacterial was taken to tube obtain turbidity visually comparable to that of 0.5 McFarland standard. The turbidity of the inoculums tube was adjusted visually by either adding bacterial colonies or by adding sterile normal saline solution to that of the already prepared 0.5 McFarland standard. The adjustment and comparison of turbidity of inoculums tube and that of 0.5 McFarland standards were performed by visually observing [31].

## Determining of antibacterial activity

**Agar disk-diffusion method:**The agar disk diffusion (Kirby-Bauer) method was conducted according [32]. Briefly, crude extracts was prepared at a concentration of 100 mg/ml with dimethyl sulphoxide (DMSO, Merck, Germany) as solvent and filter sterilized using a 0.20 mm Millipore filter. The Mueller Hinton Agar (MHA) medium was prepared and sterilized at 121°C for 15 minutes in the autoclave. Twenty milliliters of this sterilized agar medium (MHA) was poured into each 12 cm sterile Petri dishes under aseptic conditions and allowed to settle and cultured for 24 hrs at 37°C. Then cultured bacteria was emulsified in 3 ml sterile saline following the McFarland turbidity to obtain a concentration of 10<sup>8</sup> cells/ml.

One hundred micro liters (100 µl) of cell suspension with approximately 10<sup>8</sup> bacteria per milliliter was placed in Petri dishes and dispersed over agar. In the following, a sterile paper disc (6 mm in diameter) impregnated with 20 µl of the plant extracts at the concentration of 500 mg/ml and allowed to dry at 37°C for 24 hrs was placed onto the agar. A disc prepared in the same condition with only the corresponding volume of DMSO, was used as a negative control. The reference antibiotic disc (tetracycline 30 µg) was used as positive control. Each of the discs was gently placed at equidistant on top of the agar layer gently to give better contact with agar.

Plant extracts and reference drugs was allowed to diffuse for 1 hr into the plates and then incubated at 37°C for 18 hrs in inverted position. The results were recorded by measuring the zone of growth inhibition (mm) surrounding the discs. Each assay was performed in triplicates. The growth of test bacterial strains was inhibited by the tested concentrations of the crude extract by ethanol, the methanol

and chloroform of the leaves of *Nuxia congesta* in concentration dependent manner [33].

**Determination of minimum inhibitory concentration of extracts (MIC):** The crude extract of that showed antibacterial activity was subjected to serial micro broth dilution technique to determine their MIC by using resazurin as a cell growth indicator [34,35]. Under aseptic conditions, 96 well micro titter plates were used for resazurin based micro titter dilution assay. The first column of micro titer plate was filled with 100 µl stock solution (500 mg/ml) of the crude or each active solvent extract of test material except the last well in which equal amount of the respective solvent was added. Then, all the wells of micro titter plates were filled with sterilized 100 µl of MHB [36]. Two fold serial dilution of the extract or solvent fraction (throughout the row until the 10<sup>th</sup> column) was carried out by evenly mixing and transferring 100 µl test material from wells of first row to the subsequent wells along the next column of the same row using micropipette. Then, 100 µl of mixed solution of the test material and the broth was removed from the 10<sup>th</sup> column so that each well contained 100 µl of test material in serially descending concentrations. The 11<sup>th</sup> and the 12<sup>th</sup> column were used as the growth control for labeled bacterium in which the 100 µl of the solvents of the extract or that of the solvent fraction was added and diluted down the same column up to 7<sup>th</sup> row instead of the test plant material.

The resazurin solution was prepared by dissolving 0.01 gm of the resazurin powder 100 ml of autoclaved distilled water. Then, 30 µl of 0.01% w/v resazurin color indicator solution [36] was added and mixed in each well. The bacterial suspension was prepared according to CLSI guideline [33] so that the bacterial concentration was made to be approximately  $5 \times 10^6$  CFU/ml by diluting the 0.5 Macfarland standard turbidity equivalent bacterial suspensions in the ratio of 1:20 in the respective broth. Briefly, within 15 minutes of standardization of bacterial suspension, 20 µl of diluted bacterial suspension was added to each well except 7<sup>th</sup> and 8<sup>th</sup> row which were reserved as extract color contrast control and sterility control respectively, to achieve a final concentration of  $6 \times 10^5$  CFU/ml., after addition of the colorant and the bacterial suspension.

To avoid the dehydration of bacterial culture, each plate was wrapped loosely with Para film (SPEC) to ensure that bacteria did not become dehydrated. In the experiment, each micro titre plate had a set of three controls: (a) a column with all solutions with the exception of the test extract was used as growth control (b) a row with all solutions except bacterial suspension and the extract was used as sterility control (c) a row with all solutions except the bacterial inoculums which was replaced by 20 µl of MHB was used as color contrast control. Finally, the plates were incubated in temperature controlled incubator at 37°C for 24 h.

### Statistical Analyses

The results of this study was expressed as means ± standard error mean (n=3). The statistical analysis was carried out using SPSS var 20 (IBM, Armonk, NY, USA) using the one-way analysis of variance (ANOVA). The differences in the means were calculated using Turkey multiple comparisons. P values <0.05 were regarded to be significant

### Results

According to the qualitative phytochemical screening study, the ethanol and methanol crude extracts were positive for the presence of alkaloids, flavonoids, poly phenols, tannins and terpenoids, whereas,

the chloroform was confirmed for the presence of tannins, terpenoids and flavonoids (Table 1).

Tests	Ethanol	Methanol	Chloroform
Alkaloids	+	+	-
Flavonoids	+	+	+
Poly phenols	+	+	-
Tannins	+	+	+
Terpenoids	+	+	+

**Table 1:** Phyto chemical screening of *Nuxia congesta* leaf.

The methanol, ethanol and chloroform extract of the leaves of *Nuxia congesta* was subjected to a preliminary screening for antimicrobial activity against seven pathogenic bacteria *E. coli*, *E. faecalis*, *S. aureus*, *H. influenza*, *P. mirabilis*, *K. pneumonia* and *P. aeruginosa* using disc diffusion technique. The ethanol and methanol extracts of the leaves of *N. congesta* showed highest antibiotic activity towards to *E. coli* with inhibition zone of  $17.33 \pm 0.33$  mm and  $17.00 \pm 0.57$  mm at concentration of 500 mg/ml respectively.

Ethanol extract had highest inhibitory activity against *E. coli*, *E. faecalis*, and *P. aeruginosa* and minimum inhibitory showed on *K. pneumonia* and *H. influenza*. Whereas, methanol extract showed high inhibitory against *E. coli* and minimum inhibitory against *K. pneumonia*. The chloroform extracts of the leaves of *N. congesta* showed antibacterial activity against *P. aeruginosa*, *S. aureus* and *P. mirabilis*,  $8.00 \pm 0.00$ ,  $8.00 \pm 0.00$  and  $9.00 \pm 0.58$  mm respectively. And it was not active against *K. pneumonia*, *H. influenza* and *E. coli* at concentration of 500 mg/ml. The inhibitory activity of the extracts of the leaves of *N. congesta* was not as potent as the positive control (tetracycline). And negative control (DMSO) did not show any antibiotic activity (Table 2).

Bacterial Strains	Solvent used				
	Ethanol	Methanol	Chloroform	Tetracycline	DMSO
<i>E. coli</i>			-		-
<i>E. faecalis</i>					-
<i>S. aureus</i>			-		-
<i>H. influenza</i>	-	-	-	-	-
<i>P. mirabilis</i>					-
<i>P. aeruginosa</i>					-
<i>K. pneumonia</i>			-		-

**Table 2:** Diameter inhibition zone of crude extract from *Nuxia congesta* (mm).

The MIC of ethanol extracts showed overall better antibacterial activity than methanol and chloroform. It recorded minimum MIC value of 31.25 mg/ml against *P. aeruginosa* and *E. coli* and maximum value of 208 mg/ml against *K. pneumonia*. MIC of *Nuxia congesta* leaf in methanol extract ranged from 41.75 mg/ml to 208.33 mg/ml. This result indicates that methanol extract of this plant had better



antibacterial activity on the *E. coli* at 41.75 mg/ml and medium activity on *E. faecalis*, *S. aureus*, *P. mirabilis* and *P. aeruginosa* at concentration of 62.5 mg/ml and less sensitive against *K. pneumonia*. Chloroform extract of this plant had less antibiotic activity on tested bacteria ranged from 250 mg/ml to 416 mg/ml concentration crude extract and any activity was not observed on the *E. coli*, *S. aureus*, *K. pneumonia*, and *H influenza*. Ethanol and methanol extract of this plant had significant difference from chloroform extraction against all tested bacterial strains (Table 3).

Bacterial strain	Solvent used		
	Ethanol	Methanol	Chloroform
<i>E. coli</i>	31.25 ± 0.00	41.75 ± 10.37	-
<i>E. faecalis</i>	41.66 ± 10.42	62.50 ± 0.00	416.67 ± 83.33
<i>S. aureus</i>	62.50 ± 0.00	62.50 ± 0.00	-
<i>H. influenza</i>	-	-	-
<i>P. mirabilis</i>	83.33 ± 20.83	62.50 ± 0.00	416.67 ± 83.33
<i>P. aeruginosa</i>	31.25 ± 0.00	62.50 ± 0.00	250.0 ± 0.00
<i>K. pneumonia</i>	208.33 ± 41.67	208.33 ± 41.67	-

**Table 3:** Minimum inhibitory concentration (MIC) of crude extract.

## Discussion

### Phytochemical screening of crude extract of *Nuxia congesta*

Medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment and inhibit the growth of microorganism. Therefore, phytochemical tests were performed to find out the presence of biologically active chemical constituents such as alkaloids, flavonoid, phenol, terpenoid, and tannins in medicinal plants. The presences of phytoconstituents like flavonoids, saponins, tannin and phenols in the crude extract are likely to be responsible for the antimicrobial activities [37].

In the present study, methanol and ethanol extraction of crude extract showed positive response for alkaloids, Polyphenols tannins flavonoid and terpenoid tests. But chloroform leaf extract was responded positive only for flavonoid, tannins and terpenoids. This might be due to active compound found in the tested plant needs polar solvent rather than non-polar solvent [38] due to this reason, chloroform extract of tested plant exhibited less antibiotic activity.

The alkaloidal extracted from the leaf of *Prosopis juliflora* have been found to be bioactive phytochemicals which inhibited the growth of both gram positive and gram negative test bacterial strains [39]. Therefore, the presence of alkaloids in the crude extract of methanol and ethanol could contribute for their respective antibacterial activities observed in current study. Polyphenols are the other phytochemicals that involve in the antibacterial activities of medicinal plants used for different infectious diseases. The extracts of phenolic compounds isolated from *Carum carvi* have been found to have a growth inhibition effect against different gram positive and negative bacteria [40]. In present study poly phenol compound was isolated in ethanol and methanol extract. The inhibition activity of this plant could be due to this active compound found in.

### Antibiotic activity of *Nuxia congesta* plant

The antimicrobial activities of the crude extract of *N. congesta* leaf were tested by the disc diffusion method and are shown in Table 1. According to the present study, the crude extract of ethanol and methanol of study plant showed antibacterial effect against all the test bacteria except *H. influenza* with varying degrees of antibacterial activity spectrum. However, chloroform Extract showed activity only *E. faecalis*, *P. mirabilis* and *P. aeruginosa*. This antibacterial activity difference may be related to the difference in solvent used for extraction purpose. In spite of having antibacterial activities, crude extract of this plant was not potent as positive control. This might be due to the less concentration of the active principles in the crude extract of plant. And negative control (DMSO) did not show any antibiotic activity. This could be due to absence of secondary metabolites (active principle) in the negative control.

Methanol and ethanol extract of this plant had better activity on most tested bacteria while chloroform extract showed less activity against most tested bacteria. For instance, any inhibition was not observed against *S. aureus*, *K. pneumonia* and *E. coli* in chloroform extract. This indicates that Polar solvent methanol and ethanol were most successful in extracting secondary metabolites such as alkaloid and poly phenol which are responsible for the antibacterial property than the non-polar solvent chloroform [41,42].

Crude extract of the three solvents showed less antibiotic activity against *K. pneumonia* bacteria and not active at all against *H. influenza* at equal concentrations. The possible reason could be less susceptible nature bacterial species to the bioactive compounds found in this plant due to different resistance mechanism of these bacterial species and the other reason might be due to insufficient bioactive compounds in this plant to inhibit the growth this bacteria [43].

The antibacterial activities of the extract of the plant were more pronounced on the Gram-positive bacteria than the Gram-negative bacteria. This may be due to the fact that Gram-negative bacteria have an outer phospholipidic membrane carrying the structural lipo polysaccharide components, which makes their cell wall impermeable to antibacterial chemical substances. The Gram-positive bacteria on the other hand are more susceptible having an outer peptidoglycan layer which is not an effective permeability barrier. Therefore, the cell walls of Gram-negative organisms are more complex than the Gram positive [44]. But in present study gram negative bacteria was more susceptible than gram positive bacteria to extract of tested plant. For instance, *E. coli* and *P. aeruginosa* were more susceptible than *S. aureus*.

### Minimum inhibitory concentration (MIC)

Ethanol extract of tested plant recorded minimum MIC value 31.25 mg/ml and maximum value 208.33 mg/ml against *E. coli* and *K. pneumonia* respectively. This result indicates minimum MIC has higher antibiotic property whereas; the maximum MIC value has less inhibitory activity. In other word value MIC has inversely proportional zone of inhibition.

In present study, the alcoholic (ethanol and methanol) extracts exhibited minimum MIC than the corresponding chloroform. This present study is similar with the study of the extracts of *Zehneria scabra* tubers were studied [38]. Chloroform and ethanolic extracts were screened for the inhibitory activity and the results suggest that the ethanolic root extract had significant antibacterial activities against *E.*

*coli*, and *P. aeruginosa* and chloroform extract did not show inhibitory activity against all bacterial test organisms.

Ethanol extracts had better antibacterial activity than methanol and chloroform against *P. aeruginosa* and *E. coli* at 31.25 mg/ml, whereas, chloroform extracts of *Nuxia congesta* showed less antibiotic activity against tested bacteria. This indicates the solubility of active ingredients in the chloroform is low or active bio compound found in study plant is requiring polar solvent rather than non-polar solvent (chloroform).

Generally ethanol extracts of *Nuxia congesta*, displayed the highest activities (recorded minimum MIC) and followed by the methanol extract against *E. coli*, *P. aeruginosa*, *K. pneumonia* and *E. faecalis* also showed that the ethanol extracts of the leaves of *R. officinalis* and its combination with *T. vulgaris* leaves had strong inhibitory effects against *P. aeruginosa* and *E. coli*. Similar to study, in the present study ethanol and methanol extract of *Nuxia congesta* leaf had strong inhibitory against *P. aeruginosa* and *E. coli* at 31.25 mg/ml concentration. This might be due to secondary metabolites dissolved in the alcohol extract greater than in the chloroform.

## Conclusion

The present study revealed that the ethanol and methanol extraction of the leaf of *Nuxia congesta* have antibacterial activities against all most all selected pathogenic bacteria with varying antibacterial spectrum and chloroform extract of tested plant has less antibiotic properties. And also flavonoid, alkaloid, tannin, poly phenol and terpenoid bioactive chemicals were detected in ethanol and methanol extract of tested plants. Whereas, chloroform extract showed positive response for flavonoid tannin and terpenoid tests. Therefore, this study provides scientific support for the traditional use of the medicinal plant in the treatment of bacterial infections that are probably caused by the susceptible bacteria. Antibacterial evaluation of this plant in present study provide an insight into the antimicrobial properties of the extract of *Nuxia congesta* and primary platform for further phytochemical and pharmacological studies to discover new antibiotic drugs in the feature.

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