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Research Article
Qualitative Phytochemical Analysis, Antimicrobial Activity and Cytotoxic Effect of *Moringa concanensis* Nimmo Leaves

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Abstract

Background and Objective: *Moringa concanensis* Nimmo belongs to the family *Moringaceae*. This plant is abundantly seen in Perambalur district, Tamilnadu, commonly known as Kattu murungai. Around 20 types of human ailments can be cured by different parts of *M. concanensis* Nimmo plant. The main objective of this work was phytochemical analysis, antimicrobial activity and cytotoxic activity of *M.concanensis* leaves. **Materials and Methods:** The qualitative phytochemical analysis was performed as per the standard procedures previously described. The well diffusion method was used to evaluate the antimicrobial activity of *M. concanensis* leaves. The MTT assay was used to screen the cytotoxic activity of *M. concanensis* leaves. The values were expressed as Mean±SD. Significant difference have been observed using one-way Analysis of Variance (ANOVA) by SPSS software version 19.0. The $p<0.05$, was considered as significant difference. **Results:** Among all the tested extracts of *M.concanensis* Nimmo leaves, chloroform, ethyl acetate and aqueous extracts has the lowest number of phytochemicals. The ethanolic extract of the leaves was found rich source of phytochemicals as compared to the other extracts. The *Moringa concanensis* Nimmo leaves revealed that they have an effective antimicrobial activity. The *Staphylococcus aureus* possesses maximum inhibition (13.5 mm) in ethanol extract (C3). The *Trichophyton rubrum* showed 10 mm zone of inhibition in ethanol extract (C3). It possesses the good cytotoxic activity. When the extract concentration was increased, the viability of the cell lines decreased. **Conclusion:** The findings of this research work were clearly indicates that the leaves of *Moringa concanensis* Nimmo possess a noteworthy antimicrobial activity and cytotoxic activity. The further research in this plant may leads to develop a novel bioactive compounds.

Key words: *Moringa concanensis* leaves, antibacterial, antifungal activity, cytotoxic effect and phyhtochemical analysis

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Bacterial infections are still among the major cause of morbidity and mortality worldwide; the situation is complicated by the appearance and emergence of multidrug resistant strains causing treatment failures[1]. The spread of Multidrug resistant (MDR) bacteria propels the search of novel antibacterials to combat resistant phenotypes. In 2014, the World Health Organization released its 1st report on surveillance of antimicrobial resistance, revealing that this is an increasing global threat and putting our capacity to treat common nosocomial or community acquired infection at risk[2]. Botanicals constitute a good source of anti-infective compounds, in regards to the variety and diversity of their chemical structures[3-5]. According to the World Health Organization (WHO) report, approximately 80% of the world populations rely on plants or derived products for their treatment[6].

Cancer is a significant global health care problem, with an estimated worldwide incidence of 10 million new cases per year, 46% of which are in developed countries. Mortality is high, with more than 7 million deaths per year[7]. According to National Cancer Registry Programme estimates, 700,000-900,000 new cancer cases occur in India every year. The WHO has estimated that about 15 million new cancer cases will be diagnosed each year by 2020 worldwide[8]. Also by 2020, overall mortality from cancer will increase by 104% and the increase will be 5-fold higher in developing than in developed countries[9].

The plant *M. concanensis* Nimmo is indigenous to Northwest India resembling *M. oleifera* and this was abundant in Rajasthan, the dry Hills of Konkan, Andhra Pradesh and is commonly found on recent alluvial land in or near the sandy beds of rivers and streams. This plant is abundantly seen in around Perambalur district of Tamilnadu, commonly known as Kattu murungai or Peyi murungai and in Warangal district of Andhra Pradesh, commonly known as Adavi Mulaga, Konda mulga[10]. The scientific knowledge of this plant is poorly available hence, it needs scientific study to describe its medicinal properties. The present study deals with the evaluation of antimicrobial activity and cytotoxic activity of *M. concanensis* Nimmo.

MATERIALS AND METHODS

Collection and identification of plant: The healthy, matured and insect bites free leaves of *Moringa concanensis* Nimmo plant (Family-*Moringaceae*) were collected from Esanai village, Perambalur district, Tamilnadu, India (Latitude-11.2982° N, Longitude-78.8298° E) from the month of April-May, 2016. The plant sample was identified and authenticated by Dr. C. Murugan, Scientist, Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamilnadu, India. The identification number BSI/SRC/5/23/2016/Tech-152.

Preparation of plant extracts: The *Moringa concanensis* Nimmo leaves were washed, shade dried and powdered using mixer grinder. The powdered leaves (10 g) were extracted with 100 mL of selected organic solvents (aqueous, methanol, ethanol, chloroform and ethyl acetate) using soxhlet apparatus. The concentrated solvents extracts of the leaves were stored in refrigerator for further analysis.

Phytochemical analysis of *Moringa concanensis* Nimmo leaves solvent extract: The phytochemicals screening of aqueous, methanol, ethanol, chloroform and ethyl acetate extracts of *Moringa concanensis* Nimmo was subjected to different chemical tests for the detection of different phytoconstituents using standard procedures. The qualitative phytochemical analysis was performed to identify the presence of alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, glycosides, phenolic compounds, carbohydrates and proteins in the leaves extracts of *Moringa concanensis* Nimmo[11,12].

Antimicrobial activity

Test microorganisms: The test organisms used were clinical isolates *viz., Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli* and *Klebsiella nemoniae.* The human fungal pathogens like *Candida albicans* and *Trichophyton rubrum,* which were obtained from Department of Microbiology, Hindusthan College of Arts and Science, Coimbatore. The bacterial and the fungal cultures were maintained on nutrient agar medium and Potato Dextrose Agar (PDA) medium respectively.

Growth and maintenance of test microorganism for antimicrobial studies: The bacterial and fungal cultures were maintained on Nutrient Broth (NB) at 37°C and fungus was maintained on Potato Dextrose Agar (PDA) at 28°C.

Preparation of inoculum: The tested bacterial cultures were pre-cultured in nutrient broth over night in a rotary shaker at 37°C, centrifuged at 10,000 rpm for 5 min, pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically (A_{610} nm). The fungal

inoculums *Candida albicans*, *Trichophytan rubrum*, were prepared from 5-10 days old culture grown on Potato dextrose agar medium. The Petri dishes were flooded with 8-10 mL of distilled water and the conidia were scraped using sterile spatula.

Antibacterial activity: The samples were tested by the well diffusion method[13,14]. Different concentration of the extracts (C1, C2 and C3) was prepared by reconstituting with ethanol. The test microorganisms were seeded into respective medium by spread plate method 10 µL (10 cells mL^{-1}) with the 24 h cultures of bacteria growth in nutrient broth. After solidification the filter paper wells (5 mm in diameter) impregnated with the extracts were placed on test organism-seeded plates. Chloramphenicol (10 µg) used as standard for antibacterial test. The antibacterial assay plates were incubated at 37°C for 24 h. The diameters of the inhibition zones were measured in mm.

Antifungal activity: The antifungal activity was tested by well diffusion method[15]. The potato dextrose agar plates were inoculated with each fungal culture (10 days old) by point inoculation. The filter paper wells (5 mm in diameter) impregnated with 100 µg concentrations of the synthesized silver nanoparticles were placed on test organism-seeded plates. Streptocycline (10 µg) was used as positive control. The activity was determined after 72 h of incubation at 28°C. The diameters of the inhibition zones were measured in mm.

Cytotoxic activity

Cell line: The human breast cancer cell line (MCF 7) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% Fetal Bovine Serum (FBS). The cells were maintained at 37°C, 5% of CO_2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly and the culture medium was changed twice a week.

Cell treatment procedure: The monolayer cells were detached with trypsin-ethylene diaminetetra acetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of 1×10^5 cells mL^{-1}. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells $well^{-1}$ and incubated to allow for cell attachment at 37°C, 5% of CO_2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat Dimethyl sulfoxide (DMSO) and an aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum free medium. Additional 4 serial dilutions were made to provide a total of 5 sample concentrations. Aliquots of 100 µL of these different sample dilutions were added to the appropriate wells already containing 100 µL of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37°C, 5% of CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

MTT assay: 3-[4,5-dimethylthiazol-2-yl] 2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells.

After 48 h of incubation, 15 µL of MTT (5 mg mL^{-1}) in Phosphate Buffered Saline (PBS) was added to each well and incubated at 37°C for 4 h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100 µL of DMSO and then measured the absorbance at 570 nm using micro plate reader. The percentage cell viability was then calculated with respect to control as follows[16]:

$$\text{Cell viability (\%)} = \frac{\text{[A] Test}}{\text{[A] Control}} \times 100$$

$$\text{Cell inhibition (\%)} = \frac{100 - \text{[A] Test}}{\text{[A] Control}} \times 100$$

A: Absorbance value

Statistical analysis: The values were expressed as Mean±SD. Significant differences have been observed using one-way Analysis of Variance (ANOVA) by SPSS software version 19.0. The $p<0.05$, was considered as significant difference (IBM Corp. Released 2010. The IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp.)[17]

RESULTS

Phytochemical analysis in different solvent extracts of *M. concanensis* Nimmo leaves: The result of qualitative phytochemical screening of *M. concanensis* Nimmo leaves

Table 1: Results of qualitative phytochemical analysis of *Moringa concanensis* Nimmo leaves extracts

Phytochemicals	Aqueous	Ethanol	Methanol	Chloroform	Ethyl acetate
Alkaloids	++	+++	+++	++	+++
Phenolic compounds	-	++	++	-	-
Flavonoids	++	+++	++	-	-
Tannins	++	++	+	+	+
Saponins	++	++	++	+	+
Terpenoids	+	++	+	+	+
Steroids	+	++	++	+	-
Carbohydrates	+	++	++	+	+
Glycosides	+	+	+	+	+
Amino acids	+	++	+	+	++
Proteins	++	++	++	+	+

+→present in small concentration, ++→present in medium concentration, +++→Present in high concentration, -- →absent

Table 2: Results of antibacterial activity of ethanolic leaves extract of *Moringa concanensis* Nimmo

	Plant extract zone of inhibition (mm)		
Pathogenic bacteria	C1	C2	C3
Streptococcus pyogenes	07	09	12.0
Staphylococcus aureus	08	10	13.5
Escherichia coli	08	09	10.0
Klebsiella nemoniae	08	10	13.0

C1: Concentration 1-20 µL, C2: Concentration 2-40 µL and C3: Concentration 3-60 µL

Table 3: Results of antifungal activity of ethanolic leaves extract of *Moringa concanensis* Nimmo

	Plant extract Zone of inhibition (mm)		
Pathogenic fungus	C1	C2	C3
Candida albicans	06	07	09
Trichophyton rubrum	08	09	10

C1: Concentration 1-20 µL, C2: Concentration 2-40 µL and C3: Concentration 3-60 µL

extracts revealed that the leaves of *M. concanensis* have wide range of phytochemicals (Table1). Particularly, ethanol, methanol and aqueous extracts of *M. concanensis* Nimmo were good sources of different classes of phyto compounds. This indicates that these solvents are effective to isolate active biological compounds due to their high polarity.

Results of antibacterial activity of ethanolic leaves extract of *Moringa concanensis* Nimmo: The antibacterial activity of ethanolic extract of *Moringa concanensis* Nimmo leaves was depicted in (Table 2). All the concentrations of ethanolic extract showed noteworthy activity. Especially *Staphylococcus aureus, Klebsiella pnemoniae* and *Streptococcus pyogenes* showed more sensitivity in 60 µL concentration.

Results of antifungal activity of ethanolic leaves extract of *Moringa concanensis* Nimmo: In antifungal activity of ethanolic extract of *Moringa concanensis* Nimmo leaves, the *Candida albicans* and *Trichophyton rubrum* showed maximum sensitivity against 60 µL concentration (Table 3). *T. rubrum* showed a maximum inhibition zone i.e., (10 mm) and *C.albicans* showed 9 mm inhibition zone in 60 µL concentration.

Table 4: Cytotoxic activity result of ethanolic extract of *Moringa concanensis* leaves

Concentration (µg mL^{-1})	Cell inhibition (%)
18.75	3.40644
37.5	14.32571
75	25.99160
150	38.21745
300	55.52963

Result of cytotoxic activity: The cytotoxic efficacy of ethanolic extract of *Moringa concanesis* leaves is shown in the Table 4. The ethanolic extract was tested against MCF-7 cell lines. The extracts showed significant activity against the human breast cancer cell lines. When the drug concentration was increased, the viability of the MCF-7 cells was decreased. The *Moringa concanensis* ethanolic leaves extract inhibited the cell viability of MCF-7 cell lines.

DISCUSSION

Bioactive components of plants include array of compounds (e.g., tannins, lignans, coumarins, quinones, stilbenes, xanthones, phenolic acids, flavones, flavonols, catechins, anthocyanins and proanthocyanins) that could delay or inhibit the inception of degenerative diseases and increase life expectancy. Infectious diseases are also the major cause of mortality worldwide. About 50,000 people die worldwide every day because of infectious diseases[18]. Literature surveys show that plant based drugs play a promising role in the treatment of infectious diseases[19]. Flavonoids were detected in ethanolic and methanolic extracts in very high concentration and moderate concentration in aqueous extract of leaves, the flavonoids were absent in chloroform and

ethyl acetate extracts. Flavonoids belong to the group of polyphenolic compounds and are typically known for health promoting properties, such as antioxidant, antiallergic, anti-inflammatory, antimicrobial and anticancer properties[20]. They exist widely in the plant kingdom and displayed positive correlation between increased consumption of flavonoids and reduced risk of cardiovascular and cancer diseases[21].

The presence of alkaloids were observed in very high concentration in ethanol, methanol and ethyl acetate extracts and moderate concentration in chloroform and aqueous extracts of the leaves of *M. concanensis* Nimmo. Alkaloids have been reported to possess analgesic, antispasmodic and bactericidal, antimalarial and analgesic activities[22,23]. The phenolic compounds were present in medium concentration in ethanol and methanol extracts and absent in aqueous, chloroform and ethyl acetate extracts of *Moringa concanensis* Nimmo leaves. The tannins were present in medium concentration in aqueous and ethanolic extract and present in trace amount in methanol, chloroform and ethyl acetate extracts *M. concanensis* Nimmo leaves.

The extracts of *M. concanensis* Nimmo have been detected for the presence of terpenoids and saponins. The saponins were detected in medium concentration in aqueous, ethanol and methanolic extracts and trace amount in chloroform and ethyl acetate extracts. The terpenoids were detected in medium concentration in ethanolic extract and trace amount in aqueous, methanol, chloroform and ethyl acetate extracts. Terpenoids such as triterpenes, sesquiterpenes and diterpenes have been referred to as antibiotics, insecticidal, anthelmintic and antiseptic in pharmaceutical industry[24,25].

The presence of steroids were observed in all the extracts except ethyl acetate extract of *M. concanensis* Nimmo leaves on moderate concentration. The steroids were present in medium concentration on ethanol and methanolic extracts and trace amount in aqueous and chloroform extracts. The ethanolic and methanolic extract of *M. concanensis* Nimmo leaves showed the presence of carbohydrates in medium concentration and present in trace amount in aqueous, methanol, chloroform and ethyl acetate extracts. The presence of glycosides were detected in trace amount in all the solvent extracts. Moreover, glycosides commonly used to treat congestive heart failure and cardiac arrhythmia, were discovered in all the extracts of the leaves of *M. concanensis* Nimmo in small concentration[26].

The aminoacids were present in medium concentration in ethanolic and ethyl acetate extracts and trace amount in aqueous, methanolic and chloroform extracts of *M. concanensis* Nimmo leaves. All the solvent extracts of the leaves showed the presence of protein in *M. concanensis* Nimmo. The proteins were present in medium concentration in aqueous, ethanol and methanolic extract of *Moringa concanensis* Nimmo leaves. Whereas, the protein was detected trace amount in chloroform and ethyl acetate extracts. Typically, proteins are the huge group of macromolecules and act as antibiotic and antimicrobial agents.

Plants protect themselves against microbial pathogens by various defense responses including production of antimicrobial proteins which are small molecular mass antimicrobial peptides[27]. The carbohydrates and steroids were present in all the extracts in small concentration and ethanolic and methanolic extracts were traced in moderate concentration. The aminoacids was present in all the extracts. Among all the tested extracts of *M. concanensis* Nimmo leaves, chloroform, ethyl acetate and aqueous extracts have the lowest number of phytochemicals. The ethanolic extract of the leaves was found rich source of phytochemicals as compared to the other extracts.

The ethanolic extract of *Moringa concanensis* Nimmo leaves has shown remarkable antimicrobial and cytotoxic activity. It is important to investigate scientifically these plants which have been used in traditional medicines as potential source of novel antimicrobial compounds. The first step towards this goal is the *in vitro* antibacterial activity assay. In the present study, the ethanolic extract of *Moringa concanensis* Nimmo leaves was tested against various human pathogenic bacteria species like *Streptococcus pyogenes, Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*. And the fungal strains were *C. albicans* and *T. rubrum.* The antibacterial activity of ethanolic extract of *Moringa concanensis* Nimmo leaves was showed in (Table 2). All the concentrations of ethanolic extract showed a remarkable activity. Especially *S. aureus*, *K. pnemoniae* and *S. pyogenes* showed more sensitivity in 60 µL concentration. The inhibition zone length are 13.5 mm in *S. aureus* on 60 µL concentration, 13 mm in *K. pneumoniae* on 60 µL concentration and 12 mm in *S. pyogenes* on 60 µL concentration. A minimum inhibition zone was noted in *Streptococcus pyogenes* on 20 µL concentration (inhibition zone was 7 mm). In antifungal activity of ethanolic extract of *Moringa concanensis* Nimmo leaves, the *C. albicans* and *T. rubrum* showed maximum sensitivity against 60 µL concentration (Table 3). *T. rubrum* showed a maximum inhibition zone i.e., (10 mm) and *C. albicans* showed 9 mm inhibition zone in 60 µL concentration.

The cytotoxic effect of ethanolic extract of *Moringa concanensis* Nimmo leaves against MCF-7 cell lines was

assayed by MTT assay. The MTT assay is a suitable *in vitro* method for cytotoxicity against cancer cell lines and non cancer cell lines[28]. A large and increasing number of patients in the world use medicinal plants and herbs for health purposes. Therefore, scientific scrutiny of their therapeutic potential, biological properties and safety will be useful in making wise decisions about their use. The ethanolic extract of *Moringa concanensis* Nimmo leaves were tested against MCF-7 cells lines. The extracts showed significant activity against the human breast cancer cells. When the drug concentration was increased, the viability of the MCF-7 cells was decreased. The *Moringa concanensis* leaves extract inhibited the cell viability of MCF-7 cells.

Approximately, 60% of the anticancer drugs currently used have been isolated from natural products from the plants. At this time, more than 3000 plants worldwide have been reported to possess anticancer properties. This study provides an important basis for further investigation into the isolation, characterization and mechanism of cytotoxic compounds from the screened medicinal plants. It helps to carry more biological activities, including the *in vivo* studies and the statute of inhibition of cancer. Thus, these plants could be as a source for new lead structures in drug design to combat cancer.

CONCLUSION

From the above investigation, it can be conclude that the ethanolic extract of above mentioned medicinal plant part i.e. *Moringa concanensis* Nimmo leaves can be considered as a resource for potential antimicrobial and anticancer agents. The concentration 60 µL was very effective in both fungi and bacteria. The 300 µg of ethanolic extract of *M. concanensis* leaves was effective on MCF-7 cancer cell lines. The selected plant can be further exploited for the discovery of novel antimicrobial agents.

Nevertheless, the present findings may also supplement and strengthen the process of standardization and validation of herbal drugs containing active ingredients derived from the selected medicinal plants.

SIGNIFICANCE STATEMENTS

The findings of this research may help to understand the biological activities of *Moringa concanensis* Nimmo leaves. This study will help the researcher to uncover phytochemical profile of this plant.

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