

**PHYTOCHEMICAL ANALYSIS AND COMPREHENSIVE
EVALUATION OF ANTIMICROBIAL ACTIVITY OF *NANNORHOPS
RITCHIANA* LEAVES (MAZARI PALM)**

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ABSTRACT

In developing countries herbal medicines are widely used and are in high demand for primary health care because of their inexpensiveness, traditional acceptance, and with minimum side effects so very high rate of compatibility with human body. This study was aimed to estimate the antibacterial, antifungal and phytochemical analysis of *Nannorrhops ritchiana* (Mazari Palm) particularly the leaves in 95% ethanol extract with different concentrations like 100, 200, 300, 400, 500 and 600mg/ml. The antibacterial and antifungal activity was determined by disc diffusion and well diffusion method against five bacterial strains; *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Salmonella typhi* and *Klebsiella pneumonia*; two fungal strains such as *Candida albicans* and

Aspergillus niger were susceptible to the extract. The phytochemical characterization of *Nannorrhops ritchiana* showed the incidence of flavonoids, alkaloids, cardiac glycosides, glycosides, resins, saponnins, tannins, terpenoids, carbohydrates and amino acids. This is what explained the use of herbal drugs efficacy and cost effectiveness in recent years. The main scope was to study the antimicrobial analysis of *Nannorrhops ritchiana* leaves for pharmacological benefits.

KEYWORDS: *Nannorrhops ritchiana*, plant, ethanol, phytochemical, bacteria, fungi, disease.

INTRODUCTION

Trees are the gift of nature and are the heaven in this world. The increasing new inventions increase new diseases. So the mankind should learn the disease and drugs for cure. Palms are the most beneficial plants to people in the Tropics. Nannorrhops plant belongs to the family *Arecaceae*, Mazari palm is a common name of *Nannorrhops ritchiana*. It is widely present in distinct areas of the world. It is regional to the Pakistan and other countries like Afghanistan and Iran, extensively dispersed in the distinct regions of Baluchistan (Mosti *et al.*, 2006; Rashid *et al.*, 2014).

The Mazari palm can greatly bear the extreme heat and severe cold, insufficiency of water and harsh winds (Naseem *et al.*, 2005). In suitable environment it grows to the height of 5 meter or more than it. Its leaves color is green to grayish green. Young leaves of *Nannorrhops ritchiana* having sweet taste are used in livestock as laxative. Its fruit is edible and may be used for treatment of dysentery and diarrhea. It is also used in treatment of a gastrointestinal disease. From crude extracts of this plant, antifungal and cytotoxic activities have been evaluated. Its growth rate is less in infertile soil conditions, but it grows well in availability of water and fertile soils. For its better growth full sun and lime are also required (Rehana *et al.*, 2014).

Nannorrhops ritchiana is one of the robust palms, which tolerate winter frigidities to about -12°C (10°F) (possibly even -20°C or -4°F), although very hot summers required for the growth of this specie. In southern Europe and southern North America it is not widely cultivated but occasionally grown as an ornamental plant. It has a lot of fibers. It is feathery and shrubby plant, and it naturally grows well in warm and humid areas of Pakistan. It is commonly used as to form domestic substances as mats, ropes, hand fans, decoration pieces and fancy articles etc. Its large production occurs in Baluchistan. Many people are indulged in handling the leaves of this palm, especially women are playing major role in this (Rehana *et al.*, 2014).

Phytochemicals are active compounds found in fruits, vegetables, grains and any other plant foods that have been linked to reducing the risk of major chronic diseases. There are lot of evidences which suggests that Plant phytochemicals have voluminous benefits that may be greater than is currently understood. They may be used to replace the synthetic drugs against which many microorganisms have showed resistance. There is lot of hard work behind the effort to know the medicinal purposes of higher plants which would be more safer and

effective drugs with the potential to fight against pathogenic bacteria (El-Mahmood, 2010). Phytomedicines originated from plants have shown too many benefits to treat infectious diseases including viral diseases (Reddy *et al.*, 2001; Ateb and Erdourul, 2003). The root extract of *Nannorrhops ritchiana* contains alkaloids, phenols, polyphenol saponnins, tannins, anthraquinones and sterols (Kumari *et al.*, 2016).

In this study *Nannorrhops ritchiana* was used to assess the antibacterial and antifungal activity and phytochemical analysis to further evaluate the types of constituents of phytochemicals and their purification as individual groups revealed the meticulous potential of the plant to inhibit several pathogenic microbes and encourage to develop a novel and comprehensive antibacterial herbal formulation to preclude the global health issue.

MATERIAL AND METHODS

Plant collection

The leaves of *Nannorrhops ritchiana* were collected from Aziz nursery farm Pattoki, Pakistan and authenticated by Dr. Abdul Nasir Khalid, Associate Professor, Department of Botany, University of Punjab. Lahore. The plant specimen was deposited in the specially maintained garden, The University of Lahore.

Preparation of plant extracts

The plant materials of *Nannorrhops ritchiana* leaves were individually washed with water, dried and pulverized into fine powder by grinding machine and then store in airtight container. Two hundred grams of powdered plant materials of leaves was dipped in ethanol at room temperature for seven days with vigorous shaking. The plant extracts were filtered through Whatman No 1 and filtrates were dried under rotary evaporator. The residues were stored at 4°C for further use.

Determination of antibacterial activity

Cultures

In the present study, total six bacteria in which two Gram positive *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 29213), and four Gram negative *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Salmonella enterica* (ATCC 14028) and *Klebsiella pneumoniae* (ATCC 10031) and two fungus strains such as *Aspergillus niger* and *Candida albicans* were used to assess the antimicrobial properties of plant extracts.

Microbial strains were obtained from the culture collection laboratory of the Department of Microbiology at The University of Lahore.

Antibacterial susceptibility test

Two different qualitative methods were used to evaluate the antimicrobial activity as follows: disc diffusion method and well diffusion method.

Disc diffusion method

Disc diffusion method for antibacterial and antifungal susceptibility testing was carried out to assess the presence of the activity of plant extract. The bacterial cultures were adjusted to 0.5 McFarland standards then 20 μ l of culture was evenly spread on Muller Hinton agar plates while fungal culture was transferred to tubes containing 5ml normal saline and 3-4 beads and vortex to homogenize the fungal growth. 100 μ l of fungal suspension was spread on Sabouraud Dextrose agar plates. The standard concentrations of the plant extract such as 100mg, 200mg, 300mg, 400mg, 500mg, and 600mg were prepared in 1 ml DMSO. Filter paper discs were placed on media plates with sterile forceps then impregnated 5 μ l plant extract on each disc. Bacterial plates were incubated at 37°C for 24 hours while fungal plates were incubated at 25°C for 7 days to observe the antibacterial and antifungal activity. DMSO was used as negative control while Gentamicin and Nystatin were used as a positive control against bacteria and fungi respectively. The zones of inhibition were measured (in mm) according to standard protocol (Perez *et al.*, 1990).

Well diffusion method

The 20 μ l of bacterial and fungal inoculum was uniformly spread using sterile cotton swab on MH agar plates and Sabouraud Dextrose agar plates. 20 μ l of different concentrations such as 100mg, 200mg, 300mg, 400mg, 500mg, and 600mg prepared in 1 ml DMSO were added to respective wells (7 mm diameter holes cut in the agar gel, 20 mm apart from one another). The plates were incubated for 24 hour at 37°C under aerobic conditions while fungal plates were incubated at 25°C for 7 days to observe the antibacterial and antifungal activity. Tests were performed in triplicate; DMSO was used as negative control while Gentamicin and Nystatin were used as a positive control against bacteria and fungi respectively. The zones of inhibition were measured (in mm) according to standard protocol (Perez *et al.*, 1990).

Phytochemicals detection of the active components of *Nannorrhops ritchiana*

Phytochemical tests were carried out on *Nannorrhops ritchiana* leaves extract by using standard procedures to evaluate the constituents as follows (Sofowara, 1993; Trease, Evans, 1989; Harborne, 1973; Parekh and Chanda, 2007).

Detection of tannins

500mg of extract was agitated with 10 ml of distilled water and filtered. 300µl of 1% solution of ferric chloride were added to 2 ml of the filtrate. The presence of a blue-black, green, or blue- green precipitate specified the presence of tannins.

Detection of Steroids

200 mg of extract was added in 2ml of acetic acid, after cooling the solution on ice concentrated H₂SO₄ was cautiously added. Change in color from violet to blue or bluish-green showed the presence of steroidal ring i.e., a glycone protion of cardiac glycoside.

Detection of terpenoids

100mg extract was added in 99.5% ethanol. 1ml of acetic anhydride followed by concentrated hydrogen sulphate was added. Color development from pink to violet indicated the presence of terpenoids.

Detection of saponnins

Boiled 1000 mg extract with 5ml of distilled water and filtered. To the filtrate 3ml of distilled water was added and the mixture was intermittently shaken for 5 minute. In 5ml of extract solution, 5 ml of silver nitrate was added in a test tube then put in boiling water bath for 5 minutes. The appearance of silver mirror on sides of test tube indicated saponnins existence. 1-3 ml of mercuric chloride was added to 5 ml of extract, appearance of white precipitant represented a good indicator to saponnins existence.

Detection of flavonoids**NaOH Tests**

Few drops of sodium hydroxide solution were added to 2-3ml of extract in a test tube. Development of intense yellow color, on addition of few drops of dilute HCl the solution became colorless which clearly indicates the presence of flavonoids.

Detection of alkaloids

10g of extract were boiled in 50 ml water acidified with 4% HCl, filtered and 0.5ml of the supernatant was mixed with Mayer reagent in watch glass, white precipitate showed the incidence of alkaloids.

Detection of Phenol

When 0.5 ml of FeCl₃ (w/v) solution was added to 2 ml of test solution, formation of an intense color indicated the presence of phenols.

Phytosterols**Salkowski Test**

2ml of extract, 2ml chloroform and 2 ml concentrated H₂SO₄. Chloroform layer appeared red and acid layer showed greenish yellow fluorescence indicated the presence of sterols.

Lieberman-Burchard's Test

Mix 2ml extract with chloroform. Add 1-2ml acetic anhydride and 2 drops concentrated H₂SO₄ from the side of the test tube. First red, then blue and finally green color indicated the presence of sterols.

Carbohydrates Molish's Test

1 ml of extract and 2 drops of Molisch's reagent was added in a test tube and 2 ml of concentrated H₂SO₄ was added carefully keeping the test tube slightly curved. Formation of violet ring at the junction indicated the presence of glycosides.

Amino acids Ninhydrin Test

5ml of extract and 2 drops of freshly prepared 0.2% ninhydrin reagent was added and heated. The appearance of blue color indicates the presence of amino acids.

Anthraquinones

0.5g of the extract was boiled with 10 ml of H₂SO₄ and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for color changes.

RESULTS

The ethanolic extract was subjected to antibacterial, antifungal and phytochemical screening of the presence of flavonoids, alkaloids, saponins, steroids, terpenoids, tannins,

anthraquinones, phenols, phytosterols, cardiac glycosides, glycosides, resins, triterpenoids, carbohydrates, aminoacids according to standard protocol as described above. The results of antimicrobial activity through disc diffusion method and well diffusion method and phytochemical analysis were given in Table 1, 2 and 3.

During the study different concentrations obtained from 99.5% ethanolic extract of *Nannorrhops Ritchiana* screened for antibacterial, antifungal and phytochemical characterization. *Nannorrhops ritchiana* showed the maximum inhibition against bacterial isolates at highest concentration of 600 mg/ml and minimum inhibition against fungal isolates at 600mg/ml. This showed the presence of antibacterial and antifungal agents in leaves of *Nannorrhops ritchiana*.

In this study, a comparison is being done between disc diffusion method and well. *B. cereus* showed maximum inhibition (21mm) at 600mg/ml and *E. coli* showed minimum inhibition at 16mg/ml through disc diffusion method.

There is no zone of inhibition showed by *S. typhi* 100mg/ml. Maximum zone of inhibition showed by *B. cereus* through disc diffusion method 100mg/ml.

C. albicans showed maximum inhibition (4mm) at 100 mg/ml while *A. niger* showed no zone of inhibition at 100mg/ml, while (9mm) at 600mg/ml through disc diffusion method.

Table: 1. Antimicrobial activity of *Nannorrhops ritchiana* ethanolic extract against bacterial and fungal isolates by Disc diffusion method

Bacterial isolates	Zone of inhibition (mm)							
	100 (mg/ml)	200 (mg/ml)	300 (mg/ml)	400 (mg/ml)	500 (mg/ml)	600 (mg/ml)	+ve control	-ve control
<i>E. coli</i>	5	7	9	11	11	16	22	0
<i>P. aeruginosa</i>	9	11	13	15	17	20	19	0
<i>K. pneumoniae</i>	10	13	14	15	15	18	23	0
<i>S. typhi</i>	0	9	11	14	17	19	17	0
<i>B. cereus</i>	11	13	13	16	18	21	25	0
<i>S. aureus</i>	4	10	11	17	19	19	24	0
<i>C. albicans</i>	4	4	5	6	6	10	15	0
<i>A. niger</i>	0	0	6	7	8	9	17	0

B. cereus *S. aureus* showed maximum inhibition (20mm) at 600mg/ml and *E. coli* showed minimum inhibition 16mm at 16mg/ml through well diffusion method. *E. coli* and *S. typhi* showed minimum zone of inhibition 4mm at 100mg/ml and maximum zone of inhibition

showed by *B. cereus* (12mm) at 100 mg/ml through well diffusion method. *C. albicans* and *A. niger* showed no zone of inhibition (0 mm) at 100 mg/ml and 9mm at 600mg/ml.

Table: 2. Antimicrobial activity of *Nannorrhops ritchiana* ethanolic extract against bacterial and fungal isolates by well diffusion method

Bacterial isolates	Zone of inhibition (mm)					
	100 (mg/ml)	200 (mg/ml)	300 (mg/ml)	400 (mg/ml)	500 (mg/ml)	600 (mg/ml)
<i>E. coli</i>	4	9	10	13	13	16
<i>P. aeruginosa</i>	7	9	10	11	11	18
<i>K. pneumoniae</i>	6	14	16	17	17	18
<i>S. typhi</i>	4	10	12	15	18	18
<i>B. cereus</i>	12	14	16	17	18	20
<i>S. aureus</i>	6	11	13	18	19	20
<i>C. albicans</i>	0	3	4	4	5	9
<i>A. niger</i>	0	4	4	5	6	9

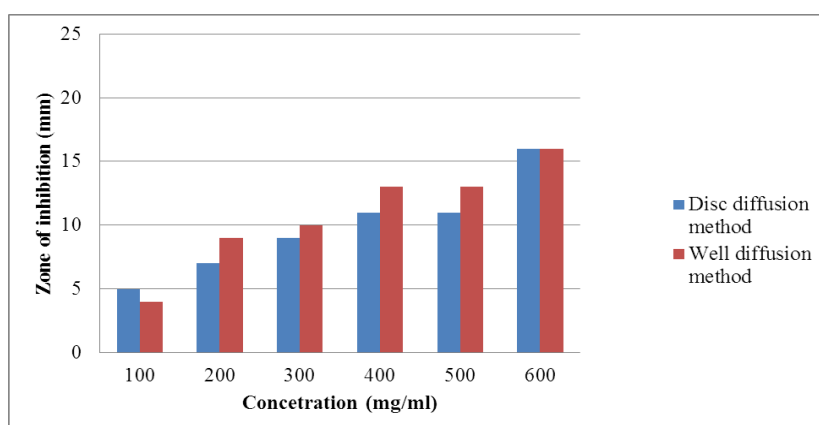


Figure 1: Antibacterial activity of *Nannorrhops ritchiana* ethanolic extract against *Escherichia coli*.

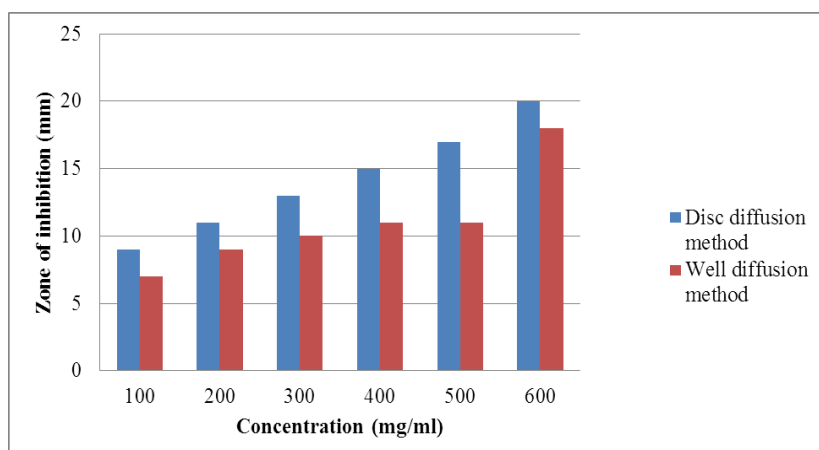


Figure 2: Antibacterial activity of *Nannorrhops ritchiana* ethanolic extract against *Pseudomonas aeruginosa*.

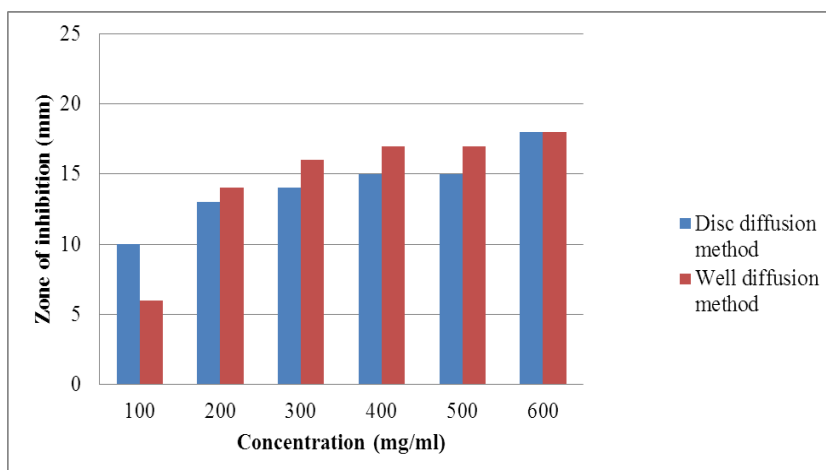


Figure 3: Antibacterial activity of *Nannorrhops ritchiana* ethanolic extract against *Klebsiella pneumoniae*

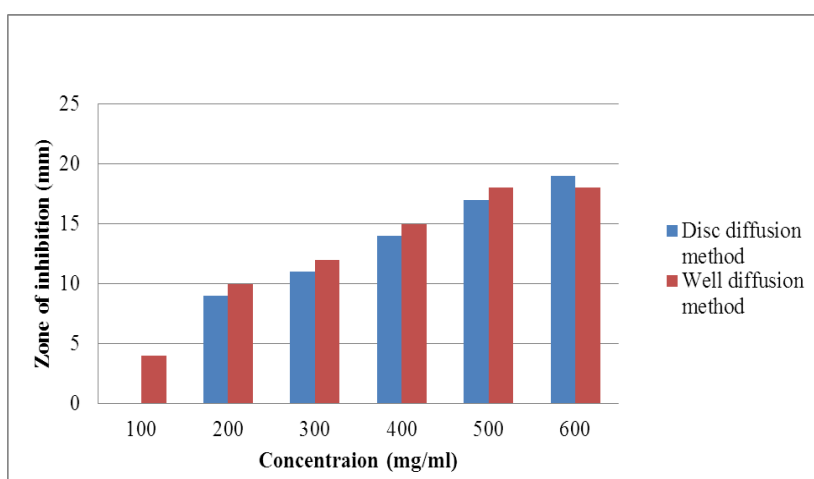


Figure 4: Antibacterial activity of *Nannorrhops ritchiana* ethanolic extract against *Salmonella typhi*

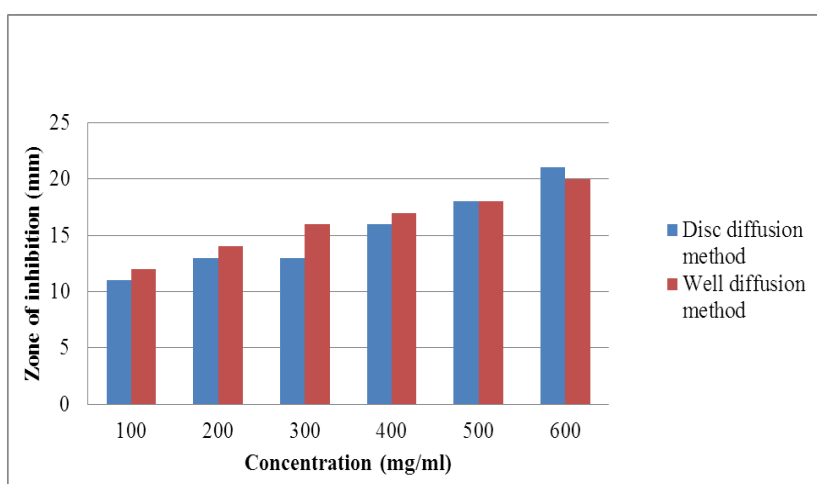


Figure 5: Antibacterial activity of *Nannorrhops ritchiana* ethanolic extract against *Bacillus cereus*

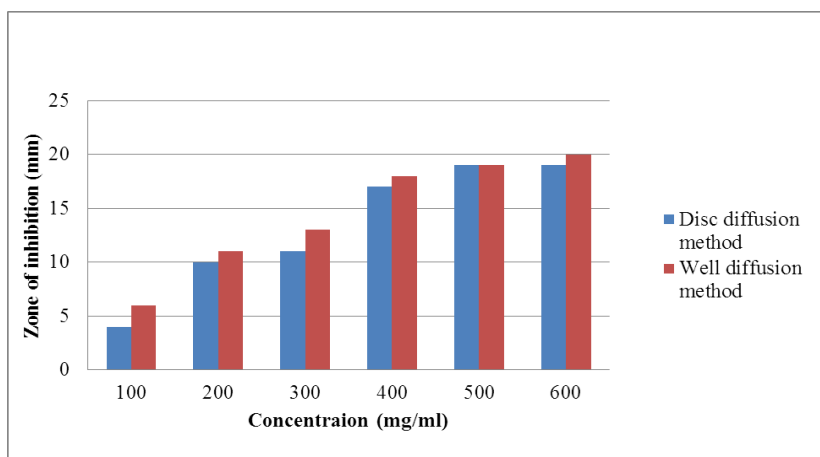


Figure 6: Antibacterial activity of *Nannorrhops ritchiana* ethanolic extract against *Staphylococcus aureus*

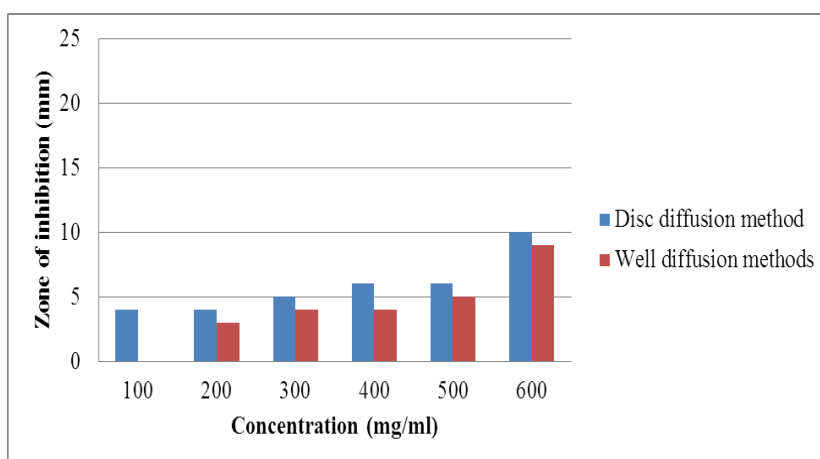


Figure 7: Antifungal activity of *Nannorrhops ritchiana* ethanolic extract against *Candida albicans*.

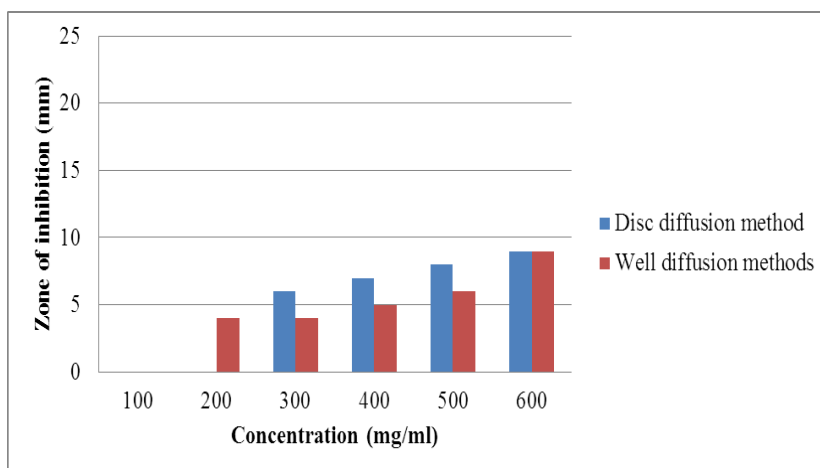


Figure 8: Antifungal activity of *Nannorrhops ritchiana* ethanolic extract against *Aspergillus niger*

The ethanolic extract of *Nannorrhops ritchiana* were subjected to phytochemical screening for the presence of alkaloids, cardiac glycosides, flavonoids, glycosides, phenols, resins, saponnins, steroids, tannins, terpenoids, triterpenoids, anthraquinones, carbohydrates, amino acids according to standard procedures as described above. The results of phytochemical analysis were given in Table 3.

Table: 3. Preliminary qualitative phytochemical analysis of various alcoholic extract of leaf of *Nannorrhops ritchiana*

Plant constituents	Ethanolic leave extract
Alkaloids	+ve
Cardiac glycosides	+ve
Flavonoids	+ve
Glycosides	+ve
Phenols	-ve
Resins	+ve
Saponnins	+ve
Steroids	-ve
Tannins	+ve
Terpenoids	-ve
Triterpenoids	+ve
Anthraquinones	-ve
Carbohydrates	+ve
Aminoacids	+ve

+: present, -: absent

DISCUSSION

All around the world, universal systems are being used which have been derived from plants and play a major role in the medication of different diseases, more research and experimentation is needed in this field to get many more benefits. For the treatment of many ailments caused by different microorganisms medical plants are used because they contain bioactive compounds which are very effective against microorganisms and provide protection system against pathogens, due to these bioactive compounds survival of plants has been raised in their environment (Sukumaran *et al.*, 2011). Against microorganisms, many reports have demonstrated the validness of old herbs so the plants provide a rich source for modern medicine to attain new principles (Evans *et al.*, 2002).

To the Scientists who are indulged in the botanical field the functioning components that are present in the medical trees are becoming attractive (Clark and Hufford, 1993). From conventional medicinal plants, different extracts have been approved. In modern era, much

attention has been focused towards the plant extracts and the constituents are separated from plant species. In developing countries, basic health needs are accomplished by the use of medicinal plants and these plants provide a new source of antibacterial, antifungal and antiviral agents that exhibit an important activity against infective microorganisms (Mingarro *et al.*, 2003 and Souza *et al.*, 2004). The compounds responsible for the antibacterial activity of this extract are not revealed.

However, fundamental phytochemical tests revealed that the compounds such as phenols, alkaloids, polyphenol saponnins, tannins, anthraquinones and sterols are present in the root extract of *Nannorrhops ritchiana* (Kumari *et al.*, 2016).

Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. Plant phenolic present in fruits and vegetables has received considerable attention because of their potential antioxidant activities (Lopez-velez *et al.*, 2003). Herbal extracts contain different phytochemicals with biological activity that can be of valuable therapeutic index. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases (Augusti and Cherian, 2008).

The crude extracts of the palm leaves was chemically and bioactive assayed for the presence of phytochemical compounds which could be responsible for their medicinal use in traditional medicine, as anti-diabetic, hyperlipidemia, and treatment of Broncho-pneumonia (Tona *et al.*, 1999).

This study showed the methanol extract of polyphenol tested positive for the presence of tannins, saponnins and trepenoids, flavonoid, alkaloid, amino acid. Tannins are a group of phenolic compounds found to form irreversible complexes with proline rich protein (Shimada, 2006; Fluck, 1973). Resulting in the inhibition of cell protein synthesis (Parekh and Chanda, 2007). Reported that tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues. Herbs that have tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery (Dharmananda, 2003). Alkaloids exhibit marked physiological effects when administered to animals and hence their wide use in medicine for development of drugs (Okwu, 2005). They produce analgesic, antispasmodic

and bactericidal effects (Stray F, 1998). Also, this study showed presence of flavonoids have been phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers used for medicinal purposes e.g. catechol, hydroquinone and resorcinol are phenolic salicylates used as analgesics, antipyretics and as internal antiseptics in medicine and surgery (Polterait, 1997). Triterpenoids and saponins showed the analgesic properties and central nervous system activities (Sayyah *et al.*, 2004; Malairajan *et al.*, 2006).

Medicinal plants are potential sources of new compounds of medicinal value and are sources of lead bioactive compounds in the drug development (Kumar *et al.*, 2006). These bioactive compounds show antimicrobial activity against a wide range of microbes. Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential has been established well (Rojas *et al.*, 2006). Since antiquity, man has used medicinal plants to treat various common infectious diseases; some of these medicines are still the part of treatment of various ailments (Black *et al.*, 2008).

In recent years, the indiscriminate use of commercial antibiotics to treat infectious diseases has resulted in the development of multiple drug resistance in both human (Kumar *et al.*, 2006). One alternative approach to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents. This situation has forced scientists to search new antimicrobial substances in various sources like medicinal plants (Edeoga *et al.*, 2005). In traditional medication many plants have been claimed for their effective or superior properties over synthetic drugs, like medicinal plants such as *bixa* spp. and *bidens* spp. have been claimed more efficient to treat infectious diseases than synthetic antibiotics by traditional healers (Rojas *et al.*, 2006). So it becomes necessary to evaluate the scientific base for the potential use of folk medicine for the treatment of infectious diseases produced by common pathogens. Medicinal plants might represent an alternative treatment in non-severe cases of infectious diseases (Shah, 2005). They can also be a possible source for new potent antibiotics to which pathogen strains are not resistant.

The traditional healing systems all around the world uses plant based antimicrobial that shows a greater untrapped and further research of plant microbial is needed. Many commercial drugs that are used in traditional modern medicine are derived from plants that are following ethno-botanical and ethno-medical knowledge (Arokiyaraj *et al.*, 2012). Medical plants have bioactive compounds which treat various ailments caused by

microorganisms. These compounds may have been evolved in plants as self-defense against pests and pathogens that help plants to establish them in their environment (Sukumaran *et al.*, 2011). Against microorganisms, effectiveness of traditional herbs has been observed so to obtain new principles plants are considered as fundamentals (Evans *et al.*, 2002). Different articles have been suggested in which phytochemicals of medicinal plants have been observed. These are used in treatment of extensive disorders that are in turn used as possible choice to artificially made drugs to treat pathogens that have exhibited resistance. To determine invulnerable, latest and valuable agents that have the tendency to fight against pathogenic bacteria (El-Mahmood, 2010), higher plants are being used

Biologically active compounds that are present in the medical plants have always been of a great interest to the scientists that are working in this field. In recent years this interest to evaluate the plants possessing antibacterial activity for various diseases is growing (Clark and Hufford, 1993). Traditional herbs have been tested from extracts that differ from each other. Medicinal plants are very important in sheltering the fundamental health in growing countries and against microorganisms that cause disease and they exhibit new antibacterial, antiviral and antifungal agents (Mingarro *et al.*, 2003 and Souza *et al.*, 2004). The compounds responsible for the antibacterial activity of this extract (*Nannorrhops ritchiana*) are not revealed.

However, fundamental phytochemical tests revealed that the root extracts of *Nannorrhops ritchiana* contained alkaloids, phenols, polyphenol saponnins, tannins, anthraquinones and sterols (Kumari *et al.*, 2016).

All around the world, universal systems are being used which have been derived from plants and play a major role in the medication of different diseases, more research and experimentation is needed in this field to get many more benefits. For the treatment of many ailments caused by different microorganisms medical plants are used because they contain bioactive compounds which are very effective against microorganisms and provide protection system against pathogens, due to these bioactive compounds survival of plants has been raised in their environment (Sukumaran *et al.*, 2011). Against microorganisms, many reports have demonstrated the validness of old herbs so the plants provide a rich source for modern medicine to attain new principles (Evans *et al.*, 2002).

CONCLUSION

The phytochemical screening of palm leaves extract demonstrated the presence of alkaloids, flavonoids, phenols, phytosterols, tannins, amino acids, terpenoids, and carbohydrates. The phytochemicals present in palm leaves extract have well known curative activity against several human pathogens and therefore could suggest the use traditionally for the treatment of various.

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