Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 5 [12] November 2016: 35-43

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.533
Universal Impact Factor 0.9804

ORIGINAL ARTICLE **OPEN ACCESS**

Antifungal and Antibacterial Potential of the Leaves Extracts of *Nannorrhops ritchiana* (Griff), *Phoenix sylvestris* (Linn.) and *Olea ferruginea* Royle

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ABSTRACT
The antimicrobial potential of the plants derived products have been regularly screened and reported in the literature. However, still good numbers of plants have been left for screening which might produce better efficacy than the earlier investigated compounds. Furthermore, resurgence and resistance issues to antimicrobial compounds also instigate to look into newer antimicrobial compounds. The present study was designed to evaluate the antimicrobial potential of crude extracts of the leaves of Nannorrhops ritchiana, Phoenix sylvestris and Olea ferruginea against three fungal strains Aspergillus flavus, Alternaria alternata, Fusarium oxysporium and six bacterial strains Ralstonia solanacearum, Xanthomonas compestris, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi and Bacillus subtellis. The efficacy of the extracts was compared with the standard antimicrobials. The plants are native to Pakistan and have a global distribution. Crude extracts were prepared according to the previously described method with four different solvents that is methanol, ethyl acetate, butanol and n-hexane. The antifungal and antibacterial activities were tested by agar tube dilution and agar well diffusion methods respectively. Results revealed the antifungal potential in tested crude extracts which were comparable to standard antibiotic, terbinafine. Order of antifungal efficacy for the selected extracts was olea ferruginea > Nannorrhops ritchiana > Phoenix sylvestris. Antibacterial efficacy of the extracts was inconsistent with highest efficacy noted with butanol extract of Olea ferruginea. The study concludes that the leaves extracts of all tested plant extracts have promising antifungal potential. However, anti-bacterial activity is limited to butanol extract of Olea ferruginea for three strains of bacteria only.
***Key words**: Antimicrobial, antifungal, antibacterial, leaves extracts, agar disk diffusion, agar tube dilution.*

Received 25.07.2016 Revised 16.09.2016 Accepted 01.11.2016

INTRODUCTION

Infectious diseases are still a great dilemma to be solved by health communities in developing countries[1]. Pathogenic Fungi cause various diseases of hair, ear, nail, skin and lungs. It includes severe infectious diseases like Aspergillosis, *coccidioidomycosis*, histoplasmosis and actinomycosis [2]. Fungal diseases are not common as bacterial but are difficult to cure than bacterial diseases [3].. More interests have been shown for the discovery of new antibacterial compounds as compared to antifungal agents. Pathogenic fungus not only harms human directly rather they are also source for damage to crops. For example *Aspergillus flavus* deteriorate many agriculture crops especially attacks on corn and peanuts before or after harvest causing ear rot and yellow mold respectively [4]. *Alternaria alternata* attacks several plant parts causes leaf blight, leaf spots and rots on 380 host species .In human it causes asthma and upper respiratory tract disease in person with weak immunity[5].. *Fusarium oxysporum* also attacks on vascular system of solanaceous plants, blockage of water passage as xylem ducts are totally occupied by the fungus[6].. Bacterial diseases are also evident in humen, animals and plants. For example *E. coli* causes various diseases like choleocystitis and urinary tract infections[7]. Similarly the bacteria *Staphylococcus aureus,* which usually causes infections in wounds also results in food poisoning and toxic shock syndrome [8,9]. *B.Subtellis* is not harmful as such but can cause food poisoning in immunocompromised person via contaminated water [10]. Due to endophytic spread, association with

weed and ability to survive in deeper layer of soil make bacterial wilt very difficult to control[11]. Black rot of cruciferous plants is another vascular disease that is caused by *Xanthomonas compestris* beginning from leaf margin to blackening of veins [12]. Moreover, *Pseudomonas aeruginosa* is common pathogenic bacteria in hospital where other effective antimicrobial agents are deficient to combat with it[13]. *Salmonella typhi* cause typhoid fever in which infected person feels weakness, constipation, abdominal pain, headache and sometime diarrhea and vomiting[14]. Fluoroquinolones, azithromycin, or third generation cephalosporins are recommended antibiotics but the disease become difficult to control due to acquired resistance of these antibiotics [15]. It is well established and evident from the literature that the excess use of antibiotics results in changes of pathogens, in addition to resistance problems they exert undesirable side effects as well[16]. Hence, there is still need for evaluating new antimicrobial agents which exhibit less side effects and more effective. In these scenario medicinal plants has been proved to contain therapeutically active constituents that conferring them as a natural product to control infectious diseases. According to WHO report, 80% of world's population uses medicinal plants as drug source for treatment of various health problems. Furthermore, it may be noted that high percentage of medicinal plants which are used in herbal medicine industry are collected from wild sources only[17].

Table 1 and 2 shows some of the plants that exhibit promising antibacterial and antifungal activities. If we go through the literature we find that plant crude extracts have been tested against different strains of bacteria and fungi. For instance, 18 medicinal plants were evaluated against nine bacterial strains *Staphylococcus aureus, S. epidermidi, Pseudomonas aeruginosa Enterococcus faecalis, Escherichia coli, Proteus vulgaris, Klebsiella pneumonia*, , *Ervinia sp. Bacillus subtilis* and a fungal strain *Candida albicans*. Out of 18 plants, *Syzygium lineare*, *Acalypha fruticosa*, *Toddalia asiatica*, and *Peltophorum pterocarpum* were recommended as potential sources of new antimicrobial agents[18]. In another study, the crude extracts of the leaves of *Kalanchoe crenata* and *Bryophyllum pinnatum* were investigated for their antimicrobial activities against Gram-negative organisms, *Citrobacter* spp *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella paratyphi, Shigella flexneri* and a gram positive bacteria *Enterococcus faecalis, Bacillus subtilis, Staphylococcus aureus,* and a fungus *Candida albicans* using agar well diffusion and broth dilution methods. All extracts were active against selective organisms except *Candida albicans*[19].

The present research work is an effort to evaluate the medicinal value of some plants collected from Takht, a small village of Tehsil Lachi District Kohat, Pakistan. The Kohat division is situated in the southern most of the capital of Khyberpakhtoonkhwa i.e. Peshawar. The area is explored for the first time for this kind of study. In this first effort we selected three medicinal plants *Nannorrhops ritchiana*, *phoenix sylvestris* and *Olea ferruginea* to assess the antimicrobial properties from unexplored area of medicinal plants against fungal and bacterial strains. Table 3 highlights some of the properties of selected medicinal plants and microbial strains used in this study. These plants were selected based on ethnomedicinal use, chemical constituents and their possible potential being antifungal and antibacterial agents proved in literature.

The outcome of the study may provide benefit to the local community, global community that prefer scientific based herbal medicine and industries utilizing plants from wild sources and in addition may lead to the development of new drug/disinfectants based on the active constituents of the plants.

Table 1. Some of the medicinal plants possessing antifungal properties

Name of plants	Extracts part	Fungi	Ref.
Terminalia prunioides, T. brachystemma, T. sericea, Terminalia gazensis, T. mollis and T.sambesiaca)	Leaves	*Candida albicans*, *Cryptococcus neoformans*, *Aspergillus fumigateus*, *Microsporum canis* and *Sporothrix schenkii*	[28]
Citrus aurantifolia (Lime fruit)	Different parts	*Aspergillus niger and Candida albican Bacteroides* spp, *Porphyromonas* spp*, and Clostridium* spp.	[29]
Bryophyllum Pinnatum and ***Kalanchoe Crenata***	Leaves	*Candida albicans*	[19]
Abrus precatorius	Leaves, stem and seed oil	*Candida albicans*	[30]
Anogeissus leiocarpus and Terminalia avicennioides	Root	*Aspergillus niger, Aspergillus fumigatus*, *Penicillium* species, *Microsporum audouinii* and *Trichophyton rubrum*	[31]
Carpolobia lutea	Root	*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis*	[32]
Cassia fistula L	Leaves	*Aspergillus niger, Aspergillus clavatus, Candida albicans*.	[33]

Glycyrrhiza glabra L.	Leaves	*Bacillus subtilis, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli,*	[34]
Cassia fistula	Leaves	*Staphylococcus aureus, Streptococcus pyogenes*; *Escherichia coli, Pseudomonas aeruginosa*	[33]
Phoenix dactylifera	leaf, fruit, seed and bark	*Staphylococcus aureus, Streptococcus pyogenes*, *Escherichia coli* and *Pseudomonas aeruginosa*	[1]
Olea europaea and *Olea ferruginea*	Crude Leaves, fruits and oil extracts	*Staphylococcus aureus, Listeria monocytogenes, Bacillus subtlilis, Bacillus cereus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumonia, Salmonella typhimurium, Enterococcus faecalis*	[25]

Table 2. Some of the medicinal plants depicting antibacterial activity

Name of plants	Extracts part	Bacteria	Reference
Anthocleista djalonensis, Nauclea latifolia Uvaria afzalii	Root	*Staphylococcus aureus, Escherichia coli*	[35]
Citrus aurantifolia	Fruit	*Staphylococcus aureus, Staphylococcus aureus, Salmonella paratyphi, Shigella flexnerii, Streptococcus faecalis, Citrobacter spp, Serratia spp, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Escherichia coli;*	[29]
Bryophyllum Pinnatum and ***Kalancho e Crenata***	Leaves	*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Shigella flexneri, Salmonella paratyphi, Citrobacter spp);* Gram-positive organisms *Staphylococcus aureus* , *Staphylococcus aureus, Enterococcus faecalis, Bacillus subtilis albicans*).	[19]
Abrus precatorius	Leaves, stem and seed oil	*Staphylococcus epidermidis, Enterococcus faecalis, Streptococcus anginosus* (*S.milleri*), *Bacillus subtilis, Corynebacterium* spp (*toxigenic strain of the mitis biotype*), *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa*	[30]
Abrus precatorius	Leaves, stem and seed oil	*Staphylococcus epidermidis, Enterococcus faecalis, Streptococcus anginosus*, *Bacillus subtilis, Corynebacterium* spp (toxigenic strain of the *mitis* biotype), *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa*	[30]
Carpolobia lutea	Root	*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis Candida albicans and Tinea capitis*	[32]
Glycyrrhiza glabra L.	Roots, Leaves	*Candida albicans*	[34]
Cassia fistula	Leaves	*Staphylococcus aureus, Streptococcus pyogenes*; *Escherichia coli, Pseudomonas aeruginosa*	[33]
Phoenix dactylifera	leaf, fruit, seed and	*Staphylococcus aureus, Streptococcus pyogenes*, *Escherichia coli* and *Pseudomonas aeruginosa*	[1]
Olea europaea and *Olea ferruginea*	Crude Leaves, fuits and oil extracts	*Staphylococcus aureus, Listeria monocytogenes, Bacillus subtlilis, Bacillus cereus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumonia, Salmonella typhimurium, Enterococcus faecalis*	[25]

MATERIAL AND METHODS

Plant Material and Extraction

The leaves of three medicinal plants were collected from Takht, a small village of Tehsil Lachi, District Kohat, Pakistan. The plants were identified by Dr. Amir. M. Khan, Assistant. Professor. Department. of Botany, Kohat University of Science and Technology, Pakistan and deposited at herbarium of Kohat University of Science and Technology Kohat, Pakistan. The plants were dried and crushed to a fine powder. 50g powder of leaves of each plant was soaked in 500ml of solvents(methanol, ethyl eacetate, butanol and n-hexane). The mixtures were kept for two weeks at room temperature. The mixtures were continuously shaked twice a day during the process. After three weeks the soaked plants material were filtered by ordinary filter paper and then by whattmann filter paper# 41. The filtrates were then processed through rotary evaporator to get semisolid extracts [20].

Antifungal assay

Agar tube dilution method as reported by Khan et al. [21]. but with slight modification was used for antifungal activity. 12mg of each extract (methanol, ethyl acetate, butanol and n-hexane) of each plant was mixed in 1ml of autoclaved distilled water to get 200µg/ml. 12mg of Terbinafine was taken in 1ml of autoclaved distilled water to prepare stock solution of antibiotic which was used as positive control. 6.5g of SDA was taken in 100ml of distilled water to prepare media for fungal activity, autoclaved and cooled to 50°C. Then 4ml of this media was taken in each test tube. Each test tube was loaded with 67 µl of test solutions. The test tubes were placed in slanting position and allowed to solidify. Triplicate test tubes were made for each test sample and fungal strains. The test tube with media and test solutions were inoculated with 4mm diameter piece of fungus of 7 days old culture. Terbinafine as positive control test tubes were also inoculated. All the test tubes were placed in incubator at 28°C for 7days. Test tubes were examined twice during process. The measurement of the linear growth of fungus was taken by measuring the linear length of fungus growth (mm) from point of origin toward the end of growth. Growth inhibition was calculated with reference to positive control. The %age inhibition of fungal growth was determined by the following formula[21].

$$\%\text{age inhibition of fungal growth} = \frac{\text{100- Linear growth in test sample(mm)}}{\text{100-Linea growth on control(mm)}} \times 100$$

Antibacterial Assay

For antibacterial assay agar well diffusion method as reported by Khan et al. [21]. but with slight modification , was used. In this method, about 2 or 3 colonies of bacteria were taken from 24h old culture and then colonies were mixed in 10ml nutrient broth media in test tubes. The test tubes were placed in incubator at 37°C for 24 h. An Autoclaved saline solution was mixed in the test tubes containing bacterial cultures and their turbidity were corrected until matched with turbidity of McFarland 0.5 $BaSO_4$ standard. This inoculum was ready for seeding nutrient agar plates. 20g nutrient agar was taken in 1L distilled water and autoclaved to prepare nutrient agar media. The media was then allowed to cool up to 45°C. 75ml media was poured into each petriplate and allowed to solidify. Bacterial strains from nutrient broth media were swabbed by an inoculating loop or swab. Holes or wells were made in each plate by a cork borer (8mm). 100µl of the test solution, and known concentration of antibiotic (chloramphenicol) were put in their respective wells. Antibiotic was taken as positive control. The plates were then incubated at 37 °C in paraffin oven. The measurement of the diameter of the zones around wells were taken and then compared with the diameter of the zones formed by antibiotic.

Statistical analysis

All the measurements were done in triplicate and statistical analysis was performed by analysis of variance (ANOVA) and mean values were compared with Duncan's Multiple Range Test**s** using SPSS version. 15 (SPSS Inc., Chicago, IL, (USA).

RESULTS AND DISCUSSION

Antifungal activity of the selected plants extracts with four different solvents; methanol, butanol, ethyl acetate and n-hexane are shown in Fig.1 to Fig.3. Methanolic and butanolic extracts of *N. ritchiana, P sylvestris and O. ferrugine*a showed promising antifungal effect against *A. flavus, A. alternata and F. oxysporum*. However, n-hexane followed by butanolic extract of *N. ritchiana* and *O. ferruginea* showed the highest efficacy. The effects were equal or comparable to standard Terbinafine antifungal effect (Fig.4 and Fig.5). The extracts obtained from other solvents showed more than 60% activity. Maximum inhibitory effect (100%) was noted against *A. alternata* with all the tested extracts except methanolic extract of *N. ritchiana* which produced 70% inhibitory effect and *P. sylvestris* where only methanolic extract revealed 100% inhibitory effect.

Anti-bacterial activity of selected plant extracts against five gram negative and one gram positive bacteria by zone formation method is shown in Table 4. The extracts did not reveal antibacterial effect except butanolic extract of *O. ferruginea* where effect was less than 50% of standard control that is chloramphenicol (Table 5). Plants possessing therapeutic potentials have a long history of traditional use. According to the World Health Organization, about four billion peoples use plants as a source of medicine for primary health care even in the modern era. Medicinal plants as herbal medications are an important component of Ayurvedic, homeopathic, naturopathic and traditional herbal therapy. About 80% peoples in developing countries use medicinal plants to cure diseases[22].. Medicinal importance of these plants depends on the concentration of active molecules which make them a rich source of different medicines. A good number of plants have been shown to possess antimicrobial potential (Table 1 to 3). But still there is a need to explore the newer antimicrobial agents from plants which might be a better source of

antimicrobials than existing ones. In addition, this may be cheaper and affordable source to the communities. In the present study, three medicinal plants were evaluated for their antifungal and antibacterial potential. The results revealed promising antifungal effect of the tested extracts (Fig.1 to Fig.3) but not the antibacterial. The tested compounds are less documented in literature for their antifungal and antibacterial activity. Furthermore, plants from the selected geographical region have not been reported earlier. It is an established fact that efficacy of the extracts depends upon the geographical region and types of solvents. Indeed the differences in efficacy of the tested extracts from the earlier reported, if any is due to the different geographical distribution, method of extractions, presence or absence of active phytochemical constituents and experiment protocol as well. In addition, it varies on the source of plant materials like roots or leaves etc. For instance, Rashid et al. [2] reported good antifungal activities and found 70-80% inhibition by extracts of roots of *N. ritchiana*. Boulenouar et al. [23]. reported antifungal effects of *P. dactylifera* L. mostly with dichloromethane extracts of rachis. Amin et al. [24] showed the antifungal potential of *O. ferruginea* crude leaves extract obtained from Khyber agency, a nearby area of our experimental specimen. They also found the n-hexane fractions were more potent than butanolic or Methanolic extracts. Methanol root extracts of *N.ritchiana* showed 60% inhibition against *A. flavus*[2] while it was 70 % in the present study. *F. oxysporum* was the most *susceptible strain* and only inhibited by n-hexane extracts of *N. ritchiana* and *P. sylvestris.* This suggested that *N. ritchiana* might have both specific compounds active against *alternaria alternata* and *A. flavus* present individually in *O.ferruginea* `against *alternaria alternata P. sylvestris* against *A. flavus.* The results indicated that the antifungal activities of the same plant of different extracts inhibit same fungal strain to various extents. Presumably due to various distributions of bioactive constituents in different extract of same plants creates this interesting situation. A comparative analysis of photochemistry of these plants might unravel these new findings. In the present study, antibacterial activity was not ascertained. Though, antibacterial activity of the selected extracts was reported in some earlier studies. For instance, butanol and methanol extracts of leaves of *O. ferruginea* were reported active against *E.coli, and P. aeruginosa*[24]. Another report from Hussain et al. [25]. also showed antibacterial activities of *Olea ferruginea* leaf. They suggested that methanol extract was efficient as antimicrobial agent against *E.coli* and *P. aeruginosa* while *B. subtellis* and S*. typhimorium* showed resistance. Kchaou et al. [26]. reported antibacterial activity of *P. dactylifera* and showed marked inhibition of zone formation against gram positive and negative bacteria. Zulqarnain et al. [27]. reported lesser antibacterial activity of *O. ferruginea* against a number of gram positive and negative bacteria. The negative antibacterial findings in our investigation may be due to different strains, different techniques for study, solvent factors and the content of active phytochemicals in our extracts.

Table 3. Ethnobotany of under tested medicinal Plants.

Plant name	Family	Local name	English name	Ethno-medicinal uses	Reference
N.ritchiana (Griff) Aitchison	Arecaeae	*Mazaray*	Mazri palm	In treating diarrhea and dysentery, commonly as purgative in treating animals.	[36]
P. sylvestris (Linn) Roxb.	Palmaceae	*Khajora*	Date Palm	Crushed fresh leaves are soaked in water overnight, then water is taken next morning in empty stomach to expel threadworms and heart problems.	[37]
O. ferruginea Royle.	Oleaceae	*Shwoon*	Olive	Oil used to treat joint pain, constipation and dandruff	[38,39]

Table 4. Table shows the antifungal and antibacterial activities of *N. ritchiana, P. sylvestris and O. ferruginea* plants extracts.

Plants	Extracts	Type of action	Reference
N. ritchiana(Griff)	Roots	Antifungal	[2]
O. ferruginea Royle.	Leaves, fruits,	Antibacterial,	[25]
O. ferruginea Royle.	Plant as whole	Antibacterial	[27]
O. ferruginea Royle.	Leaves	Antibacterial, antifungal	[24]
O. europaea Royle.	Leaves, fruits	Antibacterial,	[25]
O. europaea Royle.	Leaves	Antibacterial,	[40]
P. dactylifera L.	Rachis	Antifungal	[23]
P. dactylifera L.	Rachis	Antifungal	[23]
P. dactylifera L.	Leaves and Pit	Antifungal	[41]
P. sylvestris Roxb	Seeds	Antibacterial	[42]

P. dactylifera L.	Pit	Antifungal and antibacterial	[43]
P. dactylifera L	Dates	Antibacterial	[26]
P. sylvestris Roxb	Leaves	Antibacterial	[44]
P. dactylifera L.	Fruits and Barks	Antibacterial	[45]
P. sylvestris Roxb	Leaves	Antibacterial	[46]
O. europaea. Royle. Subspp. *Africana*	Leaves	Antibacterial and antifungal	[47]
O. europaea. Royle	Leaves	Antibacterial	[48]

Table 5. Antibacterial activity of chloramphenicol against tested bacterial strain

Bacteria	*N. ritchiana*	*P. sylvestris*	*O. ferruginea*
R.solanacearum	56±2.08	56±2.08	56±2.08
X. compestris	53.±1..66	53±1..66	53.±1..66
E.coli	56.66.±0.00	56.66.±0.00	56.66.±0.00
S. typhi	54±2.33	54.00±2.33	54±2.33
P. aeruginosa	52.66±2.33	52.66±2.33	52.66±2.33
B.subtelis	55.33±2.33	55.33±2.33	55.33±2.33

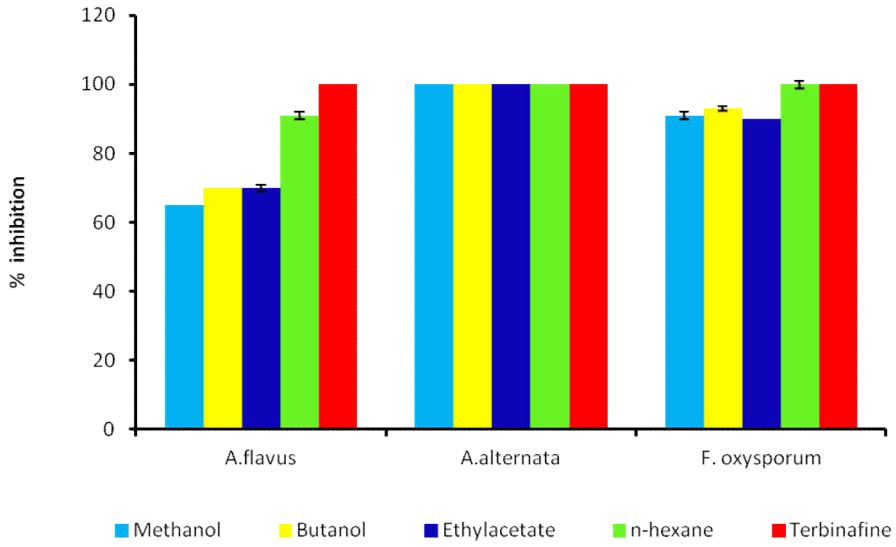

Fig.1. Antifungal activity of *N. ritchiana* leaves extracts using different solvents against three strains of fungus.

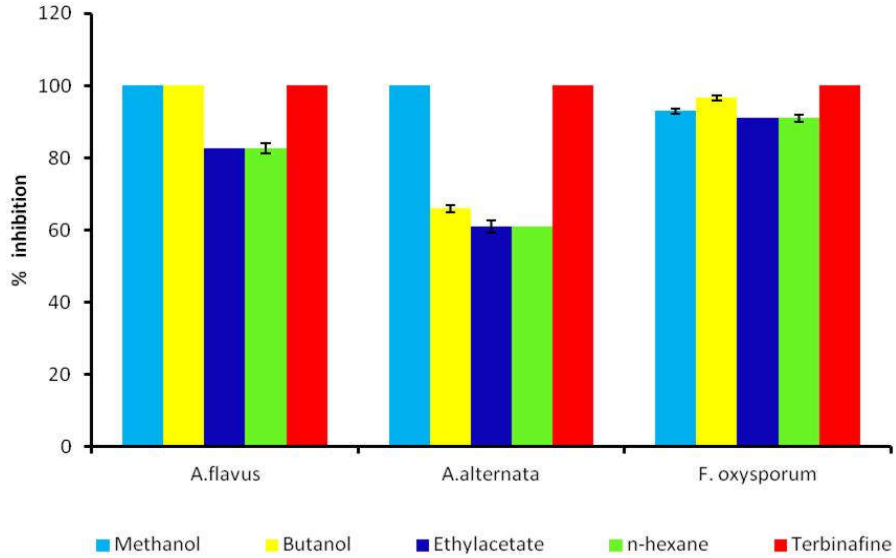

Fig.2. Antifungal activity of *P. sylvestris leaves* extracts using different solvents against three strains of fungus.

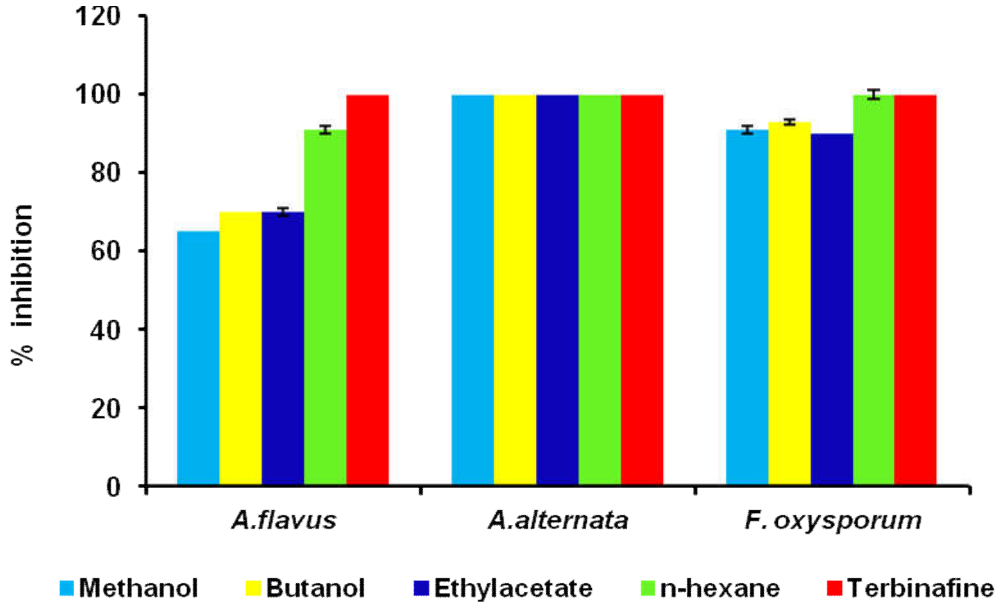

Fig.3. Antifungal activity of *O. ferruginea leaves* extracts using different solvents against three strains of fungus.

CONCLUSION

It is evident from the study that leaves extracts of *N. ritchiana* (Griff), *P. sylvestris* (Linn.) *and O. ferruginea* Royle. have promising antifungal potencies against *A. flavus, A. alternata* and *F. oxysporium.* Further work on the determination of active phytochemicals from the tested extracts and their efficacy against different strains of fungi and bacteria is recommended.

Authors' contributions: SP, AMK and MA conceived the project. SP collected, isolated the extracts and conducted the experiments. AMK, SMN and SP tabulated and analyzed the results. SMN and TY critically reviewed and prepared the manuscript.

CONFLICT OF INTEREST: The authors declare that there is no conflict of interest.

ACKNOWLEDGMENT

I sincerely thank to Mrs. Salma Perveen of Kohat University of Science and Technology, Kohat for her kind support and help during my research work.

REFERENCES

1. Al-Daihan, S., & Bhat, R.S. (2012). Antibacterial activities of extracts of leaf, fruit, seed and bark of *Phoenix dactylifera*. Afr. J. Biotechnol., 11: 10021–10025.
2. Rashid, R., Mukhtar, F. & Khan, A. (2014). Antifungal and cytotoxic activities of *Nannorrhops ritchiana* roots extract. Acta Pol Pharm., 71: 789–793.
3. Talaro, K. & Chess, B.(2014). Foundations in Microbiology: Basic Principles. In: Foundations in Microbiology: Basic Principles, 9th edn. WCB/McGraw-Hill, USA,
4. Amaike, S. & Keller, N.P. (2011). *Aspergillus flavus*. Annu. Rev. Phytopathol., 49: 107–133.
5. Wiest, P.M., Wiese, K., Jacobs, M.R., Morrissey, A.B., Abelson, T.I., Witt, W. & Lederman, M.M. (1987). Alternaria infection in a patient with acquired immunodeficiency syndrome: case report and review of invasive *Alternaria* infections. Rev. Infect. Dis., 9: 799–803.
6. Inami, K., Kashiwa, T., Kawabe, M., Onokubo-Okabe, A., Ishikawa, N., Pérez, E.R., et al. (2014).The tomato wilt fungus Fusarium oxysporum f. sp. lycopersici shares common ancestors with nonpathogenic F. oxysporum isolated from wild tomatoes in the Peruvian Andes. Microbes. Environ., 29: 200–10.
7. Ulett, G.C., Totsika, M., Schaale, K., Carey, A.J., Sweet, M.J. & Schembri MA (2013). Uropathogenic *Escherichia coli* virulence and innate immune responses during urinary tract infection. Curr. Opin. Microbiol., 16: 100–107.
8. Hennekinne, J-A., De Buyser, M-L. & Dragacci, S. (2012). *Staphylococcus aureus* and its food poisoning toxins: characterization and outbreak investigation. Microbiol. Rev., 36: 815–836.

9. Krakauer, T. & Stiles, B.G. (2013). The staphylococcal enterotoxin (SE) family: SEB and siblings. Virulence, 4: 759–773.
10. Oggioni, M.R., Pozzi, G., Valensin, P.E., Galieni, P. & Bigazzi, C. (1998). Recurrent septicemia in an immunocompromised patient due to probiotic strains of *Bacillus subtilis*. *J Clin Microbiol*., 36: 325–326.
11. Yuliar null, Nion, Y.A. & Toyota, K. (2015). Recent trends in control methods for bacterial wilt diseases caused by *Ralstonia solanacearum*. Microbes. Environ., JSME 30: 1–11.
12. Vicente, J.G. & Holub, E.B. (2013). *Xanthomonas campestris* pv. *campestris* (cause of black rot of crucifers) in the genomic era is still a worldwide threat to brassica crops. Mol. Plant. Pathol. 14: 2–18.
13. Muhammad, H., Syed, W., Iqbal, A. & Husain, B. (2010). Phytoconstituents isolated from *phoenix sylvestris* roxb. J. Basic. Appl. Sci., 6: 17–22.
14. Wain, J., Hendriksen, R.S., Mikoleit, M.L., Keddy, K.H. & Ochiai, R.L.(2015).Typhoid fever. Lancet, Lond. Engl., 385:1136–45.
15. Newton, A. (2014). Typhoid & Paratyphoid Fever - Chapter 3 - 2016 Yellow Book | Travelers' Health|CDC.http://wwwnc.cdc.gov/travel/yellowbook/2016/infectious-diseases-related-to-travel/typhoid-paratyphoid-fever. Accessed 21 Jul 2016
16. Levy, S.B. & Marshall, B. (2004). Antibacterial resistance worldwide: causes, challenges and responses. Nat. Med., 10: 122-129.
17. Das, K. (2013). Medicinal Plants : Their Importance In Pharmaceutical Sciences. Kalyani Publisher, New Delhi, 1–383
18. Duraipandiyan, V., Ayyanar, M. & Ignacimuthu, S. (2006). Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC Complement. Altern. Med., 6: 35.
19. Akinsulire, O.R., Aibinu, I., Adenipekun, T., Adelowotan, T. & Odugbemi, T. (2007). In vitro antimicrobial activity of crude extracts from plants of *Bryophyllum pinnatum* and *Kalanchoe crenata*. Afr. J. Tradit. Complement. Altern. Med., 4: 338–344.
20. Chaudhary, H.J., Zeb, A., Bano, A., Rasul, F., Munis, M.F.H., Fahad, S. & Naseem, W. (2011). Antimicrobial activities of *Sapium sebiferum* L. belonging to family Euphorbiaceae. J. Med. Plants. Res., 5: 5916–5919.
21. Khan, A.M., Qureshi, R.A., Gilani, S.A. & Ullah, F. (2011). Antimicrobial activity of selected medicinal plants of Margalla Hills, Islamabad, Pakistan. J. Med. Plants Res. 5: 4665–4670.
22. Hashim, H., Kamali, E. & Mohammed, Y. (2010). Antibacterial activity and phytochemical screening of ethanolic extracts obtained from selected sudanese medicinal plants. J. of Biosci., 2: 143–146.
23. Boulenouar, N., Marouf, A. & Cheriti, A. (2011). Antifungal activity and phytochemical screening of extracts from *Phoenix dactylifera* L. cultivars. Nat. Prod. Res. 25: 1999–2002.
24. Amin, A., Khan, M.A., Shah, S., Ahmad, M., Zafar, M. & Hameed, A. (2013). Inhibitory effects of *Olea ferruginea* crude leaves extract against some bacterial and fungal pathogens. Pak. J. Pharm. Sci., 26: 251–254.
25. Hussain, A., Qarshi, I.A., Liaqat, R., Akhtar, S., Aziz, I., Ullah, I. & Shinwar,i Z.K. (2014). Antimicrobial potential of leaf and fruit extracts and oils of wild and cultivated edible olive. Pak. J. Bot., 46: 1463–1468.
26. Kchaou, W., Abbès, F., Mansour, R.B., Blecker, C., Attia, H. & Besbes, S. (2016). Phenolic profile, antibacterial and cytotoxic properties of second grade date extract from Tunisian cultivars (*Phoenix dactylifera* L.). Food. Chem., 194: 1048–1055.
27. Zulqarnain, null, Rahim, A., Ahmad, K., Ullah, F., Ullah, H. & Nishan, U. (2015). In vitro antibacterial activity of selected medicinal plants from lower Himalayas. Pak. J. Pharm. Sci., 28: 581–587.
28. Masoko, P., Picard, J. & Eloff, J.N. (2005). Antifungal activities of six South African *Terminalia* species (Combretaceae). J. Ethnopharmacol., 99: 301–308.
29. Aibinu, I., Adenipekun, T., Adelowotan, T., Ogunsanya, T. & Odugbemi, T. (2006). Evaluation of the antimicrobial properties of different parts of *Citrus aurantifolia* (lime fruit) as used locally. Afr. J. Tradit. Complement. Altern. Med., 4:185–190.
30. Adelowotan, O., Aibinu, I., Adenipekun., E. & Odugbemi, T. (2008). The in-vitro antimicrobial activity of *Abrus precatorius* (L) fabaceae extract on some clinical pathogens. Niger. Postgrad. Med. J., 15: 32–37.
31. Mann, A., Banso, A. & Clifford, L.C. (2008). An antifungal property of crude plant extracts from *Anogeissus leiocarpus* and *Terminalia avicennioides*. Tanzan. J. Health Res., 10: 34–38.
32. Ettebong, E. & Nwafor, P. (2009). Report: In vitro antimicrobial activities of extracts of *Carpolobia lutea* root. Pak. J. Pharm. Sci., 22: 335–338.
33. Bhalodia, N.R. & Shukla, V.J. (2011). Antibacterial and antifungal activities from leaf extracts of *Cassia fistul.* An ethnomedicinal plant. J. Adv. Pharm. Technol. Res., 2: 104–109.
34. Irani, M., Sarmadi, M., Bernard, F., Ebrahimi Pour, G.H. & Shaker Bazarnov, H.(2010). Leaves Antimicrobial Activity of *Glycyrrhiza glabra* L. Iran. J. Pharm. Res. 9: 425–428.
35. Okoli, A.S. & Iroegbu, C.U. (2004). Evaluation of extracts of *Anthocleista djalonensis*, *Nauclea latifolia* and *Uvaria afzalii* for activity against bacterial isolates from cases of non-gonococcal urethritis. J. Ethnopharmacol., 92: 135–144.
36. Marwat, S., Fazal-ur-Rehman, Usman, K.A., Ghulam, S., Anwar, N., Sadiq, M. & Khan, S. (2011). Medico-ethnobotanical studies of edible wild fruit plants species from the flora of north western Pakistan (D. I. Khan district). J. Med. Plants Res., 5: 3679–3686.
37. Shabir, H., Khan, M., Rehman, H., Massod, Z., Yousa, T., Majeed, A. & Iqbal, R.(2015). Ethnomedicinal Uses of Xeric Flora in Tehsil Banda Daud Shah Collected from Distric Karak KPK Pakistan. World J. Zool., 10: 59–69.
38. Manan, Z., Razzaq, A., Islam, M. & others.(2007). Diversity of medicinal plants in Wari subdivision district Upper Dir, Pakistan. Pak. J. Plant Sci.,13: 21-28.

39. Ibrar, M., Hussain, F. & Sultan , A. (2007). Ethnobotanical studies on plant resources of Ranyal hills, District Shangla. Pak. J. Bot., 39: 329.
40. Malik, S.N.(2015). Antibacterial activity of olive (*Olea europaea*) leaves and arugula (*Eruca sativa*) seeds extract. *Int J Pharmacogn Phytochem Res* **7**, 307–310.
41. Bokhari, N. & Perveen, K.(2012). In vitro inhibition potential of *Phoenix dactylifera* L. extracts on the growth of pathogenic fungi. J. Med. Plants Res., 6: 1083–1088.
42. Kothari, V., Gupta, A. & Naraniwal, M. (2012). Comparative study of various methods for extraction of antioxidant and antibacterial compounds from plant seeds. J. Nat. Remedies., 12: 162–173.
43. Khatami, M. & Pourseyedi, S. (2015). *Phoenix dactylifera* (date palm) pit aqueous extract mediated novel route for synthesis high stable silver nanoparticles with high antifungal and antibacterial activity. IET Nanobiotechnol., 9: 184–190.
44. Ramanuj, K., Bachani, P. & Kothari, V. (2012). In vitro antimicrobial activity of certain plant products / seed extracts against multidrug resistant *Propionibacterium acnes*, *Malassezia furfur*, and aflatoxin producing *Aspergillus flavus*. Research in Pharmacy, **2**: 22–31.
45. Zehra S, Saeed A, Fatima S (2015) Antioxidant and antibacterial studies of *Phoenix dactylifera* and its varieties. Int. J. Appl. Microbiol. Biotechnol. Res., 3: 81–88.
46. Sharma, N., Sharma, A. & Singh, D. (2015). Ethno-Medicinal uses of floristic diversity of sub-tropical forests of Jammu, Jammu and Kashmir, India. Int. J Dev. Res., 5: 3945-3954.
47. Masoko, P., Picard, J., Eloff, J.N. (2005). Antifungal activities of six South African *Terminalia* species (Combretaceae). J. Ethnopharmacol., 99: 301–308.
48. Sudjana, A.N., D'Orazio, C., Ryan, V., Rasool, N., Ng, J., Islam, N., Riley, T.V. & Hammer, K.A.(2009). Antimicrobial activity of commercial *Olea europaea* (olive) leaf extract. Int. J. Antimicrob. Agents., 33: 461–463.

CITATION OF THIS ARTICLE

S Perveen, A Muhmmad Khan, M Anees , S M Nurulain, T Yasmin. Antifungal and Antibacterial Potential of the Leaves Extracts of *Nannorrhops ritchiana* (Griff), *Phoenix sylvestris* (Linn.) and *Olea ferruginea* Royle.Bull. Env. Pharmacol. Life Sci., Vol 5 [12] November 2016: 35-43