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ANTIMICROBIAL ACTIVITIES OF MOUNTAIN NEEM (*MELIA AZEDARACH)* IN THE AREAS OF WOLLEGA UNIVERSITY, NEKEMTE, ETHIOPIA.

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ABSTRACT

Mountain Neem (*Melia azedarach*) trees are found in the campus of Wollega University as ornamental plant. This study used to find out the medicinal properties of *Melia azedarach*. It used as home medicine in India and it may contains more bioactive compounds to treat various diseases such as Diabetes, Skin diseases and some plant diseases, etc., In this study the crude extracts of Mountain neem examined to determine the antimicrobial activities using Disc diffusion assay method against Five bacterial and TLC bioautography against two fungal pathogens(Bacterial pathogens: *Salmonella typhi, Bacillus subtilis, Pseudomonas aeruginosa, E.coli, and Staphylococcus aureus.* Fungal pathogens: *Candida sp., Aspergillus sp., and Botrytis ceneria).* The Ethyl acetate extract of *Melia azedarach* exhibited broad spectral activity against both bacterial and fungal pathogens. Methonolic extracts exhibited highest activity on *Aspergillus niger*, *Candida albicans* and *Bacillus subtilis*. Medium activity by aqueous extract on *E.coli.* Therefore the crude extracts of Mountain neem exhibited Antibacterial and antifungal activities. The active compound isolation and identification is necessary to find out the pharmacological important compounds to treat various diseases.

KEYWORDS: Anti microbial activity, Mountain neem, bioactivity, Anti diabetics and Medicinal plant.

INTRODUCTION

Plants are producers have been a source of food and medicine since the beginning of the human civilization. Medicinal plants are traditional and cheap medicine. There is no side effect by the usage as medicine. This is purely bio product used to kill the disease causing microorganism and treat the diseases. The basic need of human is food, shelter and dress. But now a day healthy life also included. The word "Health is Wealth" that indicates the importance of human health. We know that chemicals causing side effects, but mostly of the medicinal plants are not causing any side effects. So, the medicinal plants are very important to treat and control the diseases.

Mountain neem (*Melia azedarach*, Family: *Meliaceae*) commonly known by many names including Persian lilac, chinaberry tree and Pride of India*(Gil Nelson, 1996).* It is a large evergreen tree native to India, growing wild in the sub-Himalayan region, Indomalaya and Australasia *(Mabberley, David J., 1984). It* is widely spreads in our Wollega University campus. We know very well that is a ornamental plant variety. But that is not only ornamental plants also medicinal plant used to treat various diseases caused by bacteria, fungi, viruses, etc.,

Melia azedarach should not be confused with the *Azadirachta* trees, which are in the same family, but a different genus. The Persian lilac tree is frequently confused with Neem. However, the structure of the leaves and the colour of the flowers, white in Neem and lilac in Persian lilac, are sufficient to distinguish between the two.

The use of plant and its products has a long history that began with folk medicine and through the years has been incorporated into traditional and allopathic medicine system (R. Dubey, et.al., 2011). Since antiquity, many plants species reported to have pharmacological properties as they are known to possess various secondary metabolites like glycosides, saponins, flavonoids, steroids, tannins, alkaloids, triterpenes which are therefore, should be utilized to combat the disease causing pathogens (HH EL. Kamali., et.al., 2010). Application of plant-derived biocides in agriculture have been more popular during the past for their low health risk and feasibility. In recent years much attention has been given to nonchemical systems for seed treatment to protect them against many plant pathogens. With the advancement in science and technology, remarkable progress has been made in the field of medicine with the discoveries of many natural and synthetic drugs (R. Preethi., et.al., 2010).

Antibiotics are undeniably one of the most important therapeutic discoveries of the twentieth century that had effectiveness against serious bacterial infections. However, only one third of the infectious diseases known have been treated from these synthetic products. This is because of the emergence of resistant pathogens as a consequence of years of widespread indiscriminate use, incessant and misuse of antibiotics (VL. Enne, 2001). Antibiotic resistance has increased substantially in the recent years and is posing an ever increasing therapeutic problem. One of the methods to reduce the resistance to antibiotics is by using antibiotic resistant inhibitors from plants (C. Alagesaboopathi, 2011). Plants are known to produce a variety of compounds to protect themselves against a variety of pathogens. It is expected that plant extracts showing target sites other than those used by antibiotics might be active against drug resistant pathogens(Ahmad, et.al., 2011).

Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world. Hence, researchers have recently paid attention to safer phytomedicines and biologically active compounds isolated from plant species used in herbal medicines with acceptable therapeutic index for the development of novel drugs. Melia azedarach L., is traditionally been used as anthelmintic, antilithic diuretic, astringent and stomachic (Warrier PK, et.al., 1995). Various scientific studies reported the anticancer(Ntalli NG, et.al., 2010), antimalarial activity, analgesic and anti-inflammatory activity(Vishnukanta RAC, 2010). After scrutiny of published literature showing its medicinal importance, the present protocol has been outlined regarding the antimicrobial activity on these selected plant using different extracts. It is in view of this, that the present research was set up to evaluate the antimicrobial activity of M. azedarach, using different plant extractions against some pathogenic bacteria and fungi.

MATERIAL AND METHODS

Fresh plants were gathered from Wollega university's campuson spring season between September to November in 2016. The plant materials were identified and washed with distilled water and dried under the shade for 10-12 days. After this period, leaves and stems of the plant have been grinded and transformed to powder by a grinder. The powders were preserved in clean plastic containers, kept away from light, heat and moisture until use.

Preparation of Plant extracts- Soxhlet method

Soxhlet method used for extraction of crude compound. 1g of powder leaves blended with 50 ml of different solvents (Ethyl acetate, Methanol and water) for different periods (14, 24 and 48 h) with agitation at room temperature. After, the extracts were allowed to filtration by using a 0.45 Millipore filter paper. Then, the extracts concentrated using a rotary evaporator at 40°C under reduced pressure. Finally, the extracts were allowed to weigh and store at -20°C till their usage in the different tests (Hanan Bandar. et.al., 2013).

Procurement of microorganisms

The microbial strains investigated in the study were obtained from Biology Lab., Wollega University, Ethiopia. The strains are 5 bacterial pathogens such as *Salmonella typhi, Bacillus subtilis., Pseudomonas aeroginosa, E.coli, and Staphylococcus aureus;* 3 Fungal pathogens such as *Candida albicans, Aspergillus niger* and *Botrytis ceneria.*

Preparation of Inoculum -Test for antibacterial activity

The antibacterial assay was carried out by microdilution method in order to determine the antibacterial activity of compounds tested against the pathogenic bacteria. The bacterial suspensions were adjusted with sterile saline to a concentration of 1.0 X 10^7 CFU/ml. The inocula were prepared and stored at 4^oC until use. Dilutions of the inocula were cultured on solid medium to verify the absence of contamination and to check the validity of the inoculum. All experiments were performed in duplicate and repeated three times(Antara Sen, 2012).

Test for Antifungal activity

In order to investigate the antifungal activity of the extracts, a modified micro dilution technique was used. The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v). The spore suspension was adjusted with sterile saline to a concentration of approximately 1.0 – 10^4 in a final volume of 50 µl per disc. The inocula were stored at 4^oC for further use. Dilutions of the inocula were cultured on solid potato dextrose agar to verify the absence of contamination and to check the validity of the inoculum.

Test for antibacterial assay-Disc diffusion Assay

Disc Diffusion assay method used to detect the antimicrobial activities of neem extract. The crude extract of neem used to prepare disc for agar diffusion method. The discs were prepared using Whatman No.1 filter paper and the solvent extracts of Mountain neem were impregnated into the discs separately. Approximately 50µg of crude extracts (Ethyl acetate, Methanol and Aqueous) were impregnated separately. Muller Hinton agar plates were swabbed by muller Hinton broth containing 16 hours pathogens (*Salmonella typhi, Shigella sp., Pseudomonas aeruginosa, E.coli and Staphylococcus aureus*) separately 50 µg of Penicillin disc used as standard for Gram Positive bacteria whereas Streptomycin used as standard for Gram Negative bacteria. The prepared antibiotic discs were placed on the inoculated plates were allowed to incubation at 37^oC for 24 hrs. After incubation the results were recorded.

Test for antifungal assay-TLC Bioautography method

50µg of concentrated solvent extracts (Ethyl acetate, Methanol) and aqueous extract of Mountain neem

impregnated on the TLC (Thin layer chromatography Plate - 0.2mm thickness readymade plate, E Merck, Germany) and Antibiotic Nystatin used as standard. TLC plates were inoculated with fungal spore suspension. Fungal strains *Candida albicans*, *Aspergillus niger* and *Botrytis ceneria* were Inoculated and incubated for 5 days. The fungal spray solution was prepared using Potato dextrose broth (12g/500ml). The Chromatographic sprayer used to spray the fungal suspension on TLC plate and it was kept in 30X13X7.5 cm moisture chamber and incubated for 4 days at 25° C. After incubation calculated the zone of inhibition (Vijayalakshmi S, et al., 2008).

RESULTS

In this study 50µg of crude compounds (Aqueous extracts) of Mountain neem were exhibited potential activity on both bacteria and fungi. The solvent extract especially methanol exhibited highest activity in both bacteria and fungi (Figure 1 and 2). The inhibition size was 14 mm on *Bacillus subtilis* (Table 1) and also on *Candida albicans.* It expressed highest activity on *Aspergillus niger* inhibition was 15 mm (Table 2). The ethyl acetate exhibited highest activity 14 mm on *Pseudomonas aeruginosa* and it expressed broad spectral activity on both bacteria and fungi. Medium activity by aqueous extract on *E.coli* inhibition was 11mm. Therefore the crude extracts of Mountain neem exhibited Antibacterial and antifungal activities. The active compound isolation and identification is necessary to find out the pharmacological important compounds to treat various diseases.

Table 1: Antibacterial assay-Disc diffusion Assay.

S.No.	Strain Type	Name of the Bacteria	Zone of Inhibition in mm (Concentration of Extract 50 µg/ml)			
			Ethyl acetate	Methanol	Aqueous	Standard disc 50 µg
1	Gram + ve	*Staphylococcus aureus*	7.8	9.7	-	22
2	Gram + ve	*Bacillus subtilis*	12.6	14	5	21
3	Gram -ve	*Salmonella typhi*	10.5	6	10	19
4	Gram -ve	*Pseudomonas aeruginosa*	14	0	4	23
5	Gram -ve	*E. coli*	13.8	13	11	24

Antibiotic Discs: Penicillin for Gram + ve Bacteria and Steptomycin for Gram –ve Bacteria.

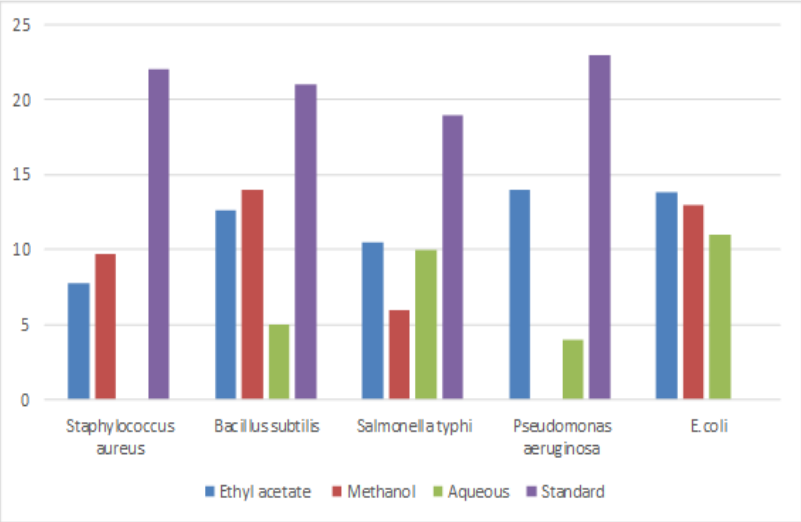

Figure 1: Antibacterial assay-Disc diffusion Assay.

Table 2: Antifungal assay-TLC Bioautography method.

S.No.	Name of the Fungi	Zone of Inhibition in mm(Concentration of Extract 50 µg/ml)			
		Ethyl acetate	Methanol	Aqueous	Standard disc (Nystatin) 50 µg
1	*Candida albicans*	12	14	7	21
2	*Aspergillus niger*	9	15	3	25
3	*Botrytis ceneria*	13	4	10	17

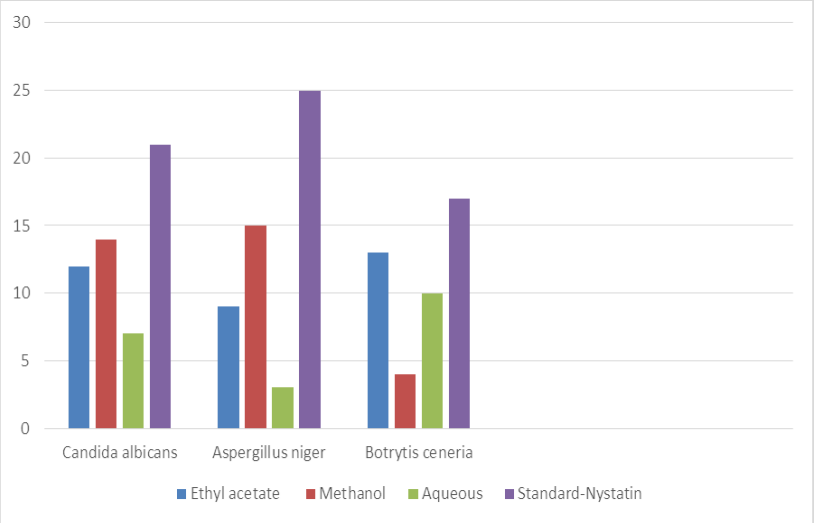

Figure 2: Antifungal assay- TLC Bioautography method.

DISCUSSION

In the present investigation, solvent extracts of *M. azedarach* was evaluated for exploration of their antimicrobial activity against certain bacteria and fungi which was regarded as human and plant pathogenic microorganism. Susceptibility of the Mountain neem extract was tested by Disc diffusion assay method and TLC bioautography.

Neem extract has shown antimicrobial activity against *E. faecalis* and *S. mutans* in previous*in vitro* studies (Dhanya Kumar NM, et.al., 2011). Prashant *et al.*, demonstrated that Neem stick extract produced maximum zone of inhibition against *S. mutans* at 50% concentration. Even at 5% concentration, Neem extract was effective against all four species of microorganisms tested in their study(Prashant GM, et.al., 2007).

Bohora *et al.*, concluded that Neem leaf extract has a significant antimicrobial effect against *E. faecalis*, *Candida albicans* and mixed culture (Bohora A, et.al., 2010). Our study has shown the leaf extract of Neem is very effective against *S. mutans* and *S. aureus* with MIC value of 125 µg. The maximum antimicrobial activity was observed on *S. mutans* at 3 mg. concentration with zone of inhibition of (24.67 ± 2.517) mm.

The alcoholic extracts of *M. azerarach* showed significant antimicrobial activity against multi-drug resistant clinically isolated microorganisms. Though, the mechanism of the action of these plant constituents is not yet fully known it is clear that the effectiveness of the extracts largely depends on the type of solvent used. The organic extracts provided more powerful antimicrobial activity as compared to aqueous extracts. This observation clearly indicates that the existence of non-polar residues in the extracts which have higher both bactericidal and bacteristatic abilities. Cowan, 1999, mentioned that most of the antibiotic compounds already identified in plants are reportedly aromatic or saturated organic molecules which can easily solubilized in organic solvents. Similar results showing that the alcoholic extract having the best antimicrobial activity is also reported by Preethi *in Leucas aspera*, *Holarrhenaantidysenterica.* Seyydnejad also studied the effect of different alcoholic viz. ethanol and methanol for antimicrobial activity and observed that this difference in the activity between different alcoholic extract is due to the difference between extract compounds in this two extract.

The study also revealed that Petroleum ether extract shows moderated and aqueous extract shows minimum antimicrobial activity. However, Murugesan showed that petroleum ether extract of plant *Memecylonum bellatum* Burm. f. shows significant antimicrobial activity. Furthermore, water extract from leaves of P. *acerifolium* had been reported to have prominent antimicrobial activity against several gram positive and gram negative human pathogenic bacteria.

The antimicrobial analysis using the agar well diffusion method and MIC value is been used by (Gurudeeban S., et.al., 2010). In the present study the MIC value of the active plant extracts obtained in this study were lower than the MBC values suggesting that the plant extracts were bacteriostatic at lower concentration but bactericidal at higher concentration (Maji S, et.al., 2010).

CONCLUSION

In conclusion, of the present investigation *Melia azedarach* contain potential antimicrobial components that may be of great use for the development of pharmaceutical industries as a therapy against various diseases. The ethyl acetate, methanol and aqueous extracts of *Melia azedarach* possess significant inhibitory effect against tested pathogens. The results of the study support the folkflore claim along with the development of new antimicrobial drugs from the plant. However, the active compound isolation and identification is necessary to find out the

pharmacological important compounds to treat various diseases.

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