International Journal of Academic Research
ISSN: 2348-7666; Vol.3, Issue-7(4), July, 2016
Impact Factor: 3.656; Email: drtvramana@yahoo.co.in

Antifungal screening of alkaloids, flavonoids & steroids of different parts of *Nerium oleander* Linn

Dr. Meenakshi Fartyal, Laboratory of Plant Tissue Culture and Secondary Metabolites, Department of Botany, University of Rajasthan, Jaipur, India

Abstract: Pathogenic fungi are found all over the world, and are a frequent cause of invasive disease in immune-compromised hosts. Besides, some other fungal species cause superficial mycosis and more serious systemic infections. *So, there is an urgent need to discover some potent antifungal agents to overcome the above problems.* The results of the study show that C. albicans found to be the most susceptible organism followed by A. flavus. T. mentagrophyte found to be the resistant organism for all the tested extracts. Steroid extract of flower showed the best activity (IZ= 12 mm, AI= 0.40±0.02, MIC= 0.312 mg/ml, MFC= 0.156 mg/ml, TA= 62.5 ml/g) against C. albicans. Steroid extract of stem (IZ= 10 mm, AI= 0.34±0.02, MIC= 0.625 mg/ml, MFC= 0.312 mg/ml, TA= 20.8 ml/g) and free flavonoid of root (IZ= 10 mm, AI= 1±0.02, MIC= 0.625 mg/ml, MFC= 0.312 mg/ml, TA= 1.6 ml/g) also showed very good activities against C. albicans. Free flavonoid of flower showed good activity (IZ= 10 mm, AI= 0.22±0.02, MIC= 0.625 mg/ml, MFC= 0.312 mg/ml, TA= 5.6 ml/g) against A. flavus. The range of MIC & MFC found to be 1.25-0.312 & 0.625-0.156, respectively. I is concluded that results reveal good antifungal potential of extracts of N. Oleander against tested microorganisms. Hence, may be explored for formation of new antifungal agents.

Keywords: Antifungal activity, Disc diffusion assay, Minimum inhibitory concentration, Total activity.

Introduction: The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem and one of the ways to overcome this problem is to encourage research to develop new drugs, which might be synthetic or natural. Since the synthetic drugs are mostly associated with side effects, hence more emphasis should be given to develop safe, natural plant based drugs. According to World Health Organization (Santos, P.R.V. *et al.,* 1995), medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency (Eloff, J.N., 1998). Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of plants. Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized, especially those related to the control of antibiotic resistant microbes. In the present study *Nerium oleander* has been selected for the study.

Nerium oleander (common name Kaner) is an evergreen shrub belongs to family Apocynaceae. It is native to Southern Europe and is widely cultivated and naturalized in Asia, Europe and North America. It is four meters in height, occurs along watercourse, gravely place and damp ravines, widely cultivated

particularly in warm temperate subtropical regions where it grows outdoors in parks, gardens and along road sides. Various medicinal properties viz. Cardiotonic, Analgesic, Antidiabetic, Anti-inflammatory, Antibacterial, Anticancer/Antineoplastic, Antifungal, Depressant, Antimitotic, Insecticidal, Larvicidal are attributed to this plant. Other properties attribute are inhibition of Nuclear factor-kappa B (NF-/cB) activation, Muscle stimulation, effective against Asthma, Seizures, Cancer, Menstrual pain, Skin problems, Warts, epilepsy, leprosy, malaria, ringworm, indigestion, and venereal disease and also cause abortions. The present investigation was undertaken to find out the antifungal potential of flavonoids, alkaloids and steroids of different parts of *N. oleander* against pathogenic fungi.

Alkaloids are known to have pharmacological effects and are used in medications, as recreational drugs or in entheogenic rituals. Literature indicates that plant alkaloids have considerable biological activity (Cowan, M.M., 1999; Okunade, A.L. *et al*., 2004). Alkaloid, flavonoid, saponin, tannin and phenol were identified in the aqueous extracts of *N.oleander* (Hussain, I. *et al*., 2011; Khan, F.A. *et al*., 2011; Hussain, J. *et al*., 2011). Alkaloids of *N. oleander* dissolved in ethanol at 1% were used in integrated pest management (Tail, G. *et al.,* 2011).

Flavonoids are potent water-soluble antioxidants and free radical scavengers, which prevent oxidative cell damage and have strong anti-cancer activity (Del-Rio, A. *et al*., 1997). It was reported that flavonoids can improve the blood circulation and lower the blood pressure (Blumenthal, M., 2003). Flavonoid, alkaloid, phenols and tannin have been reported in Nerium (Haniffa, M.A. *et al.,* 2012). Kaempferol is a flovonol type flavonoid with a wide spectrum of bioactivity. It has been isolated from *N. oleander* L. as well as a wide range of medicinal herbs (Wagner, H. *et al.,* 1955).

Steroids are frequently used as signalling molecules, represents highly concentrated energy stores, along with phospholipids function as components of cell membranes. Steroid, terpenoid, quinine, saponin, and Phenolic compounds were identified in methanolic and aqueous extract of *Nerium oleander* (Gopinath, S.M. *et al*., 2011). The roots of *Nerium oleander* yielded a new cardenolide, 12β-hydroxy-5β-carda-8, 14, 16, 20(22)-tetraenolide. Biological screening of the compound revealed antibacterial and digoxin-like cardiac activities (Mostaqul Huq, M. *et al*., 1999). Considering the rich diversity of plants, it is expected that screening and scientific evaluation of plant extracts for their antimicrobial activity may provide new antimicrobial substances. Review of the current literature reveals that no work has been carried out for extraction and screening of specific compound from selected plant. Hence, in the present work an extraction and screening for antifungal activity of extracts of *N. oleander* has been undertaken.

Material and methods:

Different parts of *N. oleander* (leaf, stem, root and flower) were collected in the month of April to June from the western parts of India (Jaipur, Rajasthan). Plants were identified by senior taxonomist at Department of Botany, University of Rajasthan and voucher specimen no: RUBL 21176 was submitted to the

Herbarium, Botany Department, University of Rajasthan.

Preparation of Extracts:

Alkaloids Extraction:

Alkaloids were extracted from different parts of the selected plant by well established method (Ramawat, K.G. *et al*., 2000).Finely powered sample (100g) of plant parts were extracted in 20ml methanol after shaking of 15 min. After filtration, filtrate kept for drying then residual mass were treated with 1% H_2SO_4 (5ml. 2 times).Extraction was then done in 10ml. Chloroform ($CHCl_3$) by using separating funnel. Organic layer of chloroform was rejected and aqueous layer was basified with 30% NH_4OH (P^H=9-10). Now again, extraction was done in 10ml. chloroform & organic layer of chloroform (lower layer) was collected in a flask and repetition of step was done with fresh chloroform. Extracts was then dried in vaccuo for further use.

Flavonoid extraction:

Selected plant parts were separately washed with sterilized water; shade dried, and finely powdered using a blender. Each sample was subjected to extraction, following the method of Subramanian and Nagarjan (1969). One hundred grams of each finely powdered sample was soxhlet extracted with 80% hot methanol (500ml) on a water bath for 24 h and filtered. Filtrate was re-extracted successively with petroleum ether (fraction I), diethyl ether (fraction II), and ethyl acetate (fraction III) using separating funnel. Petroleum ether fractions were discarded as being rich in fatty substances, where as diethyl ether and ethyl acetate fractions were analyzed for free and bound flavonoids respectively. The ethyl acetate fraction of each of the samples was hydrolyzed by refluxing with 7% H_2SO_4 for 2 h (for removal of bound sugars) and the filtrate was extracted with ethyl acetate in separating funnel. Ethyl acetate extract obtained was washed with distilled water to neutrality. Diethyl ether (free flavonoids) and ethyl acetate fractions (bound flavonoids) were dried in vaccuo and weighed. The extracts were stored at 4°c and were re-suspended in their respective solvents to get 10mg/ ml concentration for antimicrobial assay.

Steroid Extraction:

Steroids were extracted from different parts of the selected plant by well established method (Tomita, Y. *et al.,* 1970) after preliminary detection of steroids. Finely powdered sample (100g) of plant parts were extracted in petroleum ether for 2-4hr.After filtration, residual mass was treated with 15% ethanolic HCl for 4hr. Extraction was then done in ethyl acetate followed by washing in dis. water to neutralize the extract. Neutral extract was then passed over sodium sulphate to remove moisture contents and was dried in vaccuo. Chloroform was used for reconstitution of extract, filtered and dried for further use.

Selected Test Microorganisms:

Three pathogenic bacteria were screened, viz., *Candida albicans* (MTCC no. 183), *Aspergillus flavus* (MTCC no. 277) and *Tricophyton mentagrophyte* (MTCC no. 7687). The pathogens were procured from IMTECH (Chandigarh, Punjab, India). Fungal strains were grown and maintained on Sabouraud Dextrose Agar medium.

Candida albicans is major model of pathogenic yeast which is found in mouth, throat, intestine and genitourinary tract of human and considered as common constituent of bowel flora together with many bacterial species e.g. *E. coli*, *S. aureus* and *P. mirabilis*. It lives in 80% of the human population with no harmful effects, although overgrowth results in candidiasis which is often observed in immunocompromised individuals such as patients of cancer, transplant and AIDS. It is a causal agent of opportunistic oral and genital infections in humans (Enfert, C. *et al*., 2007). Superficial and mycosis infections cause local inflammation and discomfort in human beings (Pappas, P.G., 2006). Candidiasis also known as 'thrush', which is usually occur in immune compromised people (Ryan, K.J. *et al*., 2004).

Aspergillus flavus is the second leading cause of invasive and non-invasive aspergillosis (Hedayati, M.T. *et al*., 2007). The presence of *Aspergillus* in the air is a major risk factor for both invasive and allergic aspergillosis (Denning, D.W. *et al.,* 1997). *A. flavus* can cause storage problems in stored grains. It also causes diseases in economically important crops, such as maize and peanuts and produce potent mycotoxins. It can also be a human pathogen, associated with aspergillosis of the lungs and sometimes causing corneal, otomycotic and nasoorbital infections.

Tricophyton mentagrophyte is a cosmopolitan dermatophyte, belonging to a homogeneous group of fungi called the dermatophytes. The organism is found in soil, floor of swimming pools, hairs of wild boar, cats and dogs, farm animals, foot wears and from human toe webs without clinical lesions. It requires keratin for growth and can cause a variety of cutaneous (hair, nail and skin) infections in humans and animals hence considered to be anthropophilic or zoophilic in nature (Van Rooij, P. *et al*., 2006; Sanchez-Castellanos, M.E. *et al*., 2007). It causes dermatophytosis in dogs, cats, cattle and especially in rodents (George, L.K. *et al*., 1957; Houk, H.E. *et al*., 1996; Ajello, L. *et al*., 1967).

Antimicrobial assay:

'Disc Diffusion Assay' was performed for screening (Andrews, J.M., 2001). SD agar base plates were seeded with the fungal inoculum (inoculum size 1×10^7 CFU/ml). Sterile filter paper discs of Whatmann no.1 (6mm in diameter) were impregnated with 100µl of each of the extract of concentration 10mg/ml to give a final concentration of 1 mg/disc. Discs were left to dry in vaccuo so as to remove residual solvent, which might interfere with the determination. Discs with extract were then placed on the corresponding seeded agar plates. Each extract was tested in triplicate along with Ketoconazole (1mg/disc) for *T. mentagrophyte* and Terbinafine for *C. albicans* and *A. flavus* as standard drugs. The plates were kept at 4°C for diffusion of extract, thereafter were incubated at 27°C (*C. albicans* and *A. flavus* for 48 h & *T. mentagrophyte* for 5-7 days). Activity index for each extract was calculated [Table 1] by the standard formula viz.

Activity index = IZ produced by the extract/ IZ produced by standard [IZ = inhibition zone]

Determination of Minimum Inhibitory Concentration (MIC) & Minimum Fungicidal (MFC) Concentration:

Minimum inhibitory concentration (MIC) was determined for each plant extract showing antimicrobial activity against the test pathogens. 'Broth micro dilution' method was followed for determination of MIC values (Barsi, D.F. *et al.*, 2005). Plant extracts were resuspended in acetone (which has no activity against test microorganisms) to make 10 mg/ml final concentration. Two fold serially diluted extracts were added to broth media of 96-wells of micro titer plates. Thereafter 100µl inoculum (1×10^7 CFU/ ml) was added to each well. Fungal suspensions were used as negative control, while broth containing standard drug was used as positive control. Micro titer plates were then incubated at 27°C for 48 h. Each extract was assayed in duplicate and each time two sets of micro plates were prepared, one was kept for incubation while another was kept at 4°C for comparing the turbidity in the wells of micro plate. The MIC values were taken as the lowest concentration of the extracts in the well of the micro titer plate that showed no turbidity after incubation. The turbidity of the wells in the micro titer plate was interpreted as visible growth of microorganisms. The minimum fungicidal concentration (MFC) was determined by sub culturing 50 µl from each well showing no apparent growth. [Table2]. Least concentration of extract showing no visible growth on sub culturing was taken as MFC.

Total activity (TA) determination:

Total activity is the volume up to which test extract can be diluted without losing the ability to kill microorganisms. It is calculated by dividing the amount of extract from 1g plant material by the MIC of the same extract or compound isolated and is expressed in ml/g. (Eloff, J.N., 2004) [Table 3]

Results:

Secondary metabolites (alkaloid, steroid & flavonoid) were assessed for their antimicrobial efficacy by using IZ, AI (Table 1), MIC and MFC (Table 2). Quantity of extract per gram of plant material was also calculated (Table 3). In present study, 16 extracts were tested against three pathogenic organisms, including one yeast *C. albicans* & two fungi *A. flavus* and *T. mentagrophyte.* Among all the tested extracts alkaloid of all four parts & steroid of leaf found to be inactive against all the tested pathogens. *T. mentagrophyte* found to be resistant pathogen as no extract showed activity against it. *C. albicans* found to be most susceptible organism as 10 out of 16 extracts showed activity against it. Best activity was observed in steroid extract of flower (IZ= 12 mm, AI= 0.40±0.02, MIC= 0.312 mg/ml, MFC= 0.156 mg/ml, TA= 62.5 ml/g) against *C. albicans*. Steroid of stem (IZ= 10 mm, AI= 0.34±0.02, MIC= 0.625 mg/ml, MFC= 0.312 mg/ml, TA= 20.8 ml/g), Free flavonoid of root (IZ= 10 mm, AI= 1±0.02, MIC= 0.625 mg/ml, MFC= 0.312 mg/ml, TA= 1.6 ml/g) & bound flavonoids of flower (IZ= 9 mm, AI= 0.75±0.01, MIC= 0.625 mg/ml, MFC= 0.312 mg/ml, TA= 9.6 ml/g) also showed very good activities against *C. albicans* while free flavonoid of flower (IZ= 10 mm, AI= 0.22±0.02, MIC= 0.625 mg/ml, MFC= 0.312 mg/ml, TA= 5.6 ml/g) showed very good activity against *A. flavus*. Free flavonoids of leaf (IZ= 7 mm, AI= 0.70±0.01, MIC= 1.25 mg/ml, MFC= 0.625 mg/ml, TA= 1.2 ml/g), free flavonoid of stem (IZ= 8 mm, AI= 0.80±0.01, MIC= 1.25 mg/ml, MFC=

0.625 mg/ml, TA= 2.4 ml/g), bound flavonoid of stem (IZ= 7.5 mm, AI= 0.75±0.01, MIC= 1.25 mg/ml, MFC= 0.625 mg/ml, TA= 1.2 ml/g), bound flavonoid of root (IZ= 7 mm, AI= 0.70±0.01, MIC= 1.25 mg/ml, MFC= 0.625 mg/ml, TA= 1.2 ml/g), steroid of root (IZ= 8 mm, AI= 0.80±0.01, MIC= 1.25 mg/ml, MFC= 0.625 mg/ml, TA= 1.6 ml/g) and free flavonoid of flower (IZ= 7.5 mm, AI= 0.62±0.01, MIC= 1.25 mg/ml, MFC= 0.625 mg/ml, TA= 2.8 ml/g) showed good activities against *C. albicans* while bound flavonoid of leaf (IZ= 7 mm, AI= 0.16±0.01, MIC= 1.25 mg/ml, MFC= 0.625 mg/ml, TA= 2.8 ml/g), free flavonoid of stem (IZ= 7 mm, AI= 0.16±0.01, MIC= 1.25 mg/ml, MFC= 0.625 mg/ml, TA= 1.9 ml/g), steroid of root (IZ= 7 mm, AI= 0.17±0.01, MIC= 1.25 mg/ml, MFC= 0.625 mg/ml, TA= 1.6 ml/g) and steroid of flower (IZ= 7 mm, AI= 0.17±0.01, MIC= 1.25 mg/ml, MFC= 0.625 mg/ml, TA= 15.6 ml/g) showed good activities against *A. flavus*.

Among all the tested extracts, free flavonoid of stem, steroid of root, free flavonoid of flower and steroid of flower found to be the most active substances as they showed activity against 2 of the 3 pathogens. Plant extracts, which had shown activity in diffusion assay, were evaluated for their MIC & MFC values (Table 2). The range of MIC & MFC of tested extracts recorded was 1.25-0.312 & 0.625-0.156, respectively. In present study, lowest MIC value 0.312 mg/ml was recorded against *C. albicans,* indicating significant antimicrobial efficacy of tested extracts. Quantity of extract obtained per gram from plant parts & TA was calculated and recorded (Table 3). TA indicates the volume at which extract can be diluted without losing ability to kill microorganisms. High value of TA were observed against *C. albicans* (62.5 ml/g) followed by *A. flavus* (15.6 ml/g).

Discussion:

Present investigation is an effort towards the direction for getting new antifungal agents through the search of new natural sources to decrease the frequency of fungal diseases. In present study, *N. oleander* has shown antimicrobial potential against both the tested fungi. Although the plant has been studied previously for its antimicrobial activity but only restricted to the determination of IZ and that too without AI, MIC, MFC & TA evaluation. Hence could not explore the preparation of antifungal drugs. Such studies could only indicate their antifungal potential but can't replace the existing antifungal agents. In present investigation, IZ, AI, MIC, MFC & TA have been evaluated for each extract to determine their antifungal potential. Present investigation together with previous studies; provide support to the antifungal properties of *N. oleander*. Therefore it can be used as antifungal supplements in the developing countries towards the development of new therapeutic agents. Further pharmacological and clinical studies are required to understand the mechanism and the actual efficacy of these plant extracts in treating various infectious diseases.

Conclusion:

Results of the present study demonstrates the good antifungal activity of *N. oleander,* it may help in the further studies related to the treatment of infectious fungal diseases and their control.

References:

[1] Ajello, L. and Cheng, S.L. (1967), "The perfect state of *T. mentagrophyte*", Sabouraudia, Vol. 5, pp. 230-234.

[2] Andrews, J.M. (2001), "BSAC standardized disc susceptibility testing method", Journal of Antimicrobial Chemotherapy, Vol. 4, pp. 43-57.

[3] Basri, D.F. and Fan, S.H. (2005), "The potential of aqueous and acetone extracts of gall of *Quercus infectoria* as antibacterial agents", Indian Journal of Pharmacology, Vol. 37, pp. 26-29.

[4] Blumenthal M., (2003), "The ABC Clinical Guide to Herbs". Austin: American Botanical Council, page. 239.

[5] Cowan, M.M. (1999), "Plant products as antimicrobial agents", Clinical Microbiology Reviews, Vol. 12, pp. 564-582.

[6] Del-Rio, A., Obdulio, B.G., Castillo, J., Marin, R.R. and Ortuno, A. (1997), "Uses and properties of citrus flavonoids", Journal of Agricultural and Food Chemistry, Vol. 45, pp. 4505-4515.

[7] Denning, D.W., Venkateswarlu, K., Oakley, K.L., Anderson, M.J., Manning, N.J., Stevens, D.A., Warnock, D.W. and Kelly, S.L. (1997), "Itraconazole resistance in *Aspergillus fumigates",* Antimicrobial Agents and Chemotherapy, Vol. 41, pp. 1364-1368.

[8] Ellof, J.N. (1998), "Which extract should be used for the screening and isolation of antimicrobial components from plants?", Journal of Ethnopharmacology, Vol. 60, pp.1-6.

[9] Eloff, J.N. (2004), "Quantifying the bioactivity of the plant extracts during screening and bioassay-guided fractionation", Phytomedicine, Vol. 11, No. 4, pp. 370-371.

[10] Enfert C. and Hube B., (2007), "*Candida:* Comparative and Functional Genomics", Caister Academic Press.

[11] George, L.K., Roberts, C.S., Menges, R.W. and Kalpan, W. (1957), "*Tricophyton mentagrophyte* infection on dogs and cats", Journal of American Veterinary Medical Association, Vol. 130, No. 10, pp. 427-432.

[12] Gopinath, S.M., Suneetha, T.B. and Mruganka, V.D. (2011), "Chemical prophylaxis and antibacterial activity of methanolic and aqueous extracts of some medicinal plants against bovine mastitis", International Journal of Advanced Biological Research, Vol. 1, No.1, pp. 93-95.

[13] Haniffa, M.A. and Kavitha, K. (2012), "Antibacterial activity of medicinal herbs against the fish pathogen Aeromonas hydrophila", Journal of Agricultural Technology, Vol. 8, No. 1, pp. 205-211.

[14] Hedayati, M.T., Pasqualotto, A.C., Warn, P.A., Bowyer, P. and Denning, D.W. (2007), "*Aspergillus flavus*: human pathogen, allergen and mycotoxin producer", Microbiology, Vol. 153, pp. 1677-1692.

[15] Houck, H.E., Cooley, J.E., Lowitt, M.H. and Kao, G.F. (1996), "Tinea caput medusa; an unusual presentation of *Tricophyton mentagrophyte* on the scalp", Cutis, Vol. 58, pp. 48-52.

[16] Hussain, I., Khattak, M.U.R., Ullah, R., Muhammad, Z., Khan, N., Khan, F.A., Ullah, Z. and Haider, S. (2011), "Phytochemicals screening and antimicrobial activities of selected medicinal plants of Khyberpakhtunkhwa Pakistan", African Journal of Pharmacy and Pharmacology, Vol. 5, No. 6, pp. 746-750.

Table 1: Antifungal activity of extracts of *Nerium oleander* Linn. against some pathogenic fungi

Plant part	Extract	Microorganisms					
		C. albicans		*A. flavus*		*T. mentagrophyte*	
		IZ	AI	IZ	AI	IZ	AI
Leaf	A1	-	-	-	-	-	-
	S1	-	-	-	-	-	-
	E1	-	-	-	-	-	-
	E2	7	0.7±0.01	7	0.16±0.01	-	-
Stem	A2	-	-	-	-	-	-
	S2	10	0.34±0.02	-	-	-	-
	E1	8	0.8±0.01	7	0.16±0.01	-	-
	E2	7.5	0.75±0.01	-	-	-	-
Root	A3	-	-	-	-	-	-
	S3	8	0.8±0.01	7	0.17±0.01	-	-
	E1	10	1±0.02	-	-	-	-
	E2	7	0.7±0.01	-	-	-	-
Flower	A4	-	-	-	-	-	-
	S4	12	0.4±0.02	7	0.17±0.01	-	-
	E1	7.5	0.62±0.01	10	0.22±0.02	-	-
	E2	9	0.75±0.01	-	-	-	-

A1, A2, A3, A4 = Alkaloid extract of respective plant parts, S1, S2, S3, S4 = Steroid extract of respective plant parts,
E1, E2= Free & Bound flavonoids of respective plant parts, IZ=Inhibition zone in mm (value: including 6mm diameter of disc),
AI= Activity index (IZ developed by extract/IZ developed by standard), (-)= no activity, ±=SEM.

Table 2: MIC and MFC of active extracts of *Nerium oleander* Linn. against some pathogenic fungi

Plant parts & Extracts		Leaf				Stem				Root				Flower			
Microorganisms	**MIC & MFC**	**A1**	**S1**	**E1**	**E2**	**A2**	**S2**	**E1**	**E2**	**A3**	**S3**	**E1**	**E2**	**A4**	**S4**	**E1**	**E2**
C. albicans	MIC	-	-	1.25	-	-	0.625	1.25	1.25	-	1.25	0.625	1.25	-	0.312	1.25	0.625
	MFC	-	-	0.625	-	-	0.312	0.625	0.625	-	0.625	0.312	0.625	-	0.156	0.625	0.312
A. flavus	MIC	-	-	-	1.25	-	-	1.25	-	-	-	-	-	-	1.25	0.625	-
	MFC	-	-	-	0.625	-	-	0.625	-	-	-	-	-	-	0.625	0.312	-
T. mentagrophyte	MIC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	MFC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

A1, A2, A3, A4 = Alkaloid extract of respective plant parts, S1, S2, S3, S4 = Steroid extract of respective plant parts, E1, E2= Free & Bound flavonoids of respective plant parts, MIC= Minimum inhibitory concentration, MFC=Minimum fungicidal concentration, (-) = no activity.

Table 3: Quantity & Total activity of extracts of *Nerium oleander* Linn.

Plant part	Extract	Quantity of extract mg/g dwt	Total Activity(ml/g)		
			C. albicans	*A. flavus*	*T. mentagrophyte*
Leaf	A1	99	-	-	-
	S1	17	-	-	-
	E1	1.5	1.2	-	-
	E2	3.5	-	2.8	-
Stem	A2	90.5	-	-	-
	S2	13	20.8	-	-
	E1	3	2.4	1.92	-
	E2	1.5	1.2	-	-
Root	A3	33.5	-	-	-
	S3	2	1.6	1.6	-
	E1	1	1.6	-	-
	E2	1.5	1.2	-	-
Flower	A4	47	-	-	-
	S4	19.5	62.5	15.6	-
	E1	3.5	2.8	5.6	-
	E2	6	9.6	-	-

A1, A2, A3, A4 = Alkaloid extract of respective plant parts, S1, S2, S3, S4 = Steroid extract of respective plant parts,

E1, E2= Free & Bound flavonoids of respective plant parts, TA= total activity (extract per gm dried plant part/MIC of extract).