Leena Das et al. / Journal of Pharmacy Research 2015,9(7),476-479

Research Article
ISSN: 0974-6943

Available online through
www.jprsolutions.info

Antifungal and Phytochemical Analysis of *Lantana Camara*, *Citrus Limonum* (Lemon), *Azadirachta Indica* (Neem) and *Hibiscus Rosasinensis* (China Rose)

Leena Das[1] and **Suchitra Godbole**[2*]

[1]*Project Fellow, CSIR-National Environmental Engineering Research Institute (NEERI), Nehru Marg, Nagpur, Maharashtra,India.*
[2]*Department of Microbiology, Modern College of Arts,Commerce and Science, Ganeshkhind, Pune, Maharashtra,India.*

Received on:27-05-2015; Revised on: 17-07-2015; Accepted on: 08-08-2015

ABSTRACT

Lemon, Neem, Lantana Camara and China rose have been important herbs in the ayurvedic and indigenous therapeutic system. The present study was designed to investigate the preliminary phytochemical analysis and evaluation of antifungal activities of the ethanolic, methanolic and aqueous extracts of these four plants against the two fungal organisms.To evaluate their application as antifungal agents, leaves of the selected plants were used for preparing three types of extracts such as aqueous extract, ethanolic and methanolic extracts and evaluated for their in-vitro anti fungal activity against *trichophyton rubrum* and *candida albicans*. The extracts were also subjected to preliminary phytochemical analysis. The ethanolic extracts showed high antifungal activity against the test organisms compared to methanolic extracts. Aqueous extracts showed minimal antifungal activity against the test fungal pathogens. Preliminary phytochemical analysis revealed the presence of tannins, alkaloids, terpenoids, cardiac glycosides, saponins, phlobatanins and flavonoids. Results obtained in this study reveals that alkaloids, flavonoids, terpenoids, glycosides and tannins are present with high intensity in ethanolic and methanolic extract.The present study suggests that appropriate bioactive compounds may be extracted from leaves of these selected plants and used as an alternate to antibiotics.

KEYWORDS: Antifungal agent, Zone of inhibition, Lemon, *Lantana Camara*, China rose, Neem, Phytochemicals.

INTRODUCTION:

Even though pharmaceutical industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased and is an ever increasing problem. In general microorganisms such as fungi and bacteria have the genetic ability to obtain and transmit resistance to drugs which are utilized as therapeutic agent. Such a fact is cause for concern, because of the number of patients in hospitals who have suppressed immunity[1]due to new fungal strains which are multidrug resistant. Medicinal plants are known to owe their curative potentials to certain biologically active substances which exist in parts of the plant[2]. The phytochemicals present in plants are tannins, flavonoids, cardiac glycosides, terpenoids, phlobatanins and saponins. Flavonoids act as cytoplasmic poisons and also inhibit the activity of enzymes.[3]. Highly lipophilic flavonoids may also interrupt microbial membranes[4]. Tannins react with proteins to give tanning effect which is important for the treatment of inflamed cells.[5]

***Corresponding author.**
Suchitra Godbole
Department of Microbiology,
Modern College of Arts,
Commerce and Science, Ganeshkhind,
Pune, Maharashtra,India.

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decades, with more concentrated studies for natural therapies. According to WHO medicinal plants would be the best source to obtain a variety of drugs.[6] About 80% of the individuals from developed countries use traditional medicines, which has compounds derived from medicinal plants. Plants have been used to treat humans as well as animals. Therapeutic efficacies of many indigenous plants for many disorders have been described by practitioners of traditional medicines. Neem, lantana camara, lemon and hibiscus grows widely in India and other countries and used for the treatment of scurvy, ringworm ,athletes' foot, skin disorders and scalp disorders. Medicinal plants have been used in traditional treatment of skin diseases world wide.[7] In India herbal medicines have been the basis of treatment and cure for various diseases in traditional methods practiced such as ayurveda, unnani and siddha.[8] The ethano-botanical data of these plants prompted an investigation into its antimicrobial activity.

Candida albicans and *Trichophyton rubrum* can cause irritating superficial oral and vaginal infections, invasion of skin and nails (onychomycosis)[9]. Many of the fungi that cause disease in humans grow as free living mycelia in the environment but convert to yeast morphology once inside the human body in response to the raise in temperature. Collectively known as dimorphs, this group is able to cause severe and sometimes fatal infection.

Trichophyton rubrum causes tinea cruris of the groin and tinea pedis of feet and tinea corporis of arms, legs and trunk. *Trichophyton rubrum* cause tinea unguium of finger nails and toe nails[10].

Candida albicans infect the skin, nails, mucous membranes and gastrointestinal tract. In normal populations, superficial skin candidiasis occurs because of a combination of deficiency of skins barrier and an endogenous yeast behavior.

Therefore the present study was planned to determine the phytochemicals present in the plants and the antifungal potential of aqueous and alcoholic (ethanolic and methanolic) extracts of lantana camara, lemon, neem and hibiscus leaves for their efficacy against various pathogenic fungal strains.

MATERIALS AND METHODS:

Microbial cultures:

Fungal strain of *Trichophyton rubrum* (296) was obtained as standard cultures from Microbial Type Culture Collection, (MTCC), Chandigarh and *Candida albicans* was obtained from Government Medical College, Nagpur. The cultures were maintained on Sabourauds Dextrose Agar (SDA) slants at 4° C., and were subcultured at monthly intervals.

Preparation of extracts:

Lantana Camara, Neem, Lemon, Hibiscus leaves were collected from various botanical gardens and parks in Nagpur. The plant material was washed under running tap water; shade dried and powdered using mechanical grinder.

Alcoholic and aqueous extract:

Aqueous and Alcoholic (ethanolic and methanolic) plant extract were prepared by cold maceration method. 10g of dried plant part of Lemon, Neem, Lantana Camara, and Hibiscus was added to 100 ml of distilled water for aqueous extract and to ethanol and methanol for alcoholic extract and then placed in orbital shaker at room temperature at 110 rpm for 6 days. After 6 days the extracts were centrifuged and the supernatants were concentrated by evaporation in water bath at 40-50^{0}c. To the dried supernatant required amount of water was added to aqueous extract and DMSO was added to ethanolic and methanolic extracts[11].The extracts were stored in refrigerator at 4^{0}c for further use to detect antifungal activity.

Preliminary phytochemical analysis:

The preliminary phytochemical screening was followed by medicinal chemistry [12, 13, 14].

Tests for tannins and phenolic compounds: About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then was filtered. A few drops of 0.1% $FeCl_3$ was added and observed for brownish green or a blue-black coloration.

Test for alkaloids:

Dragendorffs test:

To 2 mg of the ethanolic extract, 5 ml of distilled water was added, 2M HCl was added until an acid reaction occurred. To this 1 ml of Dragendorff's reagent was added. Formation of orange or orange red precipitate indicated the presence of alkaloids.

Tests for detection of terpenoids:

(Salkowski test)

5 ml of each extract was mixed in 2 ml of chloroform, and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

Tests for saponins: About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and was filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth formation. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Tests for flavonoids: Three methods were used to determine the presence of flavonoids in the plant sample. 5 ml of dilute Ammonia solution was added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2SO_4. A yellow coloration observed in each extract indicated the presence of flavonoids. The yellow coloration disappeared on standing.

Tests for phlobatanins: -Aqueous and alcoholic extract of plant material was boiled with 1% aqueous HCL. The sample was observed for the formation of red precipitate which indicates the presence of phlobatanins.

Tests for cardiac gylcosides:

(Keller-killani test)

5 ml of plant extracts was treated with 2 ml of glacial acetic acid containing one drop of $FeCl_3$ solution. This was underlayed with 1 ml of concentrated H_2SO_4. A brown ring of the interface indicates a deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Determination of antifungal activity:

The antifungal screening was done by agar well diffusion method for the three plant extracts (Aqueous, ethanolic and methanolic) of lemon, Neem, lantana camara and hibiscus against the two dermatophytes namely *(Trichophyton rubrum and Candida albicans).*Plant extracts were dissolved in suitable solvent and then aqueous, ethanolic and methanolic plant extracts of four different plants with different concentration (10%, 20% 30%), were prepared. Sabouraud's dextrose agar (SDA) was used for the agar well diffusion method. The culture medium was inoculated with the fungal strains separately suspended in Sabourauds dextrose broth. A total of 8 mm diameter wells were punched into the agar and filled with plant extracts and solvent blanks (Distilled water and DMSO). Standard antibiotic (Fucanazole, concentration 1 mg/ml) was used as positive control and fungal plates were incubated at 30°C for 72 h. The diameters of zone of inhibition observed were measured.

RESULTS AND DISCUSSIONS:

Preliminary phytochemical analysis of aqueous, ethanol and methanol extract of leaves of neem, lantana camara, and lemon revealed the presence of flavonoids, saponins, alkaloids, terpenoids, phlobatanins, tanins and glycosides in extracts. The antifungal activity of lemon, neem and lantana camara can be attributed to the action of phytochemical compound it contains. The phytochemicals which are present in plants after extracting with required solvent are presented in the Table no (1). Mostly every bioactive compound was present in the respective three plants namely neem, lantana camara and lemon. The result of the antifungal screening of plant leaves revealed that ethanol and methanol extract represent a broad spectrum of activity. All extracts showed varying degrees of inhibition on test microorganisms. The ethanolic extract of lantana camara showed the most inhibition potential against the two dermatophytes namely *trichophyton rubrum* and *candida albicans* followed by ethanolic extract of neem and lemon. The methanolic extract of lantana camara , neem and lemon also showed satisfactory inhibition against the two test organisms. The methanolic and ethanolic extract of hibiscus showed the least inhibition against the two test organisms among the four plants. The aqueous extract had least activity against all test organisms. The antifungal activity of the extracts could be explained by presence of phenolic compounds, flavonoids, saponins, tannins, phlobatanins, glycosides and alkaloids. The mechanism of action of alkaloids is based on their ability to bind proteins thereby inhibiting cell protein synthesis. Tannins has been found to form irreversible complex with prolimerith protein leading to inhibition of protein synthesis.[15]

Zone of inhibition of 30% ethanolic extract of lantana camara, neem , lemon was the most effective against the two test organisms followed by 20% and 10% of lantana camara, neem, lemon.

The extract of hibiscus plant prepared in different solvent was found negligible against the two dermatophytes. No zone of inhibition was shown.

The data presented in Table (2) clearly indicate that alcoholic extracts of lantana camara, neem and lemon are most effective to treat fungal infections caused by T*richophyton rubrum* and C*andida albicans*.

Table 1: Phytochemicals present in different plant extracts prepared in three different solvents.

Phytochemical	Aqueous extract				Ethanol extract				Methanol extract			
	Lemon	Neem	Lantana camara	Hibiscus	Lemon	Neem	Lantana camara	Hibiscus	Lemon	Neem	Lantana camara	Hibiscus
Tannins	+	+	+	NIL	+	+	+	NIL	+	+	+	NIL
Glycosides	+	+	+	NIL	+	+	+	NIL	+	+	+	NIL
Terpenoids	+	+	+	NIL	+	+	+	NIL	+	+	+	NIL
Saponins	+	+	+	NIL	+	+	+	NIL	+	+	+	NIL
Flavonoids	+	+	+	NIL	+	+	+	NIL	+	+	+	NIL
Alkaloids	+	+	+	NIL	+	+	+	NIL	+	+	+	NIL
Phlobatanins	+	+	+	NIL	+	+	+	NIL	+	+	+	NIL

Table 2: Zone of inhibition produced by the three medicinal herbs prepared in different extract against the two dermatophytes.

FUNGAL STRAINS	CONCENTRATION	PLANT NAME	ZONE OF INHIBITION(mm)		
			AQUEOUS EXTRACT	ETHANOL EXTRACT	METHANOL EXTRACT
TRICHOPHYTON RUBRUM	10%	LEMON	0	13	10
		NEEM	0	11	10
		LANTANA CAMARA	0	12	10
	20%	LEMON	0	13	10
		NEEM	10	11	10
		LANTANA CAMARA	10	12	10
	30%	LEMON	0	10	8
		NEEM	8	12	10
		LANTANA CAMARA	8	13	12
CANDIDA ALBICANS					
	10%	LEMON	0	10	8
		NEEM	0	13	10
		LANTANA CAMARA	0	16	10
	20%	LEMON	0	8	0
		NEEM	0	13	0
		LANTANA CAMARA	0	13	12
	30%	LEMON	0	10	8
		NEEM	7	12	8
		LANTANA CAMARA	0	15	12

CONCLUSIONS:

Hence from the present study it was found that, among all the four plants Lantana camara extract which was prepared in ethanol showed the presence of alkaloids, glycosides, tannins, saponins, flavonoids with high intensity and was the most efficient against the two dermatophytes (*Trichophyton rubrum* and *Candida albicans*) than Neem, Lemon and Hibiscus with the increase in concentration. Thus the use of these plants in the treatment of pathogenic diseases associated with the infection of these pathogens is validated and can be exploited further for controlling these infections.

ACKNOWLEDGEMENT:

The authors are thankful to Department of Microbiology and Biotechnology, G.H. Raisoni Institute of Information Technology, Nagpur, Maharashtra for providing the facilities for successfully completing the research work.

REFERENCES:

1. Pinto E, Pina – Vaz C. ,SalgueiroL. et al. (2006), Antifungal activity of the essential oils of thymus pulegioides on candida,aspergillus and dermatophyte species. *J med microbiol*, 55, 1367-73.
2. Epko M.A and Etim P.C. (2009), Antimicrobial activity of ethanolic and aqueous extract of sida acuta on microorganisms from skin infections *J med plants Res* 3(9); 621-624.
3. Dhatak and Iwu,(1991). Inhibition of xanthine oxidase activity by some flavonoids, *Fitoterapia* 63; 385.
4. Tsuchiya H., Sato M., Miyazaki T., Fuziwares and Fangaki S., OhyamaM. TanataT. And Linuma M. (1996), Comparative study on the antibacterial activity of phytochemical flavonones against methicillin resistant Staphylococuss aureus, *J.ethnopharmacol*, 50;27-34.
5. Parekh and Chanda S(2007) , In-vitro antibacterial activity of crude Methanolic extract of wood fordia fruticosa flower, *Brazil J.microbial* 38:
6. Santos P.R.V. Opliveira A.C.X., Tomassini T.C.B. (1995) Control microbiogico de produtos fitoterapicos.*Rev .Farm. Bioquim* 31, 35-38.
7. Ballabh B. and Chaurasia O.P.(2007).Traditional medicinal plants of cold desert Ladakh- used in treatment of cold, cough and fever, *J.Ethnopharmacol*,112(2),341-349
8. Samy R.P, Puspharaj P.N and Gopalakrishnakone P.A,(2008).Compilation of bioactive compounds from Ayurveda, *Bioinformation* , 3,100-110
9. Vollekova A, Kostalova D, Kettmann V, Toth J.(2003).Antifungal activity of mahonia aquifolium extract and its major protoberberine alkaloids.*phytotherb Res* 17:834-37.
10. Marion Man-Ying Chan,(2002) Antimicrobial effect of resveratrol on dermatophytes and bacterial pathogens of the skin, *Biochemical pharmacology* 63,99-104.
11. Al janabiAAS(2011), Potential activity of the purine compounds caffeine and aminophylline on bacteria. *J.Glob Infect Dis* 3:133-37.
12. Sofowara A, (1982), Medicinal plants and traditional medicine in Africa,spectrum Books ltd ,Ibadan, Nigeria 193,p289.
13. Harborne J.B (1998), Analysis, 3rdedn. Phytochemical methods: A Guide to Modern Techniques of Plant Chapman and Hall, London.
14. Trease , G.E. and Evans .W.C.(2002)Pharmacognosy 12th (edn) Bailliere tindal , London p 622. Phyto chemicals.
15. Shimade .T (2006).Salivary protein as a defence against dietary tannins. *J CHEM ecol* 32(6): 1149-1163.

Source of support: Nil, Conflict of interest: None Declared