Research Article Dev *et al.*, 7(5): May, 2016:5030-5033]
CODEN (USA): IJPLCP **ISSN: 0976-7126**

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES
(Int. J. of Pharm. Life Sci.)

Antifungal Activity and Phytochemical Screening of *Lawsonia inermis* Leaves Extracts

Swami Narsingh Chandra Dev*, Kantishree De and Mohd Washid Khan
Department of Post-Graduate Studies and Research in Biological Science,
Rani DurgavatiVishwavidyalaya, Jabalpur, (MP) - India

Abstract

In the present study hexane, acetone, methanol and aqueous extracts obtained from *Lawsonia inermis* leaves were studied for their antifungal properties by using agar well diffusion assayagainst skin diseases causing fungi *viz.Candida parapsilosis, Candida albicans, Candida glabrata, Trichophytonrubrum, Cryptococcus neoformans* and *Aspergillus niger*. Methanol extract showed broad spectrum antifungal activityagainst all the tested pathogens. The phytochemical analysis of the methanolic extracts revealed the presence of terpenoids, flavonoids, glycosides, sterols and coumarins. TLC analysis exhibit best separation of different phytocostituents with the solvent system i.e. choloroform: ethanol.

Key-Words: Antifungal activity, Phytochemical, *Lawsonia inermis*

Introduction

In the series of Infectious diseases, human fungal infections are increasing due to increased immunocompromised patientsandexcess use of antibioticsresulting the development of resistance to commercial drugs. A number of metaboliteshave been shown to possess useful biological activities belonging mainly to phenolic compounds, flavonoids, fatty acids, alkaloids and terpenoids (Arif*et al*., 2009).

Lawsonia inermis, an evergreen glabrous shrub small tree, commonly known as menhadi and henna of the family Lythraceae. It is commercially cultivated in India for coloring hair and hands from thousands of years. It is also used as folkmedicinearound the world in various skin diseaseslike leprosy, scurvy, various skin and nail ailment, open wounds, ulcers, eczema and other fungal infections (Chaudhary*et al*., 2010). It makes necessary to discover natural antifungal compounds to cure fungal infections.

*** Corresponding Author**
E.mail: prof.ksde@gmail.com

Material and Methods

Plant material

The leaves of *L.inermis* were collected from Panagar block of Jabalpur (M.P.). These leaves were washed and shade-dried and then coarsely powdered by using electrical grinder. The powder was then subjected to extraction with organic solvents such as hexane, methanol and acetone by Soxhlet apparatus. Maceration method used for aqueous extraction (Ncube*et al*., 2008). The extracts were then evaporated on a water bath and the residue stored at 4˚C.The concentration (10 mg/ml) of extracts wasprepared for the bioassay and phytochemical screening.

Test organisms

All the microbial culture of human pathogens usedwere procured from Institute of Microbial Technology (IMTECH), Chandigarh. These microbes including *Candida albicans* (MTCC), *Candida glabrata* (MTCC),*Trichophytn rubrum* (MTCC), *Cryptococcus neoformans* (MTCC), *Candida parapsilosis* (MTCC), and *Aspergillu sniger* (MTCC 10188) respectively. The isolates were maintained on Sabouraud Dextrose Agar medium.

Antifungal activity

For this, Agar well-diffusion method (Perez *et al*., 2000) was followed. Four or five wells were prepared in the agar plates with the help of a cork-borer (6 mm) seeded with the test organism.60 µl of the extract (10 mg/ml) was introduced into the well separately. The plates were incubated at28°C for 48 h to 72 h.A clear zone referred as "zone of inhibition" develops around

the well if the organism is sensitive towards the test material.

Preliminary phytochemicals screening:

For qualitative phytochemical analysis of the selected extract werethe methodology of Kokate (2004) was followed.

Thin Layer Chromatography

The TLC was performed on glass TLCplates prepared by using silica gel G for TLC.After air drying andactivated in hot air oven at 110ºC for 1h.Methanolicextract was plotted on itand developed in different solvent systems and dried at roomtemperature (Table 2). Different bands wereobserved and Rf values were determined.

Results and Discussion

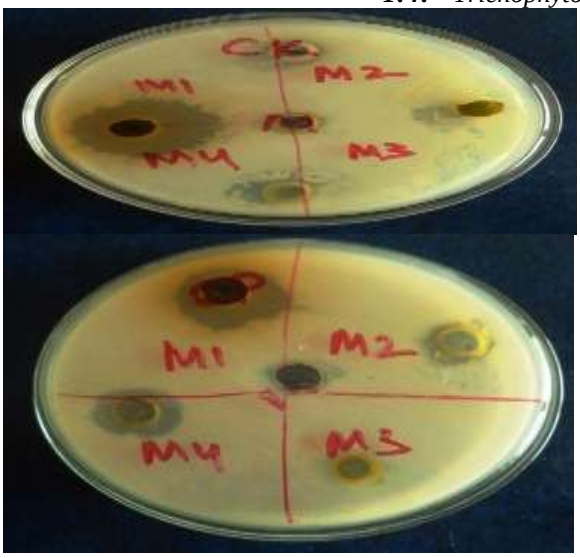
Antifungal potential

Among the different solvent extracts of *L. inermis* leaves, the broad spectrum activity was recorded in methanolic extract against all the test organisms with the maximum zone of inhibition against *C. krusie* (25 mm). Acetone extract showed activity only against *C.albicans* and *C. parapsilosis*. The hexane and aqueous extract did not show activity against any one of these pathogens. similar works were also done by (Mohammad *et al*., 2005; Mansour *et al*., 2012). Activity similar results in methanol and acetone extract were also obtained by Nair and Chanda (2007) in different plants.Varieties of antimicrobial compounds have been isolated from the methanolic extracts (Eloff, 1998; Cowan, 1999). Plant extracts in organic solvents have been found to have more antimicrobial activity in comparison to water extract (Ncube*et al*., 2008).However, negative results do not mean absence of bioactive constituents nor is that the extract is inactive. Active compound(s) may be present in insufficient quantities in these extracts to show activity with the dose levels employed (Taylor *et al*., 2001).

Table 1: Antifungal potential of leaves extract with different solvent against tested fungal pathogens

Solvent	*C. a*	*C. g*	*C. k*	*C.p.*	*A. n*	*C. n*	*T. r*
Hexane	-	-	-	-	-	-	-
Acetone	17.33±0.57	-	10.66±0.57	-	-	-	-
Methanol	18.00±0.00	22.66±0.57	25.66±1.15	20.0±1.00	18.00±1.00	17.00±0.00	10.66±0.57
Water	-	-	-	-	-	-	-
Fluconazole(+)	23.00±1.73	25.00±0.00	27.33±1.15	22.66±0.57	20.00±1.00	28.00±0.00	20.00±1.00

The abbreviated words are given respectively as ***C. a***. =*Candida albicans*, ***C.g.***= *Candida glabrata*, ***C.k***.= *Candida krusie*, ***C.p***.= *Candida parapsilosis*, ***C. n***. = *Cryptococcus neoformans* , ***A. n.*** = *Aspergillus niger*, ***T. r.*** =*Trichophyton rubrum* , **F**=Fluconozole.



(A) **(B)**

Fig. 1: Antifungal activity of methanolic extract (M1) against *C.krusie* (A) and *C. parapsilosis* (B)

Phytochemicalscreening

A significant part of the chemical diversity of plants is to protect them against microbialpathogens. Hence, they have been proven to have antimicrobial importance both *in vitro* and *in vivo*(Stuart, 2002) .Preliminary phytochemical screening revealed the presence of tannins, flavonoids, terpenoids, glycosides, fatty acids and sterols.

Thin Layer Chromatography

The solvent system content chloroform: ethanolin a ratio of 95:5 yielded a number of spots on TLC, followed by solvent systemi.e. chloroform: ethanol: acetic acid(g1).(Table 2; Fig. 2).These data would therefore be suitable to find out thephytocostituents of the plant extract for further purification of active compounds.

Table 2: Solvent system used and their separation capacity

S/No	Solvent Systems used	No. of Spots separated	Remarks
a.	Toluene: Ethyl acetate (93:7)	1	Average
b.	n-Butanol: Acetic acid: Water (4:1:5)	-	Poor
c.	Ethyl acetate: Water: Methanol (100:10:13.5)	2	Poor
d.	Benzene: Acetic acid (9:1)	2	Poor
e.	Toluene: Ethyl acetate: Methanol: Acetic acid (3:4:3:1)	-	Poor
f.	Ethyl acetate: Isopropyl alcohol: Water (65:25:10)	-	Poor
g.	Chloroform: Ethanol: (95:5)	6	Very Good
g-1	Chloroform: Ethanol: (94:5:1)	4	Good
h.	ethanol:ethyl acetate (1:2)	1	Poor
i.	Ethyl acetate: Methanol (3:7)	1	Poor

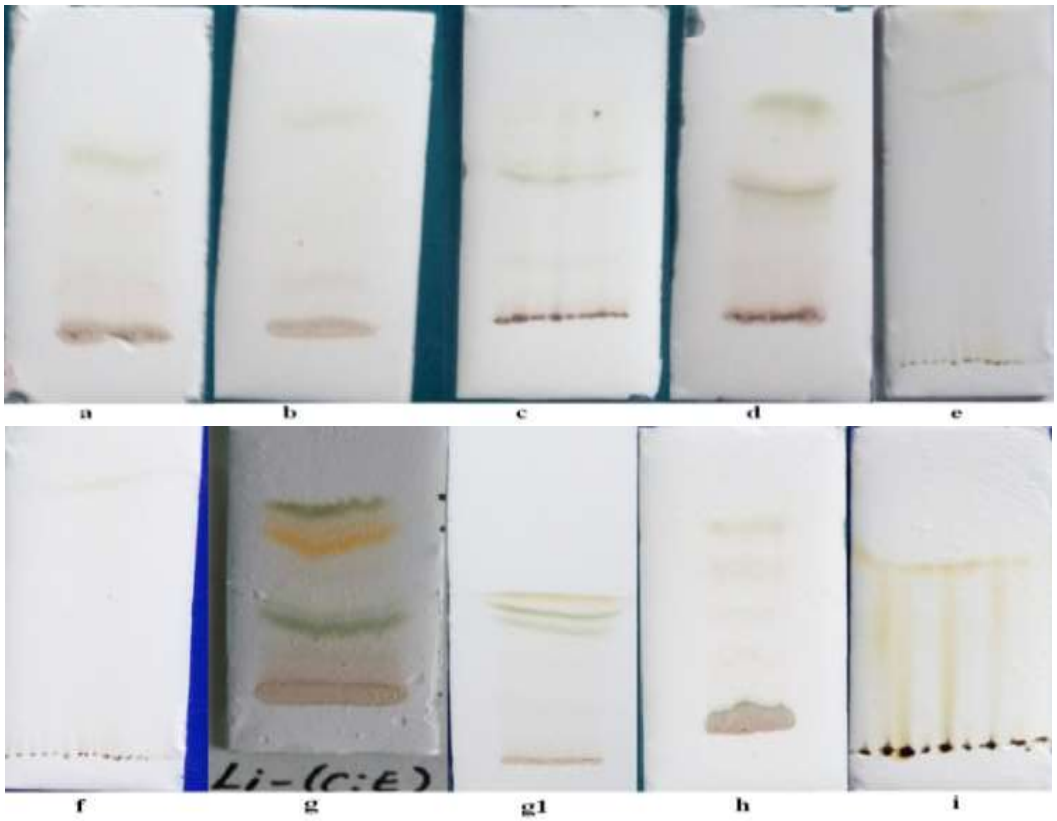


Fig. 2: TLC of methanolic extract of *L.inermis* in different solevent system

Table 3: TLC Profile of methanolic extract of *Lawsonia inermis* in solvent system (chloroform: ethanol)

S.No.	Rf value	Color of Spot in Visible light
1)	0.62	Dark green
2)	0.74	Yellow
3)	0.81	Light brown
4)	0.89	Parrot green
5)	0.91	Light brown
6)	0.94	Light yellow

The TLC profile of methanol extract of *L. inermis* showed six spots with solvent system of chloroform: ethanol (95:5) with Rf values from 0.62 to 0.94. The poor separation was observed in other solvent systems used. The results of phytochemical screening revealed the presence of terpenoids, flavonoids, tannins, fatty acids and sterols which may be the cause of antifungal activity.The above results suggest that *L.inermis*couldbe an important source of non-polar compounds with antifungal activity.

Conclusion

The study concludes that the *L. inermis* have potential antifungal activity against tested fungal pathogens. Hence this study may help to separation and purification of the active constituents to prove various biological and anti microbial activity for further scientific investigation.

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How to cite this article

Dev S.N.C, De K. and Khan M.W. (2016). Antifungal Activity and Phytochemical Screening of *Lawsonia inermis* Leaves Extracts. *Int. J. Pharm. Life Sci.*, 7(5):5030-5033.

Source of Support: Nil; Conflict of Interest: None declared

Received: 04.04.16; Revised: 14.04.16; Accepted: 10.05.16