Plant Archives Vol. 21, Supplement 1, 2021 pp. 23-27 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
doi link : https://doi.org/10.51470/PLANTARCHIVES.2021.v21.S1.006

EFFECT OF LEAF EXTRACTS OF *LAWSONIA INERMIS* LINN. ON *ALTERNARIA ALTERNATA* CAUSED LEAF SPOT DISEASE OF CHILLI

Sanjeev Meena*, Bhanu Raj Meena, Tripta Jain and Kanika Sharma
Microbial Research Laboratory, Department of Botany,
M.L.S. University, Udaipur Rajasthan, India
*Email: meenasanjeev141@gmail.com

ABSTRACT

In this study antifungal activity of crude and partially purified extracts of *Lawsonia inermis* Leaf extract has been assayed against *Alternaria alternata* which is responsible for leaf spot disease of chilli. Cold and hot extraction of leaf extract was prepared in different organic solvents, which were subsequently recycled by rotary vacuum evaporator. Antifungal activity of prepared plant extracts were determined by poison food technique. In cold extraction highestpercent extractive value was obtained with 50% alcohol extract and in hot extraction highest percent extractive value was obtained with Methanol extract. In antifungal activity 100% alcohol crude extract and partial purified acetone extract was show Maximum inhibition of *Alternaria alternata*. Mancozeb and water were used as standards. Results suggest that *Lawsonia inermis*. Leaf extract can be used to develop a bio control agent against *Alternaria alternata*. *Lawsonia inermis* plant can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals research activities.

Keywords: Antifungal activity,Plant extract, Antifungal activity, Herbal-Bioformulation, Phytochemicals, Fungicides

Introduction

Diverse types of bioactive compoundsare produced by a large numberof plants which are known to have antimicrobial properties. These bioactive compounds are also known as secondary metabolites. For the development of herbal formulation from any plant presence of significant phytochemicals is a prime importance (Mustafa *et al.,* 2017). These phytochemicals involves phenols, flavonoids, quinones, essential oils, alkaloids, sterols, thymol, coumarines and triterpenoids are untapped reservoirs of several valuable chemicals (Meena and Sharma, 2019). Herbal plant formulations have preventive effect against plant pathogenic microbes. Research need to be focused on screening of plant extracts for antimicrobial activity in search of novel compounds which can be used to control fungal diseases of plants (Savoia, 2012).

In the last decade, studies based on extraction of biologically active compounds from plant species used for medicinal purposes are intensively increased (Briskin, 2000). Several workers have been used crude extracts for determining antimicrobial activity (Srinivasan *et al.,* 2001; Kalaivani *et al.,* 2012; Klančnik *et al.,* 2010).

In hot extraction method, dried powdered plant material boil with series of solvent ranging from non polar to polar in nature (Harborne, 1984; *Kokate et al.,* 1990). Grinding of plant material resulted in coarse smaller samples leading to better surface contact with extraction solvents. This particular pre-preparation is important, as for efficient extraction to occur; the solvent must make contact with the target analytes and particle size smaller than 0.5 mm is ideal for efficient extraction (Azwanida, 2015). Different polarity of solvents play important role in extraction of active molecules of different nature. Thus, prepared extract results into partial purification of plant extract hence known as partially purified extract. Several researches have used hot extraction method for extraction of active molecules (Dai *et al,*. 2010; Harvey *et al.,* 2015; Al-Mansou*et al.,* 2014).

Prepared crude and partially purified extract contained active molecules present in particular plant material hence suitable to detect antimicrobial activity against different plant and human pathogenic microorganisms. Most suitable methods of detection of antimicrobial activity against plant pathogenic fungus are “Poison food technique.

There are several reports available on antifungal activity of plant extract against plant pathogenic fungi (Mahomoodally *et al.,* 2005) checked the anti-microbial and anti-fungal activity of different solvent extracts of *Acalypha indica* (*Euphorbeace* family) was tested against bacterial pathogens *E.coli*, *P. aeruginosa*, *K. Pneumonia* and *Staphylococcus aureus* and fungal strains *Candida albicans, Aspergillus mniger, Candida tropicalis* and *Candida kefyr*.(Locher *et al.,* 1995; Barman, 2010) The methanol extract was then subsequently fractionated using n-hexane, diethyl ether, and ethyl acetate respectively. The diethyl ether fraction was found to have the highest inhibitory activity against *T. versicolor* (46.30%) and *P. chrysosporium* (81.11%). (Elfirta *et al.,* 2018)

Hence in the present work crude as well as partially purified extract of *Lawsonia inermis*was prepared by cold and hot extraction method respectively. Crude and

fractionized partially purified extract checked for antifungal activity against test fungus.

Materials And Method

Crude and Partially Purified Extracts Preparations

The fresh leaves of *Lawsonia inermis* plantwere collected from the campus of University College of science M.L.S.U. Udaipur. The plant was submitted and identified at Herbarium of Department of Botany, University of Rajasthan, Jaipur, India as *Lawsonia inermis* Linn. and given voucher specimen number RUBL211751. The collected plant material was shade dried at room temperature. It was then ground in an electrical grinder. The ground material was passed through a sieve of mesh with size 60 to obtain a fine powder. It was then used to prepare the extract. Crude extract was prepared according to the cold extraction method (Shadomy and Ingraff, 1974). Cold extraction was prepared in water, 50% hydro alcohol and absolute alcohol. 20 gm of dried and powdered plant material was suspended in 100 ml water, a mixture of 50 ml water and 50 alcohols and 100 ml absolute alcohol separately, and kept for 48 hrs. After 48 hrs each mixture was filtered through Whatman filter paper no.1 and filtered material was vacuum dried using rotary vacuum evaporator. The dried residue was used as extract and solvent was recycled.

Percent Extractive Value

The dried extracts were weighed and their percentage in term of the dried weight of the plant material was determined by following formula:

$$\text{Percent extractive} = \frac{\text{Weight of dried extract}}{\text{Weight of dried plant material}} \times 100$$

Antifungal Activity of Crude and Partially Purified Fraction of *Lawsonia inermis*Linn. Leaf extract

Poison food technique (Groover and Moore, 1962) was used for the examining the antifungal activity of crude and partially purified fractions of *Lawsonia inermis* Linn. leaf extract. In this respect 100 mg of extract was dissolved in 10 ml acetone to prepare stock solution of 10mg/ml concentration.

9 ml molten sterile PDA culture medium was mixed with 1 ml of stock solution and this mixture was poured into pre-sterilized petri-plates (9 cm diameters) and allowed to solidify at room temperature. Petri-plates prepared in this way were inoculated aseptically with 6 mm disc of test pathogen's cultures. The petri-plates were then incubated at 28±2° C for seven days. Mancozeb, Bavistin, and only PDA culture media were used as control series along with test samples. Antifungal activities of extracts were measured as a function of increasing growth of 6 mm disc of each inoculum.

After seven day of incubation the average diameter of the fungal colonies was measured and mycelial growth in percentage was calculated by the following formula:

$$\text{Mycelial growth inhibition} = \frac{gc - gt}{gc} \times 100$$

gc= growth of mycelia colony after 7 days incubation period in control set subtracting the diameter of inoculum disc.

gt= growth of mycelia colony after 7 days incubation period in treatment set subtracting the diameter of inoculum disc.

Results

Table no. 1.1 and 1.2 depicted the results of percent extractive value of crude and partially purified fractions of leaf of *Lawsonia inermis* In case of crude extract preparations, highest percent extractive value of 19.75% was obtained with 50% hydro alcoholic extract which is followed by 100% alcoholic and 100% aqueous extract i.e. 16.25% and 10.10% respectively.

For percent extractive values of partially purified fractions, maximum value 18.25% of percent extractions observed for methanol extract and least value of 1.08% obtained with chloroform fraction. Whereas, other fractions like petroleum ether, benzene, acetone, and water fractions showed intermediate values of percent extractions i.e. 4.72%, 1.58%, 9.86%, and 4.8% respectively.

In order to check antifungal activity of crude and partially purified extract, extracts were dissolved in inert solvent i.e. acetone. It was observed to be neutral for growth of test fungus. Results of antifungal activity of crude as well as partially purified extracts were given in table no 1.3 and 1.4 respectively (Fig 1.3 A and 1.3 B). Inhibitory activity of standards and control tabulated in table no 1.5 (Fig 1.3 A). In case of crude extract, 100% alcoholic extract was observed to be best which showed 59.73% inhibition followed by 50% hydroalcoholic and aqueous extract which showed percentage inhibition of 47.08 % and 46.35 % respectively.

Among the partially purified fractions obtained, maximum mycelia growth inhibition of 71.89% was observed with acetone partially purified fraction of *Lawsonia inermis* leaf extract.

Discussion

Phytochemical screening of this plant extract reveals the presence of cardioglycosides, terpenoids, carbohydrates, proteins, phenols, quininesandtannins. Occurrence of these secondary metabolites is accountable for antimicrobial action of this plant (Gonzalez *et al.,* 2009; Singh *et al.,* 2015). There various techniques /methods are used to extraction of active ingredient from plant materials like leafs, seeds etc. (Figueiredo *et al.,* 2008; Bhatla 2018). Inhibitory activity of plant extract reasonably due to the presence of bioactive compounds specifically known as secondary metabolites. These metabolites might be interfering with metabolic activity and growth of test fungus by modulating the associated signal transduction pathways. The extraction of secondary metabolites can be done using cold or hot extraction method. Many researchers have been used cold or hot extraction method for successful expression of secondary metabolites (Grigoletto *et al.,* 2019; Chingwaru *et al.,* 2020; Darout *et al.,* 2000 Haslam, 1996). In the present study we were used *Lawsonia inermis* leaf extract to evaluate that antifungal activity against *Alternaria alternata* which caused leaf spot disease of chilli. It is interesting to know that out of crude extract, 100% alcoholic extract possess highest inhibitory activity against *Alternaria alternata.* These results hypothesized that plant extract of *Lawsonia inermis* leaf contain active ingredient which inhibited the growth of test fungus. There are many researcher have investigated antimicrobial active compound which decrease the size of

colony of fungus. Poison food technique is used commonly. This technique depends on the inhibition of microorganism's growth as an indication of sensitivity and is measured as a function of % inhibition. Several workers have used poison food technique for studying sensitivity of micro-organism against plant extracts (Girish and Prabhavathi, 2019; Nane and Thapliyal, 2000). Antifungal effects of plant extracts can be due to existence of various phytochemicals that can act alone or in synergy to inactive or execute the microorganism (Mahizan *et al.,* 2019; Yousefi *et al.,* 2017). It has been proved that there is a important relationship between extract and active compounds (Mustafa *et al.,* 2010). There are several method which are used to evaluate antifungal activity of plant extract (Satish *et al.,* 2007). In support to this hypothesis many researcher found *Lawsonia inermis* has Pharmacological activities, namely anti-inflammatory, anti-arthritic, antiulcer, anticancer, antioxidant, antidiabetic and analgesic activities has been reported in *Lawsonia* plant (Cibin *et al.,* 2012; Nag *et al.,* 2015). *Lawsonia inermis* possess antiulcer activities, hepatoprotective anti-inflammatory (Tanna *et al.,* 2009) activities, hypoglycemic and antihyperglycemic (Zaker and Nosallanejad, 2010) activities, and antimicrobial activity (Mastanaiah *et al.,* 2011; Saadabi, 2007). So we can say that *Lawsonia inermis* is very important plant. The extract of leaves of *Lawsonia inermis* shows significant antifungal activity. The result indicate that this plant *Lawsonia inermis* can be used for developing the plant extract based Bio-formulation for effective control of leaf spot disease of chilli in eco-friendly way.

Conclusion

Plant-based formulations can successfully be used in agriculture to treat plant diseases and can limit the use of chemical control agents to safer levels. Thus use of plant extract against plant pathogenic fungus is an important field of study and will be a best alternative of existing chemical antifungals in the near future.

Table 1.1: Percent extractive value of crude extract of *Lawsonia inermis* leaf extract

S.No.	Type of Extract	% extractive value
1	100% alcohol	16.25
2	100% aqueous	10.10
3	50% alcohol	19.75

Table 1.2: Percent extractive value of different partially purified fractions of *Lawsonia inermis* leaf extract

S. No.	Type of extract	% Extractive value
1.	Petroleum ether fraction	4.72
2.	Benzene fraction	1.58
3.	Chloroform fraction	1.08
4.	Acetone fraction	9.86
5.	Methanol fraction	18.25
6.	Water	4.08

Table 1.3: Antifungal activity of crude extract of *Lawsonia inermis* leaf extract against *Alternaria alternata*

S. No.	Type of extract	Growth Diameter after 7 days (mm)	% Mycelial growth inhibition
1.	100% alcoholic	33.10±1.57	59.73
2.	50% alcoholic	43.50±0.60	47.08
3.	Aqueous	44.10±1.05	46.35
4.	Control	82.20±0.20	NIL

Table 1.4 : Antifungal activity of various purified fractions of *Lawsonia inermis* leaf extract against *Alternaria alternata*

S. No.	Type of extract	Growth Diameter after 7 days (mm)	% Mycelial growth inhibition
1	Petroleum ether	34.10±0.10	58.51
2	Benzene	30.33±0.31	63.10
3	Chloroform	28.33±0.18	65.53
4	Acetone	23.10±0.61	71.89
5	Methanol	49.67±0.89	39.57
6	Aqueous	42.10±0.55	48.78

Table 1.5: Antifungal activity of standard fungicides with water control *Alternaria alternata*

S. No.	Standard fungicides and water control	Growth Diameter after 7 days (mm)	% Mycelial growth inhibition
1.	Mancozeb	25.67±1.08	68.77
2.	Water (control)	82.20±0.20	No inhibition

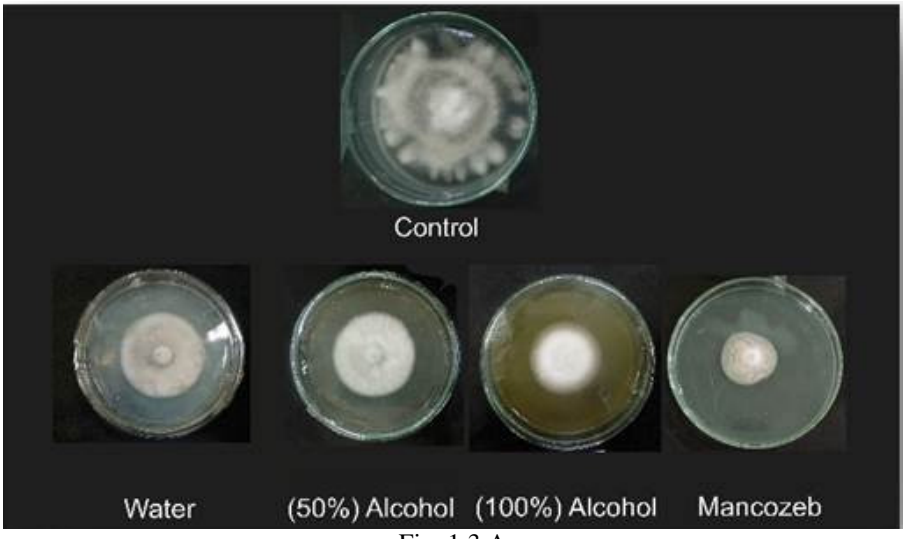

Fig 1.3 A

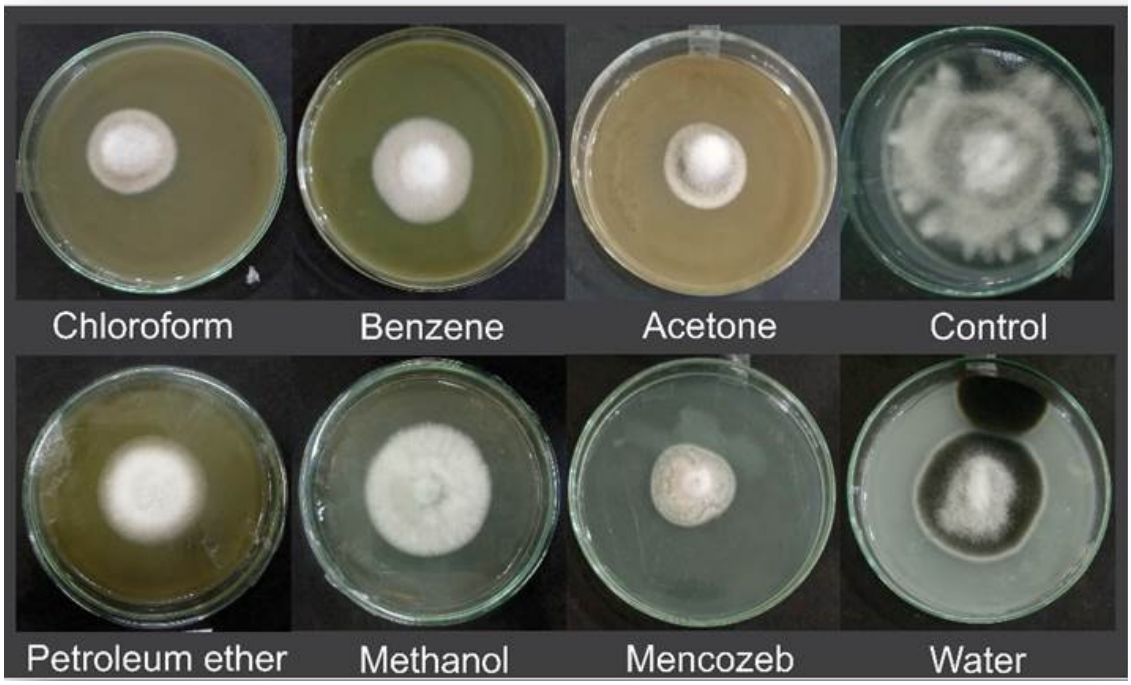

Fig 1.3 B

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