ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 08, pp.37539-37542, August, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

ANTITYPHOID ACTIVITY AND PHYTOCHEMICAL SCREENING OF DIFFERENT EXTRACTS OF *L. INERMIS* PLANT LEAVES

Ritesh Kumar Sharma, *Anjana Goel and A. K. Bhatia

Department of Biotechnology, IAH, GLA University, Mathura

ARTICLE INFO

Article History:
Received 16th May, 2016
Received in revised form
05th June, 2016
Accepted 16th July, 2016
Published online 31st August, 2016

Key words:

Antityphoid, *Lawsonia inermis*, Quinone.

ABSTRACT

Lawsonia inermis plant, generally identified as Henna, is a medicinal plant and leaves of this plant are used as dye from ancient time. The present study was conducted to study antityphoid activity and phytochemical screening of different extracts of *Lawsonia inermis* leaves. Methanol extract showed highest inhibition zone (13.74±1.52) at 20mg/disc and lowest inhibition zone (8±1) was demonstrated at 5mg/disc of hexane extract. Phytochemical screening of different extract revealed the presence of various phytoconstituents such as flavanoids, alkaloids, carbohydrates etc. Quinone is the main phytoconstituents which is responsible for antityphoid activity and presence of this compound was confirmed in all extracts while protein was absent in all extract of *Lawsonia inermis* plant leaves.

Citation: Ritesh Kumar Sharma, Anjana Goel and A. K. Bhatia, 2016. "Antityphoid activity and Phytochemical screening of different extracts of *L. inermis* plant leaves", *International Journal of Current Research*, 8, (08), 37539-37542.

INTRODUCTION

Some pathogenic microbes cause various human diseases and for prevention of these diseases discovery of antibiotics occurred in 20th century. But these antibiotics are not much efficient as some substances are capable of changing the target site of antibiotics in turn developing the resistance against antibiotic drugs (Ali *et al*., 1995). Diseases like gonorrhoea, typhoid, malaria, tuberculosis cannot be easily treated with antibiotics as they have developed drug resistance (Muhammad and Muhammad 2005, Medalla *et al*., 2011). *Lawsonia inermis,* commonly known as Henna or Mehandi, belong to family Lythraceae and have medicinal properties (Jiny *et al*., 2010 and Habbal *et al*., 2005). Different parts of this plant are used for treating different diseases like ulcer, jaundice, bleeding disorder, etc. (Borade *et al*., 2011 and Choudhary *et al*., 2010). This plant is also reported for antioxidant and immunolmodulatory (Hosein *et al*., 2007 and Mikhaeil *et al*., 2004), wound healing (Nayak *et al*., 2007), antibacterial activities (Ghosh *et al*., 2008). All these properties are due to the presence of various secondary metabolites like quinine, terpenoids, tannin, alkaloids etc. (Habbal *et al*., 2005 and Nigha *et al*., 2016). 2 hydroxy 1,4 napthaquinone, commonly known as Lawsone, is a principal colouring agent and is also respons ible for antibacterial activity and other medicinal properties (Chung *et al*., 2007, Rahmoun *et al*., 2012 and Castro *et al*., 2008). Typhoid is a host specific and systemic bacterial disease which is caused by S. enteric serotype Typhi (Suez *et al*., 2013). It is a gram–ve bacteria and is responsible for infecting 17 million humans and 600,000 deaths every year (World Health organization 2003). Typhoid fever can be treated with antibiotics but in present scenario this bacteria has developed resistance against these antibiotics (Cabrera *et al*., 2007 and Breuil *et al*., 2000). So, the alternative to antibiotics is herbal medicine as medicinal plants are the reservoirs of many active phytoconstituents, can be used as new antibacterial agent (Plotkin 1998). Thus, the present study was conducted to evaluate phytochemical screening and *in-vitro* antityphoid activity of different fractions of *L. inermis* leaves.

Corresponding author: Anjana Goel,
Department of Biotechnology, IAH, GLA University, Mathura.

MATERIALS AND METHODS

Collection of Plant material

*L. inermis Linn.*plant leaves were collected from G.L.A. University campus, Mathura and were authenticated by Dr. (Mrs.) A. S. Upadhye (Voucher no. L-081), Botany group, Plant Science Division, Agharkar Research Institute, Pune. Leaves were shade dried and were coarsely powered and packed in airtight bottle for the preparation of different extracts.

Extract preparation

Formation of different fractions of *L. inermis* leaves

These fractionations were formed as per the method of Muhit *et al*., (2011) with slight modifications. In this method 35 grams of leaves dry powder was placed in a porous cellulose thimble. The thimble was placed in an extraction chamber of Shoxhlet apparatus, above a collection of flask containing the solvent (Hexane). The flask was heated and the solvent was allowed to evapourate. Temperature was adjusted according to boiling point of the solvent. The extraction process lasted 12-15 cycles and after that solvent recovery was done. The extract formed was collected and was kept in oven for drying. Same thimble was then used for successive fractionation with ethyl acetate and methanol. All fractions isolated were dried and stored at 4^0C for further use. This fractionation was done on the basis of increasing order of polarity.

Phytochemical screening of different fractions plant leaves

Extracts were tested for the presence of active phytochemicals such as alkaloids, carbohydrates, saponins glycoside, flavonoids, triterpenoids and proteins by procedures as described by Debela, (2002). Mayer's test, Hager's test and Dragendorff's tests were performed for Alkaloids. However, Legal's test was performed for identifying glycosides. Furthermore, the prescence of tannins and polyphenolic compounds was confirmed by Ferric chloride test while Flavonoids were tested through Alkaline test. Proteins were detected by Ninhydrin and Biuret test, Steroids were identified through Salkowaski test and carbohydrates presence was tested through Biuret and fehling's test.

Characterization of *Salmonella* Typhi

We used *Salmonella typhi* (MTCC-733), biochemically characterized by MR (Methyl Red), VP (Voges-Proskauer) test, to determine the *in-vitro* antityphoid activity of *Lawsonia inermis* plant leaves. For MR test, bacterial culture was inoculated in 0.5ml sterile glucose phosphate broth and incubated for 48 hr at 35ºC followed by addition of 5 drops of methyl red in the tube. Distinct red color is positive test. Yellow is negative reaction. Red color change is due to the fermentation of glucose, in turn changing pH into acidic. In VP test, 5 ml of bacterial culture was inoculated in 2 ml of sterile glucose phosphate peptone water followed by incubation for 48 hr at 35ºC. Test is positive if eosin pink color develops (Cheesbrough 1985).

Determination of antibacterial activity by Disc diffusion method

In-vitro antityphoid activity of different fractions of plant leaves was determined by disc diffusion method (Kannahi and Vinotha 2013). Tested organism was first inoculated in nutrient agar media for 18hr followed by inoculation in 10ml nutrient agar broth.

5, 10 and 20 mg/disc of extract were loaded on filter discs and were screened against the bacterial strain on nutrient agar plates. Bacterial concentration was adjusted to 10^6 cfu/ml with the help of nephlometer. One negative control disc was also placed to nullify the effect of solvent on bacterial growth. Each bacterial strain was also screened for standard antibiotic disc (Chloromphenicol, 20mg/disc) which acted as positive control. After incubation of 24hrs at 37^0C, the plates were observed for the presence of zones of inhibition as evidence of antibacterial activity. The degree of sensitivity was determined by measuring the diameter of visible zones of inhibition to the nearest millimetres with respect to each bacterial strain and extract concentration.

Statistical Analysis

All sets of experiment were done in the triplicate form. All the results are analyzed and expressed in Mean ± S.D.

RESULTS AND DISCUSSION

For making different extracts of *L. inermis,* dried leaves were used, as phytochemical constituents are present in more concentrated form than in fresh plant leaves (Romero *et al*., 2005). Various phytocontituents were observed, which are responsible for antimicrobial activity in plants (Maurya and J. Akansha 2010). Among the various secondary metabolites present in the plant leaves extract, main phytoconstituent is Lawsone (2-Hydroxy 1,4 napthaquinone) and due to this Lawsone, plant shows antimicrobial activity (Al-Rubiay *et al*., 2008). Carbohydrates, proteins, phenols, glycosides, quinines, terpens were present in the hexane, ethyl acetate and methanol fractions of plant leaves while protein was absent in all fractions. Our results are in agreement with different researchers (Edwin 1996 and Darout 2000).

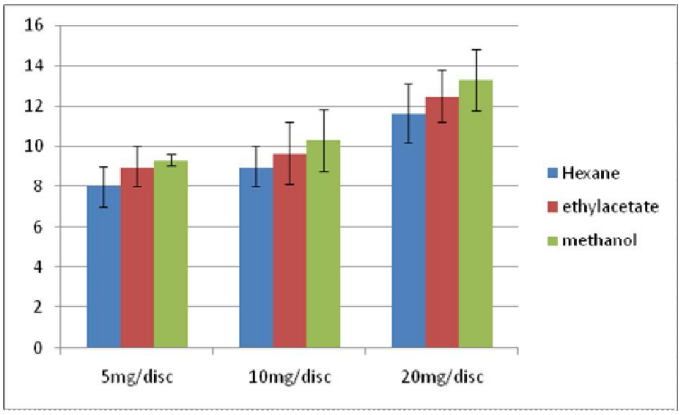
Table 1. Phytochemical constituents of different extracts of L. inermis leaves

Phytoconstituents	Hexane extract	Ethyl acetate extract	Methanol extract
Carbohydrates	+	+	+
Proteins	-	-	-
Quinones	+	+	+
Phenol	+	+	+
Terpenes	+	+	+
Glycosides	+	+	+
Flavanoides	+	+	+

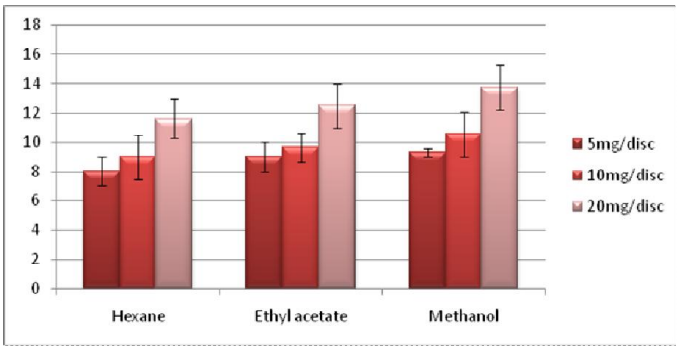
Antityphoid activity of different extract of *L. inermis* leaves is showed in Table-2. Maximum anti typhoid activity was observed against methanol extract at 20mg/disc. Graph-1 represents the results of antityphoid activity along the concentration of different extracts clearly illustrating the antityphoid activity at 20mg/disc while no antityphoid activity was observed at 5mg/disc. Whereas Graph-2 represents the antityphoid activity with individual extracts. Thus, we can conclude that this activity is in dose dependent manner as when the concentration of extract is increased, zone of

Table 2. Antityphoid activity of different extracts of *L. inermis*

Name of Bacteria	Hexane Fraction (mg/disc)			Ethyl acetate Fraction (mg/disc)			Methanol Fraction (mg/disc)		
Salmonella Typhi	5	10	20	5	10	20	5	10	20
	8±1	9±1	11.66±1.5	9±1	9.66±1.52	12.5±1.32	9.33±0.28	10.56±1.37	13.74±1.52



Graph 1. Anti-typhoid activity of different extract of *L. inermis*



Graph 2. Anti-typhoid activity of different extract of *L. inermis*

inhibition increases, confirming that our extract is dose dependent manner. As highest activity was observed in methanol extract so we can conclude that bioactive constituents are present in methanol (Choudhary *et al*., 2010).

Conclusion

In conclusion, leaves of henna have various phytochemical constituents which are responsible for the antimicrobial activity of this plant. So, in the future this plant can be used an alternative medicine for treating Typhoid and many more diseases.

Acknowledgement

The authors wish to thank the Director and HOD of Department of Biotechnology, G.L.A. University, Mathura, for providing the facilities needed during course of the study.

REFERENCES

Ali, B.H., A.K. Bashir and M.O.M. Tanira, 1995. Anti-inflammatory, antipyretic and analgesic effects of Lawsonia inermis L (Henna) in rats. *Pharmacology*, 51: 356-363.

Al-Rubiay KK, Jaber NN, Al-Mhaawe BH, Alrubaiy LK. 2008. Antimicrobial efficiency of Henna extract. *Oman Med J.,* (23):253–256.

Borade AS, Kale BN, Shete RV. 2011. A phyto pharmacological review on *Lawsoniainermis* (Linn.). *Int J Pharm Life Sci*., 2(1): 536-41.

Breuil J, Brisabois A, CasinI, Armand-Lefevre L, Fremy S & Collatz E. 2000. Antibiotic Resistance in Salmonellae Isolated from Humans and Animals in France: Comparative Data from 1994 and 1997. *Journal of Antimicrobial Chemotherapy*, 46 (6), pp.965-971.

Cabrera R, Ruiz J, Marco F, Oliveira I, Usera M, De Anta M. T., Gascon J., and Vila J. 2004. Mechanism of resistance to several antimicrobial agents in Salmonella clinical isolates causing Travellers' diarrhoea. Antimicrob Agents Chemother. 48 (10), 3934 –9.

Castro, F.A.V., Mariani, D., Panek, A.D., Eleutherio, E.C.A. and Pereira, M.D. 2008. Cytotoxicity mechanism of two naphthoquinones (menadione and plumbagin) in saccharomyces cerevisiae,‖ plos one 3 (12). e3999. Dec. 2008.

Cheesbrough, M. 1985. Medical laboratory manual for tropical countries. *Microbiology*, 2:400-480.

Choudhary, G., Goyal, S., and Poonia, P. 2010. *Lawsonia inermis* Linneaus: A phytopharmacological review. *Int. J. Pharm. Sci. Drug. Res*., 2(2): 91-98.

Chung, Y., Yoo, J., Park, S., Kim, B.H., Chen, X., Zhan, C. and Cho H, ―Dependence of antitumor activity on the electrophilicity of 2-substituted 1,4-naphthoquinone derivatives‖, *Bull. Korean Chem. Soc*., 28 (4). 691-694. Apr. 2007.

Darout I, Cristy A, Skaug N, Egeberg P. 2000 Identification and quantification of some potential antimicrobial anionic components in miswak extract. *Ind J Phar*., 32:11–14.

Edwin H. 1996. Natural polyphenols (vegetable tannins) as drugs: possible modes of action. *J Nat Prod*., 2:205–215.

Evan, C.W. 1992. Trease and Evans Pharmacology, (13 ed.). Bailliere Tindall, London, pp: 758-762.

Ghosh A, Das BK, Roy A, Mandal B, Chandra G. 2008. Antibacterial activity of some medicinal plant extracts. *J Nat Med*., 62(2): 259-62.

Habbal OA, Al-Jabri AA, El-Hag A, Al-Mahrooqi ZH, Al-Hashmi NA. 2005. In vitro antimicrobial activity of Lawsonia inermis Linn (henna): a pilot study on the omani Henna. *Saudi Med J*., 26:69-72.

Hosein HKM, Zinab D. 2007. Phenolic Compounds and Antioxidant Activity of Henna Leaves Extracts (Lawsonia inermis). *World J Dairy & Food Sci*., 2(1): 38-41.

Jiny VK, Silvipriya KS, Resmi S, Jolly CI. 2010. Lawsonia inermis (henna): a natural Dye of various therapeutic uses – a review. Inventi Impact: Cosmeceuticals. Article ID-Inventi: Cc/3/10.

Kannahi M. and vinotha K. 2013. Antimicrobial activity of *Lawsonia inermis* leaf extracts against some human *Int.J.Curr.Microbiol.App.Sci.,* 2(5): 342-349.

Maurya, R. and J. Akansha, 2010. Chemistry and pharmacology of *Withania coagulans*: An Ayurvedic remedy. *J. Pharma Pharmacol*., 62: 153-160.

Medalla F, Sjölund-Karlsson M, Shin S, Harvey E, Joyce K, Theobald L, *et al*. 2011. Ciprofloxacin-resistant *Salmonella enterica* serotype *typhi*, United States, 1999-2008. *Emerg Infect Dis*., 17(6): 1095-8.

Mikhaeil BR, Badria FA, Maatooq GT, Amer MM. 2004. Antioxidant and immunomodulatory constituents of henna leaves. *Z Naturforsch C*., 59(7-8): 468-76.

Muhammad, H. and S. Muhammad, 2005. The use of L. inermis Linn. (henna) in the management of burn wound infections. *Afr. J. Biotechnology*, 4: 934-937.

Natarajan Arivuseluan, Durai Silambarasam, Thangarul Govindam *et al*. 2011. Antibacterial activity of Mangrove leaf and bark extract against human pathogens. *Advances in Biological Research*, Vol. 5(5), 251-254.

Nayak BS, Isitor G, Davis EM, Pillai GK. 2007. The evidence based wound healing activity of Lawsonia inermis Linn. *Phytotherapy Research*, 21(9): 827-831.

Nigha M, Zafar HM, Ghaffar G. 2016. Complete Prospective of Lawsonia inermis Linn- Review. *IJIR*, 2(2): 190-197.

Plotkin M.J. 1998. Conservation, Ethnobotany and the Search for New Jungle Medicines: Pharmacognosy Comes of Ageagain. *Pharmacotherapy*, 8(5), pp. 257-262.

Rahmoun, N.M., Boucherit-Otmani, Z., Boucherit, K., Benabdallah, M., Villemin. D. and Choukchou-Braham, N. Antibacterial and antifungal activity of lawsone and novel naphthoquinone derivatives,‖ *Med. Mal. Infec*, 42 (6). 270-275. Jun. 2012.

Romero CD, Chopin SF, Buck G, Martinez E, Garcia M, Bixby L. 2005. Antibacterial properties of common herbal remedies of the southwest. *J Ethnopharmacol*., 99:253–257.

Suez J, Porwollik S, Dagan A, Marzel A, Schorr YI, Desai PT, *et al*. 2013. Virulence gene profiling and pathogenicity characterization of nontyphoidal *Salmonella* accounted for invasive disease in humans. *PLoS aOne*., 8(3): e58449.

World Health organization. 2003. The diagnosis, treatment and prevention of typhoid fever. Pp1-30.

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