ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Phytotoxic, antioxidant and Cytotoxic effect of *Holarrhena antidysenterica* Seeds extracts.

[1]Samiullah Khan, *[1]Hidayatullah Khan, [1]Muhammad Abdur Rehman Shah, [1]Fahim ullah Khan, [2]Shahnaz, [3]Rehman Ullah Khan

[1]Department of Biotechnology University of Science and Technology Bannu (28100), KPK , Pakistan.
[2]Department of Chemistry University of Science and Technology Bannu (28100), KPK , Pakistan.
[3]Department of Botany University of Science and Technology Bannu (28100), KPK , Pakistan.

Manuscript Info

Manuscript History:

Received: 13 September 2013
Final Accepted: 25 September 2013
Published Online: October 2013

Key words:
Holarrhena antidysenterica, cytotoxic, phytotoxic and antioxidant activity.

Abstract

The present study indicates the phytochemical investigations of the crude extracts of seeds of *Holarrhena antidysenterica*. The methanolic crude extract was sequentially extracted with chloroform, *n*-butanol, ethyl acetate and n-hexane. All extracts have been assessed for different pharmacological activities like antioxidant, cytotoxic and phytotoxic, to find out the therapeutic potential of this medicinally important plant. The results indicated that crude extract and different fractions of seeds of *H.antidysentrica* exhibited varied degree of antioxidant, cytotoxic and phytotoxic activities. It was examined that *H. antidysentrica* seed crude extract also inhibited plant growth. The results of phytotoxicity showed by methanolic crude and ethyl acetate fractions repressed shoot growth up to 43% and 35% respectively at concentration of 1000µg/mL. At the same concentration methanolic crude and n-butanol fraction inhibited root growth up to 32% and 29% respectively. The seed crude extracts also containing cytotoxic activities as 49% and 44% brine shrimps larvae growth are inhibited by chloroform and methanolic crude extract. The crude methanolic extract and ethyl acetate fraction indicated significant antioxidant activities in doze dependant manner and revealed maximum scavenging activity 68% and 80% at concentration of 250µg/mL.

Introduction

The medicinal plant *Holarrhena antidysenterica* belongs to family Apocynaceae commonly used as traditional medicine (Bhardwaj et al., 2005). In the Garhwal region of north-west Himalaya for the treatment of protozoan infections and fever including malaria were studied. The stem bark of the *H. antidysenterica* plant, commercially known as kurchi and kutaz in the Indian subcontinent has been investigated due to its traditional use in the treatment of amoebic dysentery, diarrhoea, asthma and bronchopneumonia (Kumar and Ali, 2000; Bhutani, 1948). The fruit extract have a potent anticancer properties (Dhar et al., 1968). In addition the plant has been reported to possess anti-helminthic, appetizing, anti-diarrhoeal and astringent. The bark of stem is found effective against various infections, antidontalgic, febrifuge, antidropsical, diuretic, in piles, colic, dyspepsia, chest affections and as a tonic in diseases of the skin and spleen properties (Chopra et al., 1982), used as an immunomodulating agent (Atal et al., 1986), larval growth inhibitor (Thappa et al., 1989), inhibit growth of various microbes and against vaginitis (Hagers, 1976). Gaur (1999) reported that the bark of the *H. antidysenterica* is used against malaria in the Garhwal region of north-west Himalaya. Similarly Dua et al., (2013) also reported anti-malarial property of steroidal alkaloid conessine isolated

from the bark of *Holarrhena antidysenterica*. Various parts of *Holarrhena antidysentrica* contain alkaloids, steroidal alkaloid, fats, tannin and resin. Various alkaloids are reported, such as 3-aminoconanines, 20-aminoconanines, 3-aminopregnans, 20-diaminopregnanes from both seeds and bark (Gopal et al., 2006). The bark has composition of bitter constituent that having stimulant value. It can be administered in both severe and persistent fever.
In the present investigation, we studied the *Holarrhena antidysentrica* seeds for variety of biological activities including antioxidant, cytotoxicity and phytotoxicity.

Material and Methods

2.1: Chemicals for Biological Activities

Chemical reagents Nitro blue tetrazolium (NBT), 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Germany, Wheat seeds, *Holarrhena antidysentrica* seeds methanolic extract and fractions of chloroform, n-butane, n-hexane and ethyl acetate, methanol, Brine shrimps, sea salt, aluminum foil.

2.2 Plant material and preparation of crude extract

Mature and fully dried seeds of *Holarrhena antidysentrica* were purchased from local supplier in District Bannu. The taxonomic identification of the plant seeds was confirmed by Prof. Abdur- Rehman, Govt. Post Graduate College Bannu, KPK Pakistan.
Fresh dried fruit of *Holarrhena antidysentrica* were grinded into fine powder with the help of pestle and mortar. The fruit powder was put into 80% methanol in such a way that the powder was completely submerged in methanol and placed room temperature for 72 h with frequent agitation and the resulting liquid was filtered (Whatman No. 3 filter paper, Whatman Ltd., England). The filtrate was placed at room temperature to evaporate the liquid content. The resulting gummy methanolic extract was put into falcon tube and lyophilized. The lyophilized sample was stored for further use.

About 100g of methanolic crude were sequentially extracted with *n*-butanol, *n*-hexane, chloroform and ethyl acetate using Soxhlet apparatus. The solvent was evaporated under reduced pressure and the fractions were then placed in a vacuum oven at not more than 40ºC for about 24 hours to remove any residual solvent. The resulting semisolid mass of each fraction was stored for assays.

Biological assays

3.1: Cytotoxic assay

Cytotoxic activity of methanolic extract of *Holarrhena antidysentrica* was found out by brine shrimps lethality bioassay [16]. 5 µg / µL (20 mg/ 4 mL) stock solution, from methanolic extract of *Holarrhena antidysentrica* seeds, was prepared in methanol. The stock solutions were diluted to 100, 250, 500 and1000 µg/ mL and set in test tubes such that control without test sample and experimental with test sample of 300, 750, 1500 and 3000 µg/3 mL. Duplicate set of test tubes were used for each concentration. They were placed at room temperature to evaporate the methanol completely. Then 10 shrimps were added to each test tube and placed at room temperature for 24 hours. Then the shrimps were counted in all test tubes by using magnifying glass and compared the results (lethality) of experimental with control.

3.2: Phytotoxic assay

Phytotoxic activity of *Holarrhena antidysentrica* seeds extracts was determined in 10 mL assay by preparing 1µg / µl solution of methanolic extract of *Holarrhena antidysentrica* seeds. Four sub solutions i.e.100, 250, 500 and 1000µg/mL of crude extract were prepared. Petri plates were washed and autoclaved. Filter papers were placed in all of them, labeled them (Control, 100, 250, 500 and 1000µg/mL). 1 mL methanol was sprayed on the filter paper in the control while 1 mL of each prepared sub solution was poured on the filter paper in the test/experimental petri plate. After complete evaporation of methanol, 9 mL distilled water were added to each petri plate. The seeds of monocot plant (maize) were soaked in water for 5 minutes and removed the floating seeds. Finally seven mature healthy seeds were set in each petri plate and placed at room temperature. After 3 days the length of root and shoot were measured with graduated ruler and then mean was taken of each. After one weak i.e. 7days the length of root and shoot were measured again and recorded.

3.3: Antioxidant assay

The Gymfi et al., (1999) procedure with some modifications was followed for this assay of DPPH (1, 1-diphenyle -2- picryle hydrazyl).100µl from each of the sample solution of 50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml, 250µg/ml and 500µg/ml were taken and mixed it with 900µl of DPPH. The same process was repeated with ascorbic acid solution. All these test tubes were incubated at 25°C for about 30 minutes in the dark because of its sensitivity to word light and checked its absorbance on spectrophotometer at 517nm. By using the following equation the potential of the samples to scavenge the DPPH free radicals was calculated;

% DPPH free radicals scavenging effect = (DPPH Ab – Sample Ab/DPPH Ab) × 100
Ab = Absorbance

STATISTICAL ANALYSIS

Percentages of inhibition were expressed as Mean ± Standard deviation from two observations in case of cytotoxicity and phytotoxicity bioassay. Antioxidant activity calculation is formulated by
% DPPH free radicals scavenging effect = (DPPH ab – Sample ab/DPPH ab) × 100
All Calculations and Table representation was performed in Microsoft Excel and Word-2010 respectively.

Table 1: Antioxidant activity results of crude extract and its fractions

Concentration (µg/mL)	Crude extract and Fractions % Scavenging					%
	Crude extract	Ethyl acetate	n-butanol	Chloroform	n-hexane	Control
50	24	33	20	12	21	100
100	38	48	28	24	34	100
150	48	58	39	30	42	100
200	60	69	46	39	51	100
250	68	80	53	47	59	100

Table 2: Cytotoxic results of crude extract and various fractions of *H. antidysentric* seeds

Concentration (µg/mL)	Crude extract and Fractions No of Shrimps died %					%
	Crude extract	Chloroform	n-butanol	Ethyl acetate	n-hexane	Control
100	12	16	8	12	18	90
250	17	17	13	14	20	100
500	23	24	19	22	34	100
1000	44	49	30	29	39	90

Table 3: Phytotoxic results of crude extract and various fractions of *H.antidysentrica* seeds.

Length (%) of shoot averagely after 3 and 7 days;

Samples	1000µg/mL % Growth Inhibition	500µg/mL % Growth Inhibition	250µg/mL % Growth Inhibition	100µg/mL % Growth Inhibition	Control %
Crude extract	43	23	09	03	100

n- butanol	28	22	17	08	100
n- hexane	33	24	07	00	100
Chloroform	26	19	08	00	100
Ethyl acetate	35	15	07	04	100

Length (%) of root averagely after 3 and 7 days;

Samples	1000µg/mL % Growth Inhibition	500µg/mL % Growth Inhibition	250µg/mL % Growth Inhibition	100µg/mL % Growth Inhibition	Control %
Crude extract	32	17	06	00	100
n- butanol	29	16	09	06	100
n- hexane	23	10	04	03	100
Chloroform	20	12	08	03	100
Ethyl acetate	25	17	10	07	100

Discussion

Pakistani medicinal flora show important role in treatment of various human problems such as nephrotoxicity (Khan et al., 2009; Khan et al., 2010; Sahreen et al., 2011), pulmonary oxidative damages (Khan et al., 2011a), cardio toxicity (Khan et al., 2011), antioxidant (Sahreen et al., 2011b) (Sahreen et al., 2011c), adrenal toxicity (Khan et al., 2011d), phytotoxicity .Recent research claims that 30% of drugs are prepared from medicinal plants (Grabley et al., 1999).

The scavenging activity of crude methanolic extract and its fractions are summarized in Table 1. Potential scavenging results are offered by ethyl acetate fraction and crude extract, 80 and 68% respectively at concentration of 250µg/ml. n-hexane, chloroform and n-butanol fractions showed 59, 53 and 47% scavenging activity at the same concentration. The present results of *H. antidysentrica* seeds crude extract and its fractions indicate that they possess potential scavenging properties and scavenge the free radicals in the form of DPPH. Similar results were also reported by Ahmad et al., (2011) for *E. prostrate* (Hagerman et al., 1998). These indicate that seeds of *H. antidysentrica* are rich in flavonoids, phenols and saponins, which are responsible for antioxidant activity.

The results of cytotoxicity furnished by *H. antidysentrica* seeds crude extract and its resultant fractions are appreciable (Table 2). Most excitable cytotoxicity of 49 and 44% were expressed by chloroform and methanolic crude extract at 1000µg/ml. While 39, 30 and 29% outcome were displayed by n-hexane, n-butanol and ethyl acetate at the same concentration. So the analysis of results was found satisfactory and showed that *H. antidysentrica* seeds containing cytotoxic activity. Marked cytotoxic activity was demonstrated by the chloroform fraction and the methanolic extract, 49 and 44% respectively against brine shrimps. These results are very much similar and potentially supported by the research work of Keawpradub et al., (2005).

The results of phytotoxicity by *H. antidysentrica* seeds crude extract and its resultant fractions are shown in (Table 3). Most potent results were presented by methanolic crude and ethyl acetate fractions that repressed shoot growth up to 43 and 35% respectively at concentration of 1000µg/ml. The contribution of n-hexane, n-butanol and chloroform also carry good sense as they restricted shoot growth up to 33, 28 and 26% respectively.

At the same concentration methanolic crude and n-butanol fraction inhibited root growth up to 32 and 29 % respectively. 25, 23 and 20% inhibition was offered by ethyl acetate, n-hexane and chloroform respectively. All the results were expressed in % inhibition of growth.
Our plant seed extracts phytotoxic efficiency is strongly supported by Javaid (2009) for *Withania somnifera* and *Datura alba* which efficiently restricted the growth of root and shoot of *Rumex deutatus* L. It was observed that *Withania somnifera* and *Datura alba* containing active ingredients that are responsible for this activity. Consistent results were also examined by Kordali et al., (2008) which showed both seedling and roots inhibition due to the occurrence of phenolic compounds from Turkish origanum essential oil.

Conclusion

The results highlight the fact that seeds of *H. antidysentrica* have potent cytotoxic, antioxidant and Phytotoxic effects.

Acknowledgement

This study was carried out by Samiullah Khan in the research laboratory of Biotechnology Department, University of Science and Technology Bannu (28100), KPK, Pakistan.

References

Ahmad, M., Shah, A.S., Khan, R.A., Khan, F.U., Khan, N.A., Shah, M.S. and Khan M.R. (2011): Antioxidant and antibacterial activity of crude methanolic extract of *Euphorbia prostrata* collected from District Bannu (Pakistan). African Journal of Pharmacy and Pharmacology., 5: 1175-1178.

Atal, C.K., Sharma, M.L., Kaul, A., Khajuria, A. (1986): Immunomodulating agent of plant origin. Indian Drugs., 18:133-141.

Bhardwaj, S. and Ghakar, S.K. (2005): Ethnomedicinal plants used by the tribal's of Mizoram to cure cut and wound. Indian Journal of Traditional Knowledge., 4: 75-80.

Bhutani, K.K. (1984): Proceedings of National symposium of applied biotechnology of medicinal, aromatic and timber yielding plants University of Calcutta, India., 387-392.

Chopra, R.N., Chopra, I.C., Handa, K.L., Kapur, I.D. (1982): Chopras Indigenous drugs of India Academic Press, New Delhi, India. 342.

Dhar, M.L., Dhar, M.M., Dhawan, B.N., Mehrotra, B.N., Ray, C. (1968): Screening of Indian plants for biological activity. Ind. J. Exp. Biol., 6: 232-37.

Dua Virendra, K., Gaurav, V., Bikram, S., Aswathy, R., Upma, B., Dau Dayal, A., Gupta, N.C., Sandeep, K. and Ayushi, R. (2013): Anti-malarial property of steroidal alkaloid conessine isolated from the bark of Holarrhena antidysenterica. Malaria Journal., 12:194

Gaur, R.D. (1999): Flora of the District Garhwal of North West Himalaya: Transmedia Publication. Srinagar,(Garhwal).PP.159

Grabley, S. and Thiericke, R. (1999): Bioactive agents from natural sources: trends in discovery and application. Adv. Biochem. Eng. Biotechnol., 64: 101-54.

Gyamfi, M.A., Yonamine, M., Aniya, Y. (1999): Free-radical scavenging action of Medicinal herbs from Ghana: *Thonningia sanguinea* on experimentally- induced liver injuries. Gen Pharmacol., 32: 661-667.

Hagerman, A.E., Riedle, K.M., Jones, G.A., Sovik, K.N., Hartzfeld, P.W. (1998): High molecular weight plant poly phenolic (tannins) as Biological antioxidants. J. Agric. Food Chem., 46: 1887-1892.

Hagers 1976) Handbuch der Pharmazeutischen Praxis. Springer-Verlag, Berlin. New York.; 5:92-95.

Hazra, B., Biswas, S., Mandal, N. (2008): Antioxidant and free radical scavenging activity of *Spondias pinnata.* BMC Complement Altern Med., 8: 64-4.

Javaid, A. (2009): Role of effective microorganisms in sustainable agricultural productivity. In: Advances in sustainable agriculture. Springer (article in Press).

Keawpradub, N., Dej-adisai, S., Yuenyongsawad, S. (2005): Antioxidant and cytotoxic activities of Thai medicinal plants named Khaminkhruea: *Arcangelisia flava, Coscinium blumeanum* and *Fibraurea tinctoria.* J. Sci. Technol., 27: 455-467.

Khan, M.R., Rizvi, W., Khan, G.N., Khan, R.A., Sahreen, S. (2009): Carbon tetrachloride-induced nephrotoxicity in rats: Protective role of *Digera muricata* J. Ethnopharmacol., 122: 91-99.

Khan, R.A., Khan, F.U., Ahmad, M., Shah, A.S, Khan, N.S., Khan, M.R, Shah, M.S. (2011): Phytotoxic and antibacterial assays of crude methanolic extract of *Mentha longifolia* (Linn.) Afri. J. Pharm. Pharmacol., 4: 175-200.

Khan, R.A., Khan, M.R., Sahreen, S., Bukhari, J. (2010): Prevention of CCl4-induced nephrotoxicity with *Sonchus asper* in rat. J. Food Chem. Toxicol., 23: 1304-1321

Khan, R.A., Khan, M.R., Sahreen, S., Jan, S., Bokhari, J., Rashid, U. (2011a): Phytotoxic characterization of various fractions of *Launaea procumbens.* Afr. J. Biotechnol., 10: 5377-5380.

Khan, R.A., Khan, F.U., Ahmad, M., Shah, A.S., Khan, N.S, Khan, M.R., Shah, M.S. (2011): Phytotoxic and antibacterial assays of crude methanolic extract of *Mentha longifolia* (Linn.) Afri. J. Pharm. Pharmacol., 4: 175-200.

Khan, R.A., Khan, M.R., Sahreen, S., Jan, S., Bokhari, J., Rashid, U. (2011): Prevention of CCl4 induced adrenal oxidative stress in rat by *Sonchus asper*. J. Med. Plants Res., 5: 3347-3350.

Kordali, S., Cakir, A., Ozer, H., Cakmakci, R., Kesdek, M., Mete, E. (2008): Antifungal, Phytotoxic and insecticidal properties of essential oil isolated from Turkish *Origanum acutidens* and its three components, carvacrol, thymol and p-cymene. Bioresour. Technol., 99: 8788-8795.

Kumar, A. and Ali, M. (2000): A new steroidal alkaloid from the seeds of Holarrhena antidysenterica. Fitoterapia., 71:101-104.

Meyer, N.R., Ferrigni, J.T., Putnam, L.B., Jacobson, D.E., Nichols, J.L., McLaughlin. (1998): Brine Shrimp: A convenient general bioassay for active plant constituents. Plant Medica., 45: 31-34.

Sahreen, S., Khan, M.R., Khan, R.A. (2011): Phenolic compounds and antioxidant activities of *Rumex hastatus* D. Don. Leaves. J. Med. Plants Res., 5: 2755-2765.

Sahreen, S., Khan, M.R., Khan, R.A. (2011): Estimation of flavonoids and evaluation of protective effect of *Carissa opaca* Stapf ex Haines fruit against CCl4 induced nephrotoxicity in rat. Food and Chemical Toxicology., 10: 54.

Sahreen, S., Khan, M.R., Khan, R.A. (2011): Hepatoprotective effects of methanol extract of *Carissa opaca* leaves on CCl4-induced damage in rat. BMC Compl. Altern. Med., 11:48

Thappa, R.K., Tikku, K., Saxena, B.P., Vaid, R.M., Bhutani, K.K. (1989): Conessine as a larval growth inhibitor, sterilant, and antifeedant from Holarrhena antidysenterica. Insect Sci Appl., 2:149-156.