IJABR

International Journal of Advanced Biotechnology and Research (IJABR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-10, Issue-1, 2019, pp514-518
http://www.bipublication.com

Research Article

Biological activities of Curcuma longa

Saifullah Mehsud[1*], Musarrat Rauf[2] and Rabia Samad[3]

[1]Dept. of Pharmacy, Abbottabad University of Sci. & Tech, Havelian,KPK, Pakistan.
[2]Dept. of Dentistry, Ayub Medical College, Abbottabad,.KPK, Pakistan
[3]Dept. of Dentistry, Ayub Medical College, Abbottabad.,KPK, Pakistan

Corresponding Author: Saifullah Mehsud, Assist. Professor (Pharmacy)
Abbottabad University of Sci. & Tech, Havelian,
Abbottabad (KPK), Pakistan.
Email address:* saifmehsud@gmail.com. Cell# 03335547606

[Received: 25/11/2018; Accepted: 15/02/2019; Published: 16/02/2019]

ABSTRACT

The aim of this study was to assess the crude extract of Curcuma longa against an in vitro biological activities insecticidal, antileishmanial, antibacterial, antifungal, cytotoxicity(brine shrimp lethality) and phytotoxic activities. Plant samples at highest doses was found to possess excellent phytotoxic activity against Lemna minor. Antifungal activity was significantly observed with 90% inhibition against Trichophyton longifusis and Microsporum canis while it proved to be active with LD_{50}=191.561 with upper limit =309.42 and lower limit =30.203 and moderate insecticidal activity. Ethanlic extract did not display any significant antibacterial and cytotoxic (brine shrimp lethality) activity and antileishmanial activity.

Keywords: *Curcuma longa*, insecticidal, antileishmanial, antibacterial, antifungal cytotoxicity (brine shrimp lethality) and phytotoxicity.

INTRODUCTION

The use of medicinal plants for the treatments of many diseases is associated to folk medicine from different parts of the world (Thomson, 1978). Natural products from some plants, fungi, bacteria and other organisms, continue to be used in pharmaceutical preparations either as pure compounds or as extracts (Srivastava, et al., 1996). Plant extracts possess potential antioxidant, analgesic and antidiarrheal activities, which rationales in folk medicine (Sanjib Saha et al., 2012). Turmeric (Curcuma longa) is a rhizomatous herbaceous perennial plant of ginger family, Zingiberaceae which is native to tropical South Asia.It is often pronounced as Turmeric. Curcuma longa is commonly used in the treatment of diabetes by ayurvedic physicians. Curcumin is biological component isolated from the rhizome of Curcuma longa that possess anti-hyperglycemic activity (Arun N and Nalini N, 2002, Hamid et al., 2012), hypolipidemic action (Suresh Babu P and Srinivasan K., 1997) and anti-renal lesion effect (Suresh Babu P and Srinivasan K., 1998). The use of curcumin is recommended for prevention of advanced glycated end products (AGE)

accumulation and associated complication of diabetes (Sajithlal GB et al., 1998).Practitioner of traditional medicines believe that curcumin powder is beneficial against many diseases including biliary disorders, diabetes, hepatic disorders, rheumatism,sinusitis, cancer and Alzheimer disease (Stockwell, 1988).

MATERIAL AND METHODS

Collection and preparation of plant material:

Fresh leaves of the plant sample were collected and air-dried. Then ground with a grinder to a powder, the powdered material was 2 Kg. crude extract of air-dried powder was subjected to cold extraction with 96% ethanol for 8 days and then filtered through filter paper. The ethanol extract was concentrated in a vacuum 40^0 C rotary evaporator, and air-dried at room temperature to give a final yield of 105 gm. (H. Sadraei *et al.*, 2003).

Insecticidal bioassay

Insecticidal studies was carried out by exposing the insects to test sample by a contact method using filter paper. Solutions of crude extracts of Curcuma longa was prepared by dissolving in 3-4 ml organic solvents (ethanol/methanol) and absorbed on filter papers placed in petri dishes having diameter equal to the internal diameter of petridishes. One filter paper was absorbed by the same organic solvent (methanol) used to dissolve the samples, to be used as check for determination of solvent effect and solvent was evaporated overnight. Next day 10 adults of same size and age were transferred to petri dishes containing samples.

A check batch of insects was transferred to petri dishes containing solvent only, (which now evaporated overnight).and another batch supplemented with reference insecticide 'Coopex' in same quantity was used. All the insects were kept without food throughout 24 hours-exposure period. Mortality counts were done after 24 hours exposure period.

Results were calculated as Mortality mean percent.

Leishmanicidal bioassay

Parasite culture

The promastigote culture of Pakistani leishmania spp (L. major) obtained from the suspected lesions of Cutaneous Leishmaniasis patients and inoculated on the liquid phase of Novy- McNeal-Nicolle (NNN) medium then cultured in Roswell Park Memorial Institute (RPMI) 1640 media supplemented with 10% fetal bovine serum (Kalter., 1994). The cultures were made at least in duplicates for each case. Parasites cultivated in bulk were aseptically sediment down at 3000rpm, counted by the help of improved neubauer chamber under the microscope and diluted with the fresh medium to a final concentration of 2.0 x 10^6 parasites/ml. the compound to be checked were dissolved in appropriate solvent and then diluted to a final concentration of 1.0mg/ml of PBS (phosphate buffered saline, pH 7.4 containing 0.55 MeOH, 0.5% DMSO). In a 96 well microtiter plate, 90ul of the experimental compound was added in culture and serially diluted so that minimum concentration of the compound is 0.1ug/ml. 10ul of PBS (phosphate buffered saline, pH 7.4 containing 0.55 MeOh, 0.5% DMSO) was added as negative control while glucantime, amphotericin B, pentamidine and ampicillin to a final concentration of 1.0mg/ml was added separately as positive control. The plate was incubated between 21-22^{o}C in dark for 3-6 days. The culture was examined microscopically on an improved neubauer chamber and IC_{50} values of compounds possessed antileishmanial activity were calculated.

Antibacterial activity

The antibacterial activity was determined by agar well diffusion method. A loopful of a 10^4-10^6 suspension of a 24h old broth of each bacterium was streaked on the surface of Muller-Hinton agar (BBI-USA) plates. Wells were dug in the agar with help of sterile dimethyl sulfoxide (DMSO). Dilutions of stock solution containing 50, 100, 150 and 200ug were prepared in DMSO and 10 ul of each dilution was added in respective wells. The plates were then incubated at 37^{o}C for 24h and zone of inhibitions were measured in

millimeters (mm) and compared with control (Att-ur-Rehman et al, 1991). Antibacterial activity was studied against Eschericia coli, Bacillus subtilis, Shigella flexenari, Stphylococcus aureus, Pseudomonas aeruginosa and Salmonella typhi. Ampicillin, Tobramycin and Amoxacilline were used as standard drugs. The samples of curcuma longa were subjected to antibacterial screening.

Antifungal activity

The antifungal activity of the crude extracts of Curcuma longa was determined by measuring the minimum inhibitory concentrations of the extract using agar well diffusion assay. The assay was then carried out according to the method of Hufford et al., 1975. The extract was tested at final concentration of 78-50%. A 24 h culture of Candida albicans, diluted to give a final concentration of 10^5 cells per ml, was used, while standard spore suspensions of 10^3 from the other test fungi were utilized in the Minimum Inhibitory Concentration (MIC) assays. After inoculating the Sabouraud's dextrose agar (previously incorporated with the desired extract concentration with fungi, the plates were incubated at 30^oC for 5 days. Control plates containing each of test fungi separately without the addition of the anti-microbial extracts were similarly set up. The Minimum Inhibitory Concentration was regarded as the lowest concentration of the extract that did not permit the growth of any of the test fungi after the period of incubation.

Phytotoxicity Screening

This test was performed according to the modified protocol of McLaughlin et al, 1988. The test fractions were incorporated with sterilized E-medium at different concentrations i.e. 10, 100, 1000 ug/ml in methanol. Sterilized conical flasks were inoculated with fractions of desired concentrations prepared from the stock solution and evaporated overnight. Each flask was inoculated with 20ml of sterilized E-medium and then ten Lemna minor each containing a rosette of three fronds were placed on media. Others flasks were supplemented with methanol serving as negative control and reference inhibitor i.e Parquet serving as positive control. Treatment was replicated three times and the flasks incubated at 30^oCin Fisons Fi-tron 600H growth cabinet for seven days,9000 lux intensity, 56+10rh(relative humidity) and 12 hours day length. Growth of Lemna minor in fraction containing flask was determined by counting the number of fronds per dose and growth inhibition was calculated with reference to negative control Mc Laughlin et al., 1988

Cytotoxic Activity (Brine Shrimps Lethality Bioassay)

The procedure of Mayer et al was adopted to determine the lethality of plant extracts to brine shrimp. Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds (Meyer et al., 1982). Using the protocol of (Meyer et al, 1982) brine shrimp (Artemia salina larvae) eggs were hatched in a shallow rectangular plastic dish, filled with artificial seawater, which was prepared by mixing a commercial salt mixture (Instant Ocean, Aquarian System, Inc., Mentor, OH, USA) with double distilled water. An unequal portion was made in plastic dish with the help of perforated device. An approximately 50mg of eggs were sprinkled into the large compartment, which was darkened while the smaller compartment was opened to ordinary light. After two days a pipette collected naupil from the lighted side. A sample of the test fraction was prepared by dissolving 20mg of each fraction 2ml of methanol. From this stock solution, 1000, 100, 10ug/ml was transferred to12 vial; three for each dilution, and three vials were kept as control having 2 ml of methanol only. The solvent was allowed to evaporate overnight. After two, when shrimp larvae were ready, 1 ml of sea water was added to each vial along with 10 shrimps and the volume was adjusted with sea water to 5ml per vial. After 24 hours, the number of surviving shrimps counted. Data was analysed by a Finney computer program to determine LD_{50} (Finney, D. J et al, 1971)

In vitro Antifungal activity of Curcuma longa

The crude extract of Curcuma longa was tested for their antifungal activity. The crude extract showed

significant activity with 90% inhibition against Trichophyton longifusis and Microsporum canis. But it showed no activity against the other pathogens used in the study. However, essential oils of N. juncea, with major constituent of neptalactone, have shown antifungal and antibacterial properties (Inoue et al., 2001; Tripathi et al., 2004, 2008; Kobaisy et al., 2006). Results are tabulated in Table 1.

In vitro Cytotoxic activity of Curcuma longa

For further study on plants in the same way the Brine shripms were also subjected to Curcuma longa extract for lethality assay, which is most suitable method for screeningof cytotoxic (bioactive) principles. The fresh crude extract was subjectedto lethality assay and results were calculated using Finney computer program. It proved to be active with LD_{50}=191.56 with upper limit=309.42 and lower limit=30.203. The results are tabulated Table 2.

In vitro Antibacterial activity Curcuma longa

The crude extract of Curcuma longa was subjected to antibacterial bioassay using agar well diffusion method. Curcuma longa crude extract was also subjected to antibacterial bioassays using agar well diffusion method. The result indicates there is non-significant activity against Bacillus subtilis and Staphylococcus aureus.The results indicated that there is non-significant activity against Bacillus subtilis and Staphylococcus aureus. The results for crude are tabulated in Table 3.

In vitro* Antileishmanial Activity of *Curcuma longa

The crude extract of *Curcuma longa* was screened for their antileishmanial activity. The crude extract showed no leishmanicidal activity. The crude *Curcuma longa* was screened for their antileishmanial activity. The crude extract showed to leishmanicidal activity. Results are tabulated in table 4.

Phytotoxic activity of Curcuma longa

Similarly the crude extract of Curcuma longa was studied for their cytotoxic activities. Curcuma longa crude showed significant activity in higher doses i.e. with 1000ug/ml, which is 100% for crude. At 2nd dose i.e. 100ug/ml Curcuma longa crude showed low activity against Lemna minor. In the lower doses i.e. with 10ul the crude sample of Curcuma longa did not show any significant activity. All the results are tabulated in Table 5. The results showed that the samples used for analysis have significantly inhibited the Lemna minor growth in 1000ug/ml doses. The results indicates the Curcuma longa is phytotoxic against Lemna minor, so, it can be utilized in standardization, evaluation and development of new natural herbicides/weedicides.

Insecticidal Activity of Curcuma longa by contact toxicity method

The crude extract of the plant was tested against the said three species of common grain pests. Results were noted after 24hours. All the insects were checked and the sample shows 40% motility against *Rhyzopertha dominica* 60% mortality rate against *Callosbruchus analis* were noted. In case of Curcuma longa the crude extract (sample) shows 40% motility against Rhyzopertha dominica and 60% motility against Callosbruchus analis were noted. This indicates that there is low insecticidal activity of Curcuma longa against Rhzopertha dominica and Callosbruchus analis species of common pests. So there is need to evaluate and standardize this plant for further investigation of insecticidal compounds. Results are shown in table 6.

CONCLUSION.

The data revealed that *Curcuma longa* extract contains active compounds, which could serve as alternative agents as the drug therapy in the control of parasitic diseases. Thus more investigation should be done *in vivo*in laboratorial infected animals. This would help us in obtaining a novel drug that potentially be less toxic and more cost-effective against parasite various pathogens. It is concluded that Curcuma longa used in these studies showed significant as well as low activities therefore there is need to evaluate

and standardize these natural products/plants for further investigation.

ACKNOWLEDGEMENTS

The authors are grateful and wish to express thanks to Faculty of Pharmacy Gomal University D.I. Khan, Khyber Pakhtunkhwa, Pakistan and HEJ Research institute Karachi, Pakistan for their gratitude and making the facilities available for the research.

REFRENCES

1. Arun N and Nalini N (2002). Efficacy of turmeric on blood sugar and polyol pathway in diabetic albino rats. Plant foods for human Nutrition, 57:41-51
2. H. Sadraei, G. Asghari, A.A Hekmatti., (2003). Antispasmodic effect of hydroalcholic extract of Pychnocycha spinose. Journal of Ethnopharmacology 86; 187-190.
3. Hamid I., Baharullah K., Sultan A, Ali R, Muhammad I, Muhammad NA, Hameed UR, Saqib W, Abdul W. (2012). Comparative efficacy of Aloe vera and Tamarix aphylla against cutaneous leishmaniasis. International jounal of Basic Medical Sciences and Pharmacy.; 2: 42-45.
4. Inoue S, Uchida K, Yamaguchi H, Miyara T, Gomi S,Amano M. (2001). Karakuoram Himalaya district and their antifungal activity by vapor contact. J. *Essential Oil Research* 13: 68-72
5. Kobaisy M, Tellez MR, Dayan FE, Mamonov LK, Mukanova GS, Sitapaeva GT, Gemejieva NG. (2005). J Essential Oils Res; 17: 704-707.
6. Sanjib Saha, MD Khirul Islam, MD Anisuzzaman, MD Mahadhi Hassan, Farooq Hossain, Chinmoy Talukder. (2012). Evaluation of antioxidant, analgesic anti diarrheal activity of *Phoenix paludosaroxb* leaves. . International jounal of Basic Medical Sciences and Pharmacy.; 2: 46-52.
7. Sajithlal GB, Chitra P and Gowri C (1998). Effect of curcumin on advanced glycation and cross-linking of collagen in diabetic rats. Biochemistry and Pharmacology, 56: 1607-1614
8. Srivastava J., J. Lambert and N. Vietmeyer. (1996). Medicinal plants. An expanding role in development. World Bank Technical. 320.
9. Suresh Babu P and Srinivasan K (1997). Hypolipidemic action of curcumin, the active principle of turmeric Curcuma longa in streptozocin induced diabetic rats. Molecular and Cellular Biochemistry, 166: 169-175.
10. Suresh Babu P and Srinivasan K (1998). Amelioration of renal lesions associated with diabetes by dietary curcumin in streptozocininduced diabetic rats. Molecular and Cellular Biochemistry, 181: 87-96.
11. Stockwell, C., (1988).Nature's pharmacy London, United Kingdom. Century Hutchison Ltd.
12. Thomson, W.A.R (1978). Medicines from the earth. Maidenhead, United Kingdom. McGraw-Hill Book Co.
13. Tripathi P, Dubey NK, Shukla AK. 2008. Use of some essential oils as post-harvest botanical fungicides in the management of grey mould of grapes caused by *Botrytis cinerea. World J Microbiology & Biotechnology 24: 39-46.*
14. Tripathi P, Dubey NK, Banejri R, Chansouria JP.N. 2004. Evluation of some essential oils as botanical fungitoxicants in management of post-harvest rotting of citrus fruits. World J Microbiology & Biotechnology 20: 317-321.