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ORIGINAL ARTICLE

IN VITRO STUDIES ON *LUFFA CYLINDRICA* SEEDS FOR ANTHELMINTIC AND ANTIOXIDANT PROPERTIES

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Abstract:

Purpose: To investigate the antioxidant and anthelmintic activities of ethanolic, methanolic and chloroform extracts of Luffa cylindrica seeds commonly used in South Asia, South Europe and Africa traditional medicine for the treatment of various ailments.
Methods: The extracts were obtained by successive extraction (using Soxhlet Apparatus) using Petroleum-ether (60-80°c), Benzene, Solvent ether, Chloroform, Acetone, Ethanol and Methanol as solvent. The antioxidant effect of the extracts was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Superoxide Scavenging Assay. The anthelmintic activity of the extracts was tested against Indian Earthworm (Pheretima posthuma) . Various concentrations of Ethanol, Methanol and Chloroform extract (40, 60, 80 mg/ml) of leaves of Luffa cylindrica were tested in the bioassay, which involve determination of time for paralysis and time for death of worms. Mebendazole was included as references standard.
Results: The ethanolic extract showed significant in vitro antioxidant activity in comparison to the standard antioxidant (ascorbic acid) methanolic, chloroform extract. The drug has been found to have anthelmintic activity in the ethanolic extract, methanolic extract and the chloroform extract but the most significant activity has been found in the ethanolic extract. It was found to be comparable with the standard drug Mebendazole.
Conclusion: These results suggest that Luffa cylindrica have potent antioxidant and anthelmintic activities. Further study is necessary for isolation and characterization of the active antioxidant agents which can be used to treat various oxidative stress-related diseases.

KEY WORDS:

Luffa cylindrica, Antioxidant activity, Anthelmintic activity, Free radicals.

INTRODUCTION

Medicinal plants are traditionally used to prevent disease, maintain health and to cure ailments [1]. It is estimated that about 80% of the population in africa use traditional medicine to meet their healthcare needs. This is because, they produce wide array of phytochemicals most of which are used by the plant as a chemical defence against predators. Plants with these compound such as peptides, flavonoids , alkaloids, phenols and tannins have been reported to possess strong biological activities which are now gaining much

Title: "IN VITRO STUDIES ON *LUFFA CYLINDRICA* SEEDS FOR ANTHELMINTIC AND ANTIOXIDANT PROPERTIES, Source: Review of Research [2249-894X] Madan singh[1], Bhanu Priya[2], Amit Kumar Singh[3] and Neelam Soni[4] yr:2014 | vol:3 | iss:10

importance [2]. Free radicals have been claimed to play an important role in affecting human health by causing several chronic disease such as cancer, diabetes, aging, atherosclerocis, hypertension, heart attack and other degenerative disease [3]. These free radicals are generated during body metabolism. Exogenous intake of antioxidant can help the body scavenge free radicals effectively. Nowadays there is a noticeable interest in antioxidants, especially in those which can prevent the presumed deleterious effects of free radicals in the human body and to prevent the deterioration of fats and other constituents of foodstuff. In both cases there is a preference for antioxidants from natural rather than from synthetic sources [4]. The importance of the reactive oxygen species (ROS) has attracted increasing attention over the last decade. ROS include free radicals (O2-), hydroxyl radicals (OH-), hydrogen peroxide (H2O2) and singlet oxygen (O2) along with various forms of activated oxygen [5,6]. They are involved in various physicochemical processes and disease such as aging [7], Cancer [8], atherosclerosis [9].

Helminthes derived from the Greek word "helminthes" meaning "worm". Helminthes is a broad categorical term referring to various types of parasitic worms that reside in the body [10]. The world health organization reveals that over two billion people are suffering from parasitic worm infections [11]. It is estimated that by the year 2025, about 57% of the population in developing countries will be influenced [12]. Anthelmintic are drugs that may act locally to expel worms from GIT or systemically to eradicate adult helminthes or development forms that invade organs and tissues [13]. Anthelmintic from the natural sources may play a key role in the treatment of these parasite infections. Increasing problems of development of resistance in helminthes against anthelmintics have led to proposal of screening medicinal plants for their anthelmintic activity [14, 15].

Luffa cylindrica belongs to the family cucurbitacea was originated in South Asia, South Europe and Africa and used in medicine, make up, slippers, hat sand wallpaper [16]. *Luffa cylindrica* is a sub-tropical plant, which requires warm summer temperate and long frost free growing season when grown in temperate regions. It is an annual climbing which produces fruit containing fibrous vascular system. It is difficult to assign with accuracy the indigenous areas of *Luffa* species [17]. The plant is reputed to have anti-tubercular and antiseptic properties. The extract of seeds has been used in snake bites, convulsions, cramps, tetanus and also in the treatment of syphilis [18]. The fruit is used in dropsy, nephritis, chronic bronchitis [19]. The seeds are considered as emetic and cathartic. The seed oil is reported to be used for skin infections. In the form of tincture, the fruit used in the treatment of ascites, jaundice, billiary and intestinal colitis and also in enlarged spleen and fever [20]. *Luffa cylindrica* has been reported to posses both medicinal and nutritional properties [21].

EXPERIMENTAL

Plant material and its extraction

The seeds of plant *Luffa cylindrica linn*. were procured in the month of april 2011 from the campus of R.K.D.F. College, Bhopal, Madhya Pradesh and were positively identified and confirmed by the botanist Dr. Anil Prakash, Department of Biotechnology, Barkatullah University, Bhopal. The dried seeds of plant were washed with water to remove soil and other adhering dirt under shade. Seeds were converted into moderately coarse powder by mechanical grinder, whereas the dried uniform seed powder was used for the extraction of active constituents of the plant, determination of ash value, extractive values, and loss on drying and phytochemical investigation. The coarsely powdered plant material (about 60 gm) was defatted with Petroleum Ether (60-80°C). The defatted plant material was again subjected to successive extraction with Petroleum ether (60-80°C), Benzene, Solvent ether, Chloroform, Acetone, Ethanol and Methanol using Soxhlet apparatus. The solvent was removed in vaccum at 40°C by using rotatory evaporator (Rota Evaporator Buchi Type) which gave Dark green, Green, Light green, and Green colour of all extracts respectively. The extracts were concentrated to dry mass.

Determination of antioxidant activity
Free radical scavenging activity (DPPH method)

Different concentrations of solvent extracts 10-100 mcg/ml were prepared in methanol. DPPH (0.004% in methanol) was used as free radical. Equal volume of different concentrations of solvent extracts and DPPH were mixed in clean and labeled test tubes separately and the tubes were incubated at room temperature in dark for 30 minutes. The optical density was measured at 517nm using UV-Vis Spectrophotometer (Shimadzu, UV-1700, Japan). The degree of stable DPPH decolorization to DPPHH (reduced form of DPPH) yellow indicated the scavenging efficiency of the extract. The % reduction and IC50 were calculated. The free radical scavenging activity (FRSA) (% antiradical activity) was calculated

using the following equation.

$$\% \text{ Antiradical activity} = \frac{\text{Control absorbance - Sample absorbance}}{\text{Control absorbance}} \times 100$$

Superoxide scavenging activity by alkaline DMSO method

This activity was measured by the reduction of NBT. Extracts were dissolved in DMSO to give the concentration of 10-100 mcg/ml. Alkaline DMSO (1 ml alkaline DMSO containing, 5 mM NaOH in 0.1 ml water and 0.9 ml Dimethyl sulfoxide) and NBT (25 mg of nitro-blue tetrazolium was dissolved in 25 ml of Dimethyl sulfoxide to give concentration of 1 mg/ml). To the reaction mixture containing 0.1 ml of NBT (1 mg/ml solution in DMSO) and 0.3 ml of the extract and standard in DMSO, 1 ml of alkaline DMSO (1 ml DMSO containing, 5 mM NaOH in 0.1 ml water) was added to give a final volume of 1.4 ml and the absorbance was measured at 560 nm. Extracts (10-100 µg/ml) were added to a hydrogen peroxide solution (0.6ml, 40mM). 300 µl of plain DMSO, 0.1 ml NBT solution and 1 ml alkaline DMSO was mixed and absorbance was taken at 560 nm and this was taken as control reading. The percentage of super oxide radical scavenging by the Luffa cylindrical extracts and standard compounds were calculated as follows [22-24]

$$\% \text{ Inhibition of NBT} = \frac{\text{Test Absorbance} - \text{Control Absorbance}}{\text{Test Absorbance}} \times 100$$

***In-vitro* Anthelmintic Activity**

Animal

Indian adult earthworms (*Pheretima posthuma*) were used to study anthelmintic activity. Earthworms were collected from the moist soil area of Bhopal and washed with normal saline to remove all faecal matter. The earthworms of 3-5cm in length and 0.1- 0.2cm in width were used for all experimental protocols.

Grouping of Animals

- Group 1 Control Group (Normal Saline)
- Group 2 Standard Group (Mebendazole)
- Group 3 Ethanol Group (40,60 & 80 mg/ml concentration)
- Group 4 Methanol Group (40,60 & 80 mg/ml concentration)
- Group 5 Chloroform Group (40,60 & 80 mg/ml concentration)

Evaluation of anthelmintic activity

The anthelmintic activity was carried out on adult Indian earthworm known as Pheretima posthuma due to its anatomical and physiological resemblance with the intestinal round worm parasites of human beings. Ethanolic, methanolic and chloroform extracts from the seeds of *Luffa cylindrica Linn*. were investigated for their anthelmintic activity against *Pheretima posthuma*. The anthelmintic assay was carried as per the method of Patil et al, 2010 with minor modifications. Group of test organisms each containing six earthworms of approximately equal size were released into 10 ml of desired preparation. The dose suspensions were prepared using carboxy methyl cellulose sodium (1% CMC), which is non-toxic and non-irritant used in oral and other formulations. Each group was treated with the following vehicle (1% CMC in normal saline) and solution of ethanol and methanol (40, 60, 80 mg/ml each) extracts of *Luffa cylindrica Linn* seeds. Mebendazole (40, 60, 80 mg/ml in 1% CMC) was used as standard reference. All drugs and extract suspensions were freshly prepared before starting the experiment. Observations were made for the time taken for paralysis and death of individual worms. Paralysis was said to occur when the worms were not able to move even in the normal saline. Death was conducted when the worms lost their motility followed with fading away of their body color. [25]

RESULTS

Phytochemical screening

The Qualitative screening of phytochemical compounds in ethanolic extract of *Luffa cylindrica* seeds revealed the presence of alkaloids, tannins, carbohydrates, steroids and cardiac glycosides and methanol extract revealed the presence of alkaloids, tannins, carbohydrates and cardiac glycosides.

Table 1 Preliminary Phytochemical Screening

S.N.	Chemical Tests	Phytoconstiuents	Methanol Extract	Ethanol Extract
1.	Hager's test	Alkaloids	+ve	+ve
	Wagner's test		+ve	+ve
	Mayer's test		-ve	-ve
2.	Treated with: Lead acetate	Tannins	+ve	+ve
	Dil. Potassium Permanganate solution		+ve	-ve
	Dil. Iodine solution		-ve	+ve
3.	Sudan Red III Test	Volatile oils	-ve	-ve
4.	Salkowski test	Steroids	-ve	+ve
5.	Molish test	Carbohydrates	+ve	+ve
	Non-Reducing polysaccharides:			
	Iodine test		-ve	+ve
	Tannic acid test for starch			
	Reducing sugars:		-ve	-ve
	Fehling's test			
	Benedict's test		+ve	+ve
			+ve	+ve
6.	Ninhydrin test	Amino acids	-ve	-ve
	Tyrosine test		-ve	+ve
	Cysteine test		-ve	-ve
7.	Legal test	Cardiac Glycosides	+ve	+ve
	Keller-Killiani test		+ve	+ve
8.	Borntrager's test	Anthraquinone	-ve	-ve
	Modified Borntrager's test	Glycosides	+ve	+ve

+ve = Present, -ve = Absent

Antioxidant assays

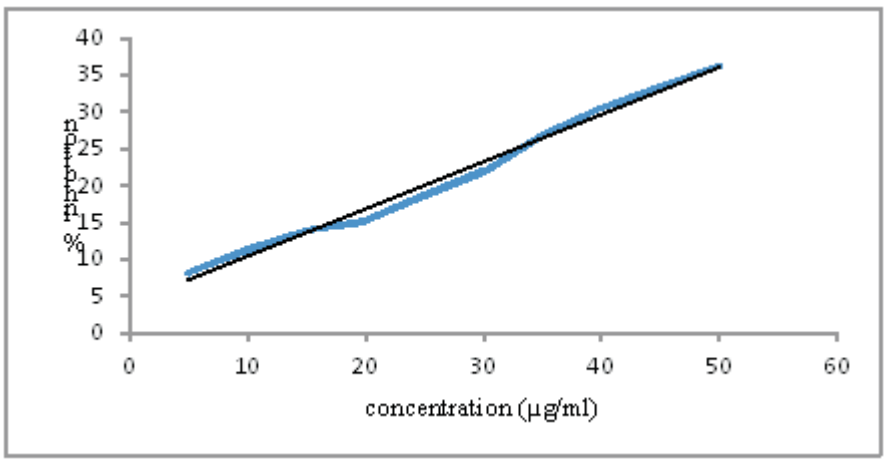
DPPH Radical Scavenging Activity

DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of the DPPH radical is determined by the decrease in its absorbance at 517 nm induced by antioxidants. The absorption maximum of a stable DPPH radical in ethanol was determined at 517 nm. The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidant molecules and radical progresses, which results in the scavenging of the radical by hydrogen donation. Graph 2 & 3 illustrate increase in scavenging of DPPH radicals in dose dependent manner due to the scavenging ability of the *Luffa cylindrica* ethanolic and methanolic extracts. IC50 value of ascorbic acid was found to be 13.34µg/ml. IC50 value of ethanolic, methanolic and chloroform extracts were found to be 63.49µg/ml, 43.84µg/ml and 35.49 µg/ml.

Table 2 - % Inhibition of DPPH by Ascorbic acid at different concentration

Concentration (µg/ml)	% Inhibition
10	8.16
20	11.23
30	13.92
40	15.23
50	18.69
60	21.84
70	26.89
80	30.56
90	33.42
100	36.24

Graph 1 - % Inhibition of DPPH by Ascorbic acid at different concentration

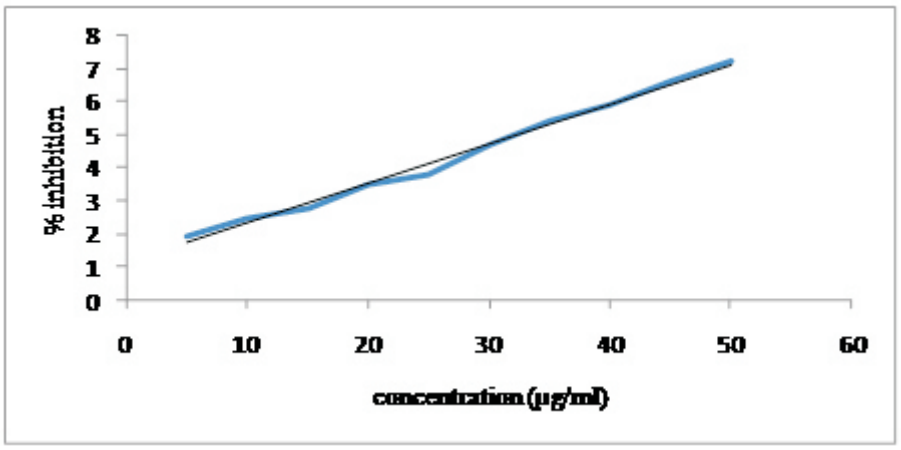


IC/50 – 13.34 (µg/ml)

Table 3 - % Inhibition of DPPH by ET at different concentration

Concentration (µg/ml)	% Inhibition
10	1.93
20	2.46
30	2.78
40	3.48
50	3.82
60	4.7
70	5.42
80	5.89
90	6.59
100	7.22

Graph 2 -% Inhibition of DPPH by ET at different concentration

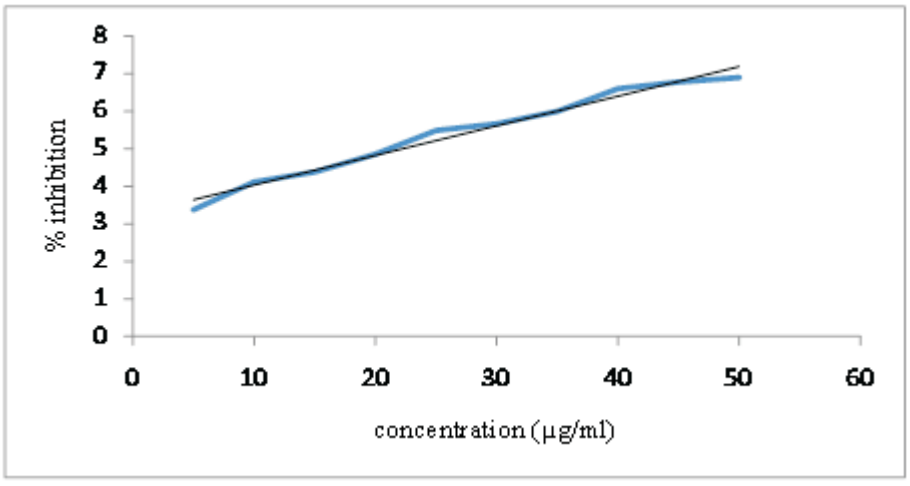


IC/50 – 63.49(µg/ml)

Table 4 - % Inhibition of DPPH by MT at different concentration

Concentration (µg/ml)	% Inhibition
10	3.38
20	4.12
30	4.38
40	4.86
50	5.49
60	5.67
70	6.00
80	6.60
90	6.78
100	6.90

Graph 3 -% Inhibition of DPPH by MT at different concentration

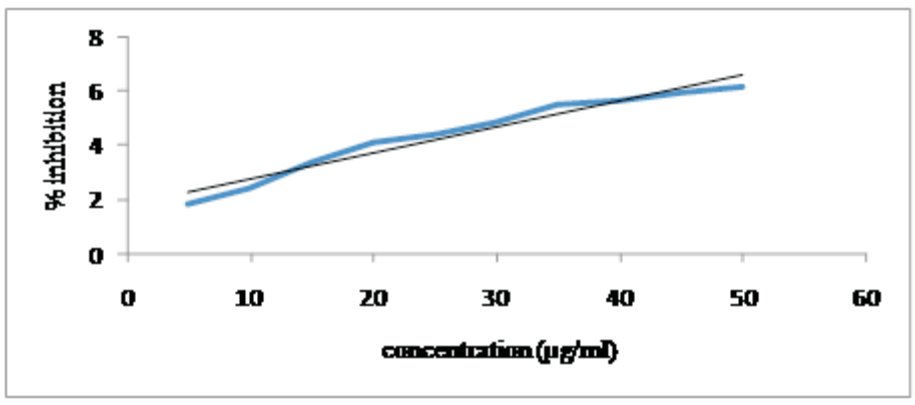


IC/50- 43.84(µg/ml)

Table 5 - % Inhibition of DPPH by Chloroform at different concentration

Concentration (µg/ml)	% Inhibition
10	1.86
20	2.46
30	3.38
40	4.12
50	4.38
60	4.86
70	5.49
80	5.67
90	5.95
100	6.15

Graph 4 -% Inhibition of DPPH by Chloroform at different concentration



IC/50- 35.49 (µg/ml)

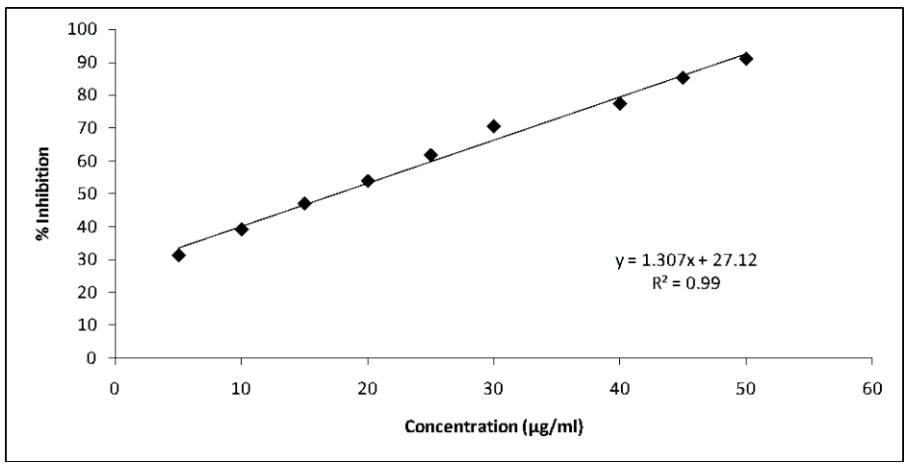
Superoxide scavenging activity by alkaline DMSO method

Superoxide radicals are known to be very harmful to the cellular component. Superoxide free radical was formed by alkaline DMSO which reacts with NBT to produce coloured diformazan. The ethanolic extract of *Luffa cylindrica* superoxide radical and thus inhibits formazan formation. Graph 4 & 5 illustrate increased scavenging of superoxide radicals in dose dependent manner due to the scavenging ability of the *Luffa cylindrica* ethanolic extract. IC50 Values of ethanolic, methanolic and chloroform extracts were found to be 26.94 µg/ml, 16.40µg/ml and 12.50 µg/ml.

Table 7– Superoxide scavenging activity of ET at different concentration

Concentration (µg/ml)	% Inhibition
5	31.37255
10	39.21569
15	47.05882
20	53.92157
25	61.76471
30	70.58824
40	77.45098
45	85.29412
50	91.17647

Graph 6 - Superoxide scavenging activity of ET at different concentration

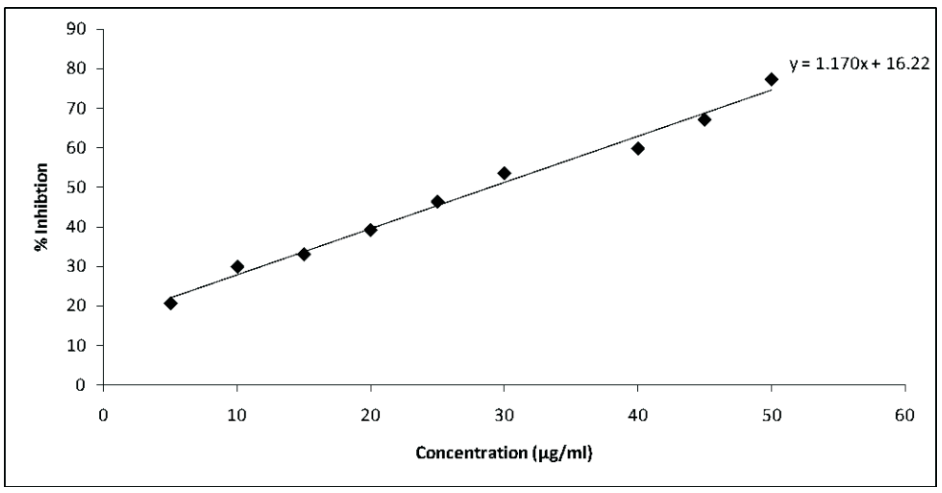


IC/50- 26.94(µg/ml)

Table 6 – Superoxide scavenging activity of MT at different concentration

Concentration (µg/ml)	% Inhibition
5	20.61856
10	29.89691
15	32.98969
20	39.17526
25	46.39175
30	53.60825
40	59.79381
45	67.01031
50	77.31959

Graph 5 - Superoxide scavenging activity of MT at different concentration

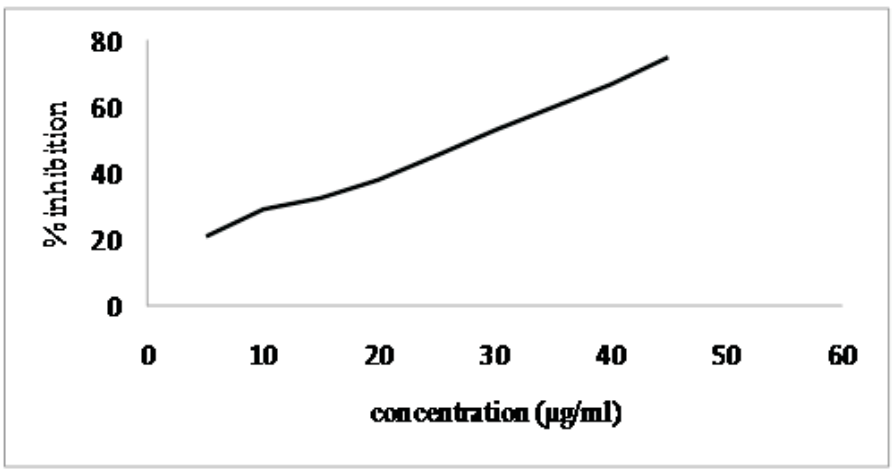


IC/50 – 16.40 (µg/ml)

Table 8– Superoxide scavenging activity of Chloroform at different concentration

Concentration (µg/ml)	% Inhibition
5	20.71856
10	28.89760
15	32.56989
20	38.19526
25	45.39174
30	53.15080
40	59.69010
45	67.01031
50	75.31959

Graph 7- Superoxide scavenging activity of Chloroform at different concentration



IC/50 – 12.50(µg/ml)

Anthelmintic activity evaluation

Ethanolic, Methanolic and Chloroform extracts in dose dependent manner showed anthelmintic

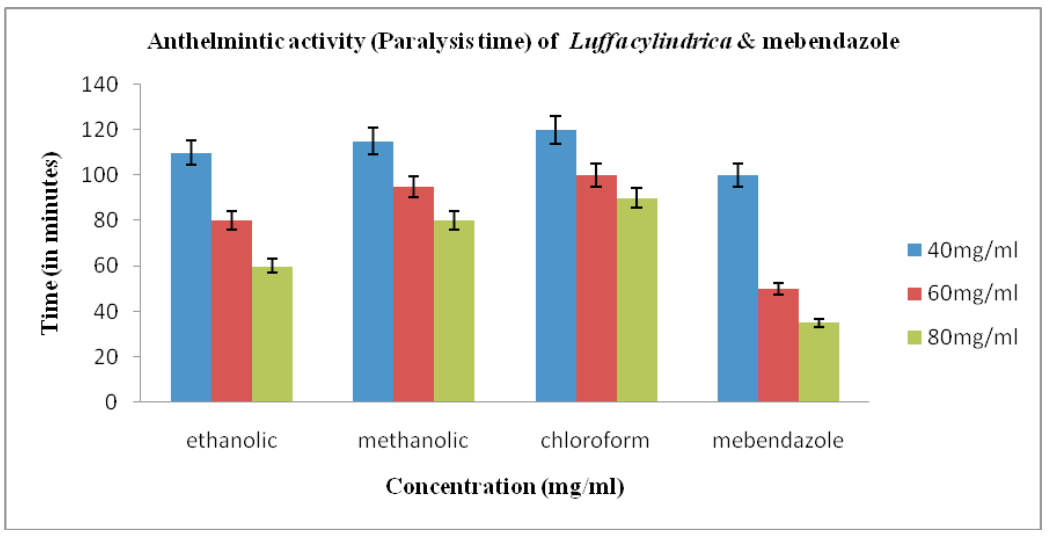
activity. Results were comparable with standard drug Mebendazole.

Table 9 - Anthelmintic activity of Ethanolic, Methanolic and Chloroform extract of Luffa cylindrica seeds.

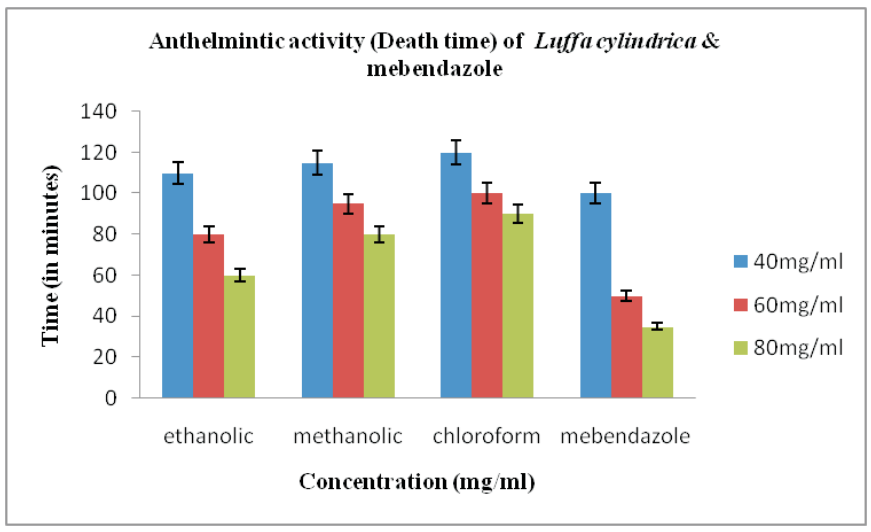
Treatment	Concentration (mg/ml)	Paralysis Time (in min)	Death Time (in min)
Ethanolic extract	40 mg/ml	40 ± 0.86	70 ± 0.57
	60 mg/ml	35 ± 0.28	50 ± 0.86
	80 mg/ml	30 ± 0.57	45 ± 0.57
Methanolic extract	40 mg/ml	35 ± 0.40	90 ± 0.28
	60 mg/ml	25 ± 0.57	70 ± 0.51
	80 mg/ml	28 ± 0.28	60 ± 0.34
Chloroform extract	40 mg/ml	110 ± 0.86	120 ± 0.57
	60 mg/ml	80 ± 0.51	90 ± 0.51
	80 mg/ml	65 ± 0.34	70 ± 0.28
	40 mg/ml	45 ± 0.28	100 ± 0.86
Mebendazole	60 mg/ml	35 ± 0.40	50 ± 0.46
	80 mg/ml	25 ± 0.40	35 ± 0.28
Control (Normal Saline)	–	–	–

Where P = Time taken for paralysis of worms (in min)
D = Time taken for death of worms (in min)

Graph 8: Anthelmintic activity of ethanolic and methanolic extracts of Luffa cylindrica



Graph 9:



DISCUSSION

The present study of *Luffa cylindrica* herb might be useful to supplement information in regards to its identification parameters assumed significantly in the way of acceptability of herbal drugs in present scenario of lack of regulatory laws to control quality of herbal drugs.

The phytochemical analysis of crude extracts indicated the presence of major phytocompounds, including phenolics, flavonoids, alkaloids, glycosides, steroids which may have been responsible for the observed antioxidant activity. Parasitic helminths affect animals and man, causing considerable hardship and stunted growth. Hundreds of millions if not billions of human infections by helminthes exist worldwide and increased world travel and immigration from the developing countries. However tremendous advances has been made during the previous decade and substantial number of synthetic precursors have been derived to cope up the damage caused by parasite, but unfortunately no effective medicine has been developed so far.

CONCLUSION

The DPPH free radical scavenging activity and Super oxide scavenging activity by alkaline DMSO method of ethanolic and methanolic extracts was determined. The ethanolic extract showed significant *in vitro* antioxidant activity in comparison to the standard antioxidant. The ethanolic extract was found to exhibit significant antioxidant activity as compared to the methanolic and chloroform extract extract.

The drug has been found to have anthelmintic activity in the ethanolic extract, methanolic extract and the chloroform extract but the most significant activity has been found in the ethanolic extract. It was found to be comparable with the standard drug Mebendazole. Further studies using in- vivo model are required to find out and establish effectiveness and pharmacological rationale for the use of seeds as anthelmintic drug. Further studies for isolation of active constituent from the ethanolic extract to establish the mechanism of action is required.

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REFERENCES:

1.Kalemba D, Kunicka A. Antibacterial and antifungal properties of essential oils. Cure Med Chem 2003;10:813-829.

2.Oyedemi SO, Bradley G, Afolayan AJ. In-vivo and in-vitro antioxidant activities of aqueous stem bark extract of Strychnos henningsii (Gilg). Afri J pharm and pharmacol 2010;4(Pt 2):70-78.
3.Raguveer C, Tandon RV. Consumption of functional food and our health concerns. Pak J Physiol 2009;5(Pt 1):76-83.
4.Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol 2004;26(Pt 2):211-219.
5.Rangari VD; Pharmacognosy and phytochemistry.;1sted.Career publications, Nashik,;2003.677-687.
6.Blois MS. Antioxidant determinations by the use of a stable free radical. Nature 1998; 29:1199-1200.
7.Finkol T, Holbnok NJ. Oxidant oxidative stress and the biology of aging. Nature 2000;408(Pt 6809):239-247.
8.Senthil K, Aranganathur S, Nalio N. Evidence of oxidative stress in the circulation of ovarian Cancer patients. Clin Chim Acta 2004;3(Pt 39):27-32.
9.Upston JM, Kritharides L, Stocker R. The role of vitamin E in athrosclerosis. Prog Lipid Res 2003;42:405-422.
10.Patel J, Kumar JS, Qureshi MS, Jena PK. Anthelmintic activity of Ethanolic extract of whole plant of Eupatorium odoratum L. Int J of Phytomed 2010;2(Pt 2):127-132.
11.Mulla WA, Thorat VS, Patil RV, Burade KB. Anthelmintic activity of leaves of Alocasia indica Linn. Int J of Pharmatech Res 2010;2(Pt 1):26-30.
12.Clewes CAN, Shaw C. Parasite. British Med Bull 2000;56:193-208.
13.Mohamed AAER, Ismail MAN, Mohamed AO, Moustafa FM. Cytotoxic effects of albendazole, antiparasitic drug on the liver of the rat; subchronic study. Egypt Jl of Biolog1999;1:16-29.
14.Aswar M, Aswar U, Watkar B, Vyas M, Wagh A, Gujar KN. Anthelmintic activity of Ficus berighalensis. Int J of Green Pharm 2008;2:120-172.
15.Iqbal Z, Lateef M, Ashraf M, Jabbar A. Anthelmintic activity of Aretemisio brevifolia in sheep. Journal of Ethanopharmacol 2004;93:265-268.
16.Jin HK, Hyeong JJ. Phytopthora Rot on Luffa cylindrica caused by phytopthora nicotianae. The Plant Patho J 2006;22(Pt 3):211-214.
17.Bal KE, Baly Lallam A. Gross morphology and absorption capacity of cell-fiber from the fibrous vascular system of loofah (Luffa cylindrical). Textile Res J 2004;74:241-247.
18.Stephens JM. Gourd Luffa-Luffa cylindrical, Luffa aegyptica and Luffa acutangula. J Hortic Sci Univ Florida 2003;3:19-21.
19.Chopra RN. Glossary of Indian medicinal plant, CSIR, New Delhi,1956,279.
20.Anonmymors, The wealth of India, vol.VI, CSIR, New delhi.1962,231.
21.Nagao T, Lanaka R, Iwase Y, Hanazone H, Okabe H. Studies on the constituents of Luffa acutangula Roxb. Pharm Bull 1991;39:599-606.
22.Patil AP, Patil VV, Patil VR. In-vitro free radicals scavenging activity of Madhuca indica Gel. Pharmacologyonline 2009;2:1344-1352.
23.Yerra R, Senthikumar GP, Gupta M. Studies on in vitro antioxidant activities of methonal extracts of Mucuna pruriens (Fabaceae) seeds. Eur Bull Drug Res 2005;1:31-39.
24.Sanja SD,Sheth NR, Patel NK, Patel Dhaval, Patel B. Characterization and evaluation of antioxidant activity of Portulaca oleracea. Int J Pharm Pharm Sci 2009; 1:74-84.
25.Wahid AM, Varad ST, Rohan VP, Kishor BB. Anthelmintic activity of leaves of Alocasia indica Linn. Int J Pharm Tech Res 2010;2(1):26-30.

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