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Phytomedicinal Potential of *Luffa cylindrica* (L.) Reom Extracts

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Only 100mg/ml of ethanol and water extract of fruit of *Luffa cylindrica*(L.)Reom., was invariably found to be potential enough in inhibiting skin infecting bacterial strains *Staphylococcus aureus*, *Staphylococcus epidrmidis, Micrococcus leutius, Pseudomonas aeruginosa and Eshcherichia coli* and fungal strains *Candida albicans mtcc-183, Candida tropicalis mtcc-184, Aspergillus niger mtcc-281, Trichophyton rubrum mtcc-294, Epidermophyton flocossum mtcc-613 and Microsporum canis mtcc-327.* However, these extracts were bactericidal as exhibited no growth even after incubation for more than 48 hours which adds value to its phytomedicinal property of these extracts. 250-500 µl/ml of seed oil could prevent the growth of all the microbial strains used for testing. The aqueous (250, 500 mg/kg) extract of fruit of *Luffa cylindrica*(L.)Reom., was evaluated for its hypoglycemic potential in normoglycemic rats followed by alloxan and glucose loaded hyperglycemic rats by single oral administration. The study report showed that the plant extract significantly ($p<0.05$) reduced blood glucose level both in normoglycemic rats induced by alloxan and oral glucose loaded methods till the end of 10h and 2h respectively during the course of experiment. The maximum fall of blood sugar level among the test drug, was observed in aqueous extract treated group (48.9%), and followed by ethanolic extract (37%) and seed oil (10.25%) at the end of 10h. The oral glucose tolerance test of the test substances revealed significant fall of blood sugar level and maximum fall was observed in seed oil treated group (50%), followed by aqueous extract (40.7%) and ethanolic extract (22%) at the end of 2h of the study

Key words: *Luffa cylindrica*(L.)Reom, Antidermatophytic, Hypoglycemic, Plant extract, Glibenclamide, Insulin.

Renewed interest in search of potential drug candidates of natural origin has motivated scientific community for exploration of different plant resources, the medicinal values of which have been restricted to ethnological uses and needs experimental validation. Science of drug development and disease treatment has long been realized plants as one of the potential source of treatment material against several human ailments. Pharmaceutical formulations with multiple activities have been of recent choice for treating several diseases including diabetes. The complicacy of the diseases like, diabetes further increases when it gets associated with several types of skin infections caused by bacteria, fungi or yeast, bringing about great health hazards and global socio-economic loss. The latest WHO estimate for the number of people with diabetes mellitus, worldwide, is projected to reach 346 million by 2030[1]. Estimated global healthcare expenditures to treat and prevent diabetes and its complications

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are expected to total at least 561 billion in 2030[1]. This calls for jumping into still untapped natural resources to find newer molecules to fights against such diseases.

Plants belonging to family cucurbitaceae have a rich history of treating common people suffering from diabetes of which many plants have been experimentally verified and reported to possess anti-diabetic activity [2]. Previous studies have reported antibacterial and antifungal activities of the ethanolic extracts of *Luffa cylindrica*(L.)Reom., fruit and in vitro antioxidant activity of *Luffa cylindrica*(L.)Reom., seed oil[3-4]. These reports however, exclude findings on their anti-dermatophytic activity. *Luffa cylindrica*(L.)Reom.,, an Indian Cucurbitaceous plant is selected in the present work for two best reasons; first being, it is a closely related species of anti diabetic plant *Luffa acutangula* and no *in vitro* or *in vivo* anti-diabetic study has so far been carried out by now using this plant. Secondly, as preliminary reports are available on this plant to exhibit antimicrobial activity but lacks any activity reports with reference to dermatophytes, [3-5] which are common source of infections in diabetic patients. Besides, available reports on anti-oxidant activity of Luffa extracts strengthen the criteria of selection of this plant to study these desirable properties [4-6]. Therefore, an attempt has been taken to assay the antidiabetic and antidermatophytic potential of seed oil and aqueous and ethanolic extracts of fruits of *Luffa cylindrica*(L.)Reom.,.

MATERIAL AND METHODS

Plant materials

The fruits and seeds of the plant, collected from Nayagarh District, Odisha, India, were identified to be *Luffa cylindrica*(L.)Reom., by Dr. P.C. Panda, taxonomist, Regional Plant Resources Center (RPRC), Bhubaneswar,India.

The collected seeds were washed thoroughly with water, air dried for a week at 40^0c. The seed coat was removed mechanically from the seeds and the kernels were pulverized using electronic grinder. The powdered seeds were extracted with hexane under Soxlet extraction procedure [4]. The solvent was distilled off and the oil was separated.

Preparation of Extract

The fruits of *Luffa cylindrica*(L.)Reom., were collected and dried under shade and then powdered using a mechanical grinder. The dried fruits were pulverized and subjected for successive extraction using ethanol and water for 6 hrs by using Soxlet apparatus [3]. The extracts were evaporated to dryness under reduced pressure using a Rota evaporator. The portion of the residue was re-dissolved in DMSO and their antimicrobial efficiency was noted.

Microorganisms Used

The following bacterial strains were obtained from Medical Microbiology departments, Institute of Medical Science and Sum Hospital, Sikhsa O Anusandhan University, Bhubaneswar, India and fungal strains were obtained from IMTECH, Chandigarh, India. The bacterial strains staphylococcus aureus , *Staphylococcus epidrmidis, Micrococcus leutius, Pseudomonas aeruginosa and Eshcherichia coli and fungal strains Candida albicans* mtcc-183*, Candida tropicalis* mtcc-184*, Aspergillus niger* mtcc-281*, Trichophyton rubrum* mtcc-294*, Epidermophyton flocossum* mtcc-613 *and Microsporum canis* mtcc-3270 were used as microbial strains for study.

Determination of Minimum Inhibitory Concentration

To measure the MIC values, micro-broth dilution method was used [7]. The extracts were serially diluted 2-fold in Mueller Hinton Broth medium to obtain various concentrations of the stock, 100, 50, 25, 12.5 and 6.25 mg/ml and were assayed against the test organisms.

Determination of Minimum Bactericidal and Fungicidal effect

Equal volume of the various concentration of each extract and Mueller Hinton broth were mixed in micro-tubes to make up 1ml of solution [7]. 1ml of McFarland standard of the organism suspension was added to each tube. The tubes were incubated aerobically at 37^oC for 24 hours. Two control tubes were maintained for each test batch. These include tube-containing extract without inoculums and the tube containing the growth medium and inoculum. The MBC/MFC was determined by sub culturing the test dilution on Mueller Hinton Agar and further incubated for 24 hours. The highest dilution that yielded no single bacterial/fungal colony was taken as the Minimum bactericidal/fungicidal concentration.

Determination Minimum Killing Time

This experiment was designed to determine the time required to kill the bacteria *in vitro* [8]. Equal volume of the each extract (at MIC level) and Mueller Hinton broth were mixed in micro-tubes to make up 1ml of solution. 1ml of McFarland standard of the organism suspension was added to each tube and incubated at 37^{0}C. One loop of the sample from the above test tubes were sub cultured on to a Mueller Hinton Agar plates at 0, 5, 10, 15, 30, 45, 60, 90,120,180 min intervals and incubated overnight.

Antidiabetic activity

Animals

The healthy Wistar albino rats, weighing 150–200g body weight of either sex were selected and housed in acrylic cages in standard laboratory conditions and were fed standard rodent diet with water *ad libitum*. The experiments on animals were conducted in accordance with the standard experimental procedure and the animals were used as per the experimental protocol duly approved by the Institutional Ethical Committee of the School of Pharmaceutical Sciences, Siksha 'O' Anusandhan University, Bhubaneswar, Orissa with registration No. IAEC 1171/C/08/CPCSEA.

Screening for antidiabetic activity

The Screening for antidiabetic activity was conducted as per the method described by Dash et al.(2001).The test samples were suspended in 25% Tween 20 in distilled water.

Glibenclamide (10mg/kg) was used as reference control during the study. The oil was administered by proportionately (based on density) diluting with olive oil through oral route.

Study on normoglycemic animals

The animals were fasted for 18 h, but were allowed to free access of water during course of the experiment. At the end of the fasting period, taken as zero time (0 h), blood was withdrawn (0.1ml) from the tip of the tail of each rat under mild ether anesthesia. Blood glucose levels were measured using clinical glucometer (Accu-Check Active®, Roche). The normal rats were then divided into five groups of six animals each. Group I served as solvent control and received only vehicle (2 ml/kg) through oral route, Group II received glibenclamide (10 mg/kg) and served as reference control. Groups III, IV and V received the aqueous extract, ethanolic extract and oil of fruits of *Luffa cylindrica*(L.)Reom.*,l*, each at doses of 200 mg/kg. Blood glucose levels were examined after 1, 2, 4, 6, 8 and 10 h of administration of single dose of test and control samples.

Study on alloxan induced diabetic animals

The acclimatized animals were kept fasting for 24 h with water *ad libitum* and injected intraperitoneally a dose of 150 mg/kg of alloxan monohydrate in normal saline. After 1 h, the animals were provided feed *ad libitum*. The blood glucose level was checked before alloxanisation and 24 h after alloxanisation as above. Animals were considered diabetic when the blood glucose level was raised beyond 200 mg/ ml of blood. This condition was observed at the end of 72 h after alloxanisation. The animals were segregated into five groups of six rats in each. Group I served as solvent control and received only vehicle (2 ml/ kg) through oral route. Group II received glibenclamide (10 mg/kg). Groups III, IV and V received the aqueous extract, ethanolic extract and oil of fruits of *Luffa cylindrica*(L.)Reom.*,*, each at doses of 200 mg/kg in a similar manner. Blood glucose level of each rat was estimated at 1, 2, 4, 6, 8 and 10h, respectively.

Oral glucose tolerance test

In order to select the optimal dose of the extract to be used in this study, the glucose tolerance test was first performed in normal rats using single doses of the extract (200 mg/kg) during 120 min. After acclimatization, rats were divided into five experimental groups (n = 5): Group I, normal rats administered with *Luffa cylindrica*(L.)Reom.*,* aqueous extract (200 mg/kg); Group II, normal rats received ethanolic extract (200 mg/kg); Group III, normal rats received oil extracts (200 mg/kg); Group IV, normal rats received glibencamide (10 mg/kg) and Group V, normal rats received vehicle (NaCl 0.9% w/v +DMSO 3%).

Normal rats fasted overnight received glucose (2 g/kg b.w.) 30 min before the administration of the extract, the vehicle and the standard drugs. Blood samples were collected from the tail vein before the administration of glucose and at 0 min, 30, 60, 90 and 120 min later. Blood glucose levels were measured using clinical glucometer (Accu-Check Active®, Roche).

Statistical analysis

All the results were analyzed statistically using one-way analysis of variance (ANOVA)

followed by Dunnet's t-test. A *p*-value less than 0.05 is considered significant. All the results are expressed as Mean ± S.E.M for six animals in each group.

RESULTS AND DISCUSSION

100 mg/ml of water extract has been found to be sufficient enough to inhibit all the bacterial culture and two fungal strains *Trichophyton rubrum* and *Epidermophyton flocossum in vitro.* It required only 500 µl/ml amount of seed oil to prevent the growth of all the microbial strains used for testing except *Micrococcus leutius* and Microsporum canis which required even lesser amount of the oil ie 250 µl/ml (table 2). Ethnolic extract need 50 to 100mg/ml only to inhibit the growth of these microorganisms. This indicates ethanolic and water extracts can be considered to be potential prospect molecule to treat skin infecting pathogens. The fact that, these extracts at their MIC value show bactericidal property, as exhibited no growth in culture of these pathogens even after incubation for more than 48 hours, adds value to these extracts to its phytomedicinal property.

Table 1. Determination of MIC against bacteria using three extracts of *Luffa cylindrica(L.)Reom.,l*

Name of the microorganism	Seed oil (µl/ml)	Ethanolic extract(mg/ml)	Water extract(mg/ml)	Ciprofloxacin(mg/ml) 1
Staphylococcus aureus	500.0	100.0	100.0	0
Staphylococcus epidermids	500.0	100.0	100.0	6.25
Micrococcus leutius	250.0	50.0	100.0	12.5
Pseudomonas aeruginosa	500.0	100.0	100.0	3.125
E.coli	500.0	100.0	100.0	6.25

Table 2. Determination of MIC against fungal strains using three extracts of *Luffa cylindrica(L.)Reom.,l*

Name of the microbes	Minimum Inhibitory Con.(mg/ml)			
	Seed oil (µl/ml)	Ethanolic extract (mg/ml)	Water extract (mg/ml)	Fuconazole (mg/ml) 1
Candida albicans	500.0	50.0	0	6.25
Candida tropicalis	500.0	50.0	0	6.25
Trichophyton rubrum	500.0	100.0	100.0	0
Epidermophyton flocossum	500.0	100.0	100.0	0
Microsporum canis	250	50.0	50.0	12.5

Table 3. Effect of various extracts of *Luffa cylindrica(L.)Reom.,* on blood glucose level in normoglycemic animals

Groups & treatment	Blood Glucose Levels (mg/dl)						
	0hr	2hr	4hr	6hr	8hr	10hr	% decreases at 10hr
Solvent control (2ml/kg)	95.66±0.66	92.5±0.76	89.66±0.55	88.16±0.79	86.5±1.17	85.16±0.79	
Glibencamide (10mg/kg)	91.83±0.90	85.33±1.20^{b}	78.5±0.76^{b}	64.5±0.99^{c}	55.16±1.35^{c}	43.33±1.17^{c}	52.81
Aqueous (200mg/kg)	117.83±3.91	109.5±1.25	86.83±0.79^{a}	62.16±6.46^{c}	61.5±0.76^{c}	60.16±0.94^{c}	48.94
Ethanol (200mg/kg)	92.33±1.87	90.83±0.47	74.5±5.11^{b}	63.66±4.50^{c}	59.66±0.66^{c}	58.16±0.87^{c}	37.00
Oil (200mg/kg)	97.5±2.60	94.5±2.33	92.33±1.60	89.83±1.51	88.5±1.47	87.5±1.72	10.25
F	21.58**	46.25**	9.46**	14.79**	197.20**	269.96**	

Table 4. Effect of the various extracts of *Luffa cylindrica(L.)Reom.,* in alloxan induced diabetic rats

Teatment & Dose	Blood Glucose Levels (mg/dl)											% decrease
	0hrs	1hrs	2hrs	3hrs	4hs	5hs	6hrs	7hrs	8hrs	9hrs	10hrs	at 10hr
Solvent (2ml/kg)	224.33±1.14	222.83±0.70	220.5±0.76	219..33±1.28	217.66±1.05	214.66±0.88	211..5±0.76	208.33±1.05	206..33±0.88	205.16±1.30	202.83±1.19	
Glibencamede (10ml/kg)	228.83±0.47	226.66±0.80	224.33±1.35	223.5±0.76	184.16±0.60c	151.83±0.79c	138.16±0.94c	126.5±0.76c	113.5±0.99c	103.33±0.88c	98.16±0.94c	57.10
Aquous extracts (200mg/kg)	227.5±0.76	224.66±1.08	222.5±0.76	221.16±0.70	208.33±2.40c	195.16±0.87c	183.16±0.94c	169.16±1.13c	163.16±1.16c	157.33±0.88c	154.5±1.28c	32.08
Ethanolic extracts 200mg/kg)	226.66±0.76	224.33±0.88	222.33±1.40	221.5±0.88	213.66±0.71	203.83±0.90c	198.33±0.88c	183.66±1.14c	176.16±0.79c	169.33±0.88c	158.33±0.98c	30.14
Oil (200mg/ml)	226..33±0.88	223.16±0.87	1221.16±1.13	221.5±0.92	217.5±0.88	216.66±0.98	212.66±2.72	209.16±1.13	206.5±1.05	204.83±1.13	200.16±0.94	11.56
F	3.92**	2.97*	1.71	2.51	114.63**	877.14**	444.95**	1037.75**	1510.33**	1652..36**	1553.87	

Table 5. Effect of various extracts of *Luffa cylindrica(L.)Reom.,* on tolerance of oral glucose in normal rats

Treatment & doses	Initial glucose levels	Blood Glucose Levels (mg/dl)					
		0h	0.5h	1h	1.5h	2h	% age decreases at 2h
Control (2ml/kg)	116.16±0.94	128.5±1.17	123.33±0.88	119.33±0.88	121.16±0.60	116.16±0.87	
Glibencamide(10mg/kg)	127.5±1.11	132.16±0.94	114.33±1.11^{c}	96.83±0.98^{c}	81.5±0.76^{c}	54.3±1.14^{c}	57.38
Aqueous extracts (200mg/kg)	110.16±4.43	137.5±5.42	114.83±2.54^{b}	103.33±2.71^{b}	96.5±1.56^{c}	81.5±2.43^{c}	40.72
Ethanolic extracts (200mg/kg)	97.16±5.87^{b}	116.33±1.17^{a}	97.5±0.56^{c}	95.16±1.10^{c}	96.16±1.27^{c}	90.5±1.87^{c}	22.20
Oil extracts(200mg/kg)	98.83±5.66a	121.16±3.09	94.83±0.79^{C}	94.16±7.09^{c}	68.5± 7.57^{c}	60.5±6.64^{c}	50.06
F	8.95**	8.40**	79.38**	8.99**	31.00**	55.23**	

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test; (F-value denotes statistical significance at *p<0.05, **p<0.01); (t-value denotes statistical significance at ap<0.05, bp<0.01 and cp<0.001 respectively, in comparison to group-I).

The experimental results of the effect of various extracts and oil of seeds of *Luffa cylindtrica* in normoglycemic rats (Table 3) showed that blood glucose levels decrease significantly ($p<0.05$) with effect from 4h onwards till the end of 10h, including the standard drug glibenclamide. The maximum fall of blood sugar level among the test drug, was observed in aqueous extract treated group (48.9%), and followed by ethanolic extract (37%) and seed oil (10.25%) at the end of 10h. However at the same time the standard drug registered a fall of blood sugar level of 52.8%. The result of the study indicates that the seeds of *Luffa cylindrica(L.)Reom.,* may have hypoglycemic effect.

The test extracts and seed oil was again tested in alloxan induced hyperglycemic animals and the result of the study showed that all test substances progressively decreases the blood sugar level in a significant extent ($p<0.05$). The maximum fall of blood sugar level was shown by the standard drug treated group (57%), followed by aqueous extract (32%), ethanol extract (30%) and seed oil (11.5%), as shown in table 4. The findings of the study revealed that the extracts and oil of test seeds have property to decrease the blood sugar level in hyperglycemic animals. The said property may be due to pancreatic and/or extra pancreatic action of the test substances.

The oral glucose tolerance test of the test substances revealed significant fall of blood sugar level and maximum fall was observed in seed oil treated group (50%), followed by aqueous extract (40.7%) and ethanolic extract (22%) at the end of 2h of the study (table 5). The fall of blood sugar level in excess of oral glucose administered rats, suggest that the seeds of *Luffa cylindrica(L.)Reom.,* may affect glucose absorption in the intestine or may have pancreatic/ extra pancreatic action or combination of any of the effects.

It is generally accepted that alloxan treatment causes permanent destruction of â-cells and impairment of renal function; and sulfonylureas are known to lower the blood glucose level by stimulating â-cells to release insulin [10]. However, the statistically significant antihyperglycemic as well as hypoglycemic activities shown by the aqueous and ethanolic extract of *Luffa cylindrica(L.)Reom.,* seed in both single dose treated normoglycaemic and hyperglycaemic models might suggest that the said effect be due to extra pancreatic and extra-intestinal action of the test extract [11]. The hypoglycemic effect comparable to glibenclamide suggested that the extract may act by regenerating the â-cells in alloxan-induced diabetes [12]. And the decreased activity in glucose level in OGTT might be, due to a decrease in the rate of initial glucose absorption when plant fiber is given orally with glucose [11].

CONCLUSION

In this study, both aqueous and oil extract of *Luffa cylindrica(L.)Reom.,* fruits seemed to have hypoglycemic potential as evident from several animal model and *in vitro* assay and may be further studied for beneficial therapeutic effects in diabetic. As , in addition its extracts have potential anti dermatophytic activity against a range of skin infecting pathogens, such plants can definitely serve as an important source of multiple properties like anti dermatophytic, antidiabetic and antioxidant etc.

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