Available online at www.ijpsdr.com

International Journal of Pharmaceutical Sciences and Drug Research 2015; 7(4): 340-344

Research Article

ISSN: 0975-248X
CODEN (USA): IJPSPP

Wound Healing, Phytochemical and Antimicrobial Properties of *Luffa cylindrica* (Linn.) Seed Extracts

Bassey S. Antia[1*], Emmanuel E. Essien[1], Jude E. Okokon[2], Iyiade G. Alalade[1]

[1]*Department of Chemistry, University of Uyo, Akwa-Ibom, Nigeria*
[2]*Department of Pharmacology and Toxicology, University of Uyo, Akwa Ibom, Nigeria*

ABSTRACT

The study investigates the wound healing and antimicrobial activities of extracts from *Luffa cylindrica* (Linn) seeds. The seed extracts (n-hexane, chloroform, diethyl ether, ethyl acetate, butanol and methanol) were evaluated for wound healing properties in white albino rats using the full thickness skin excision model. Thirty-two female wistar albino rats were randomly divided into four groups. Animals in the test groups were treated topically with 1 ml of 200mg/ml of the extracts for 17 days post-surgery. The standard and control groups were treated with Neobacin® powder (neomycin and bacitracin) and sterile distilled water respectively. Significant (P<0.05) wound contractions were observed across the groups. Diethyl ether extract had the most prominent wound healing activity while chloroform was least active. The median lethal dose (LD_{50}) of the methanol seed extract administered intraperitoneally in wistar albino mice was found to be 24.5 mg/kg. Different concentrations of the various extracts of *L. cylindrica* seeds were evaluated for antimicrobial activity against selected wound pathogens (*Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli, Proteus vulgaris* and *Candida albicans*) using agar well diffusion method and the minimum inhibitory concentrations (MIC) of each were determined. The seed extracts were active against gram positive, gram negative and fungus; the M.I.C. of various extracts of seeds extracts range from 0.04-0.6 g/ml. Furthermore, phytochemical studies revealed the presence of saponins, tannins, flavonoids, carbohydrates, cardiac glycosides, deoxy-sugar, terpenes, phlobatannins and alkaloid. The way the different extracts functioned on the selected wound pathogens depended on the phytochemicals present in the extract.

Keywords: *Luffa cylindrica,* wound healing, antimicrobials, excision wound model, agar well diffusion.

INTRODUCTION

Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, neutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and clinical entities for synthetic drugs. [1]

Luffa cylindrica (Linn.) (Smooth loofah or sponge gourd) is an annual climbing plant that belongs to the family Cucurbitaceae. It is an herbaceous plant and thrives commonly with twining tendrils. [2] The fruit has a network of fibres surrounding a large number of flat blackish seeds. In traditional medical practice, the fruits are reported to have anthelmintic, carminative, laxative, depurative, emollient, expectorant, tonic and galactogogue effects. [3-4] Earlier studies have shown that the plant possesses a number of medicinal properties including anti-inflammatory [5], anaesthetic,

***Corresponding author: Dr. B. S. Antia,**
Department of Chemistry, University of Uyo, Akwa-Ibom, Nigeria; **E-mail:** antiabassey@yahoo.com
Received: 30 April, 2015; **Accepted:** 08 June, 2015

anti-cancer [6], hepatoprotective [7], antimicrobial [8] and enzyme-inhibitor effect. [9] The seeds are said to have healing properties and also effective in the treatment of asthma, sinusitis and fever [10]; seed oils are employed in cosmetics for its anti-tumour, anti-inflammatory and antimicrobial activities in the treatment of skin diseases. However, there has been paucity of detailed scientific information on wound healing and antimicrobial activity of *L. cylindrica* seed extracts on some clinical isolates that can help ascertain some folkloric claims. This work, is therefore, aimed at examining the wound healing potentials of *L. cylindrica* seed extracts in correlation with the *in vitro* growth inhibition of selected wound pathogens.

MATERIALS AND METHODS

Sample Collection and Preparation

Mature dried fruits of *L. cylindrica* pods were collected in the month of March from the wild in Abak Local Government Area of Akwa Ibom State, Nigeria. The sample was identified and authenticated by Dr. (Mrs.) M. E. Bassey of the Department of Botany, University of Uyo, Nigeria and a voucher specimen was deposited at the Herbarium of the department. The pods were beaten with a wooden club to free the seeds for easy removal. The seeds released from the pods were washed, sun-dried, dehulled and pulverized using a laboratory manual grinder.

Preparation of Extracts

The pulverized seed sample was macerated in n-hexane for 72 hours and fixed oils obtained by distillation. The oil-free residue was successively extracted with solvents of increasing polarity: chloroform, diethyl ether, ethyl acetate, n-butanol and methanol. The filtrates were concentrated at 40°C to obtain the crude extracts.

Experimental Animals

Thirty nine (39) wistar albino mice of either sex weighing 20-25 g of 70 days were used for acute toxicity studies. Thirty two (32) adult female albino rats (wistar strain) weighing 150-220 g were also employed for wound healing evaluation. The animals were procured from the animal house, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo. The animals for acute toxicity studies were fasted 24 hours prior to drug administration to avoid food-drug interaction but were allowed access to water *ad libitum.* The animals for wound healing evaluation were fed standard rat ration (Grower's mash) and water *ad libitum.* The animals were kept in wooden cages under standard environmental condition (25 ± 2°C) at a natural day and night cycle. Permission and approval for animal studies were obtained from the College of Health Sciences, Animal Ethics Committee, University of Uyo, Uyo.

Determination of LD_{50}

Median lethal dose (LD_{50}) was determined using Lorke's method. [11] Thirty nine albino mice of wistar strain were randomized and divided into groups of thirteen of three mice per group. The methanol extract was administered intraperitoneally (*i.p*) in a dose range of 10-5,000 mg/kg and observed for 24 hours.

Wound Healing Activity

Excision Wound: The animals were anaesthetized prior to and during creation of wounds with ether. The dorsal fur of the animals was shaved and the anticipated area of the wound to be created was outlined with methylene blue using a circular stainless stencil of approximately 14 mm in diameter. An excision wound was inflicted by cutting away full thickness along the marking on the depilated back.

Experimental Design: The animals were randomly divided into eight groups of four rats each. Group I –VI were treated with n-hexane, chloroform, diethyl ether, ethyl acetate, butanol and methanol extracts respectively. Group VII were treated with Neobacin® powder as standard while group VIII were treated with sterile water as control. The extracts (1ml) were applied once daily to treat different groups of animals. Wound closure was assessed by tracing the wound every alternate day of post-wounding using transparent tracing paper and a permanent marker. The tracing paper was placed on a graph sheet, measured and the areas recorded in mm^2. [12]

Microorganism Used

Antimicrobial tests were carried out using bacteria and fungus obtained from clinical samples isolated from the medical laboratory section of St. Luke's Hospital, Anua, Uyo, Akwa Ibom State. The microorganisms were *S. aureus, P. aeruginosa, K. pneumonia, P. vulgaris, E. coli* and *C. albicans.* All the bacteria were suspended in nutrient broth and the fungus in Saboraud dextrose agar (SDA).

Evaluation of Antimicrobial Activity

Antimicrobial activity of *L. cylindrica* seed extracts was evaluated by agar well diffusion method. [13] Freshly prepared 24 hours culture was spread on to the sterile nutrient agar plates. Spreading was done using swab which was spread on to the plates uniformly with wells punched into the plates using a sterile cork borer of 5 mm diameter. Streptomycin and nystatin were used as standards for antibacterial and antifungal assays respectively. Different concentrations of the extracts were used in screening for antimicrobial activity (0.1, 0.2, 0.3 and 0.4 g/ml). Inoculated bacterial culture was incubated at 37°C for 24 hours and fungal culture at 31°C for 48 hours. [14] Antimicrobial activity was determined by measurement of zone of inhibition around each well in plate. The M.I.C for the extracts was determined by broth dilution method. [14] Different extracts of various dilutions were incubated at 37°C for 24 hours and the results were recorded.

Phytochemical Analysis

Preliminary phytochemical screening for saponins, tannins, flavonoids and carbohydrates was conducted using standard methods by Trease and Evans [15]; cardiac glycosides, phlobatannins, anthraquinones, deoxy-sugars, terpenes and alkaloids were also determined following the methods of Sofowora. [16]

Statistical Analysis

Results of all the estimations were indicated in mean ± SEM. Difference between the groups was statistically determined by analysis of variance (ANOVA) with Dunnett's test multiple comparisons using SPSS package. The level of significance was set at $P<0.05$.

RESULTS AND DISCUSSION

Phytochemical Screening

The result of the preliminary phytochemical screening (Table 1) reveals that methanol extract contains high amount of saponins, alkaloids and phlobatannins; butanol extract (alkaloids and deoxy-sugars); diethyl ether extract (deoxy-sugars, cardiac glycosides, alkaloids and carbohydrate) and chloroform extract (deoxy-sugars and cardiac glycosides). Anthraquinones were not detected in any of the extracts studied. Muthumani *et al.* [8] made a similar observation showing the presence of sugar, protein, alkaloids, sterols, flavonoids and glycosides as major constituents of *L. cylindrica* seeds. A number of triterpenoids and fibrinolytic saponins have been isolated and characterized from seeds [17-18] and fruits [19] of *L. cylindrica.* The flavonoid, apigenin [20] has also been identified.

Acute Toxicity

The result of the median lethal dose (LD_{50}) of methanol extract of *L. cylindrica* seeds is presented in Table 2. The methanol extract (which contains high amount of saponins, alkaloids and phlobatannins) caused dose-dependent mortality where the limit test dose of 5000 mg/kg body weight was used. No test substance related mortality was observed between 10-20 mg/kg body weight while 100% mortality was recorded from 30-5000 mg/kg body weight. The median lethal dose (LD_{50}) of the methanol extract of the seeds was determined to be 24.5 mg/kg body weight. The toxicity observed might be due to the intraperitoneal route of administration (higher bio-availability) as the methanol extract contains high concentration of saponins which when injected into the blood stream are highly toxic because of their ability to lyse erythrocytes *in vivo* consequently reducing the oxygen-carrying capacity of the blood [21], but when administered orally becomes comparatively harmless. Ilango *et al.* [22] established a median lethal dose (LD_{50}) of 3000 mg/kg body weight for methanol extract (containing glycosides, saponins, and carbohydrates) of fruit pulp of *Momordica balsamina* (Cucurbitaceae) in albino rats. Disparity in results obtained might be from the different experimental animals used (mice and rats) and route of administration (intraperitoneal and oral). Anamika *et al.,* [18] reported that *L. cylindrica* seeds contain various triterpenoid saponins, some of them highly toxic and also shown to demonstrate *in vivo* immunostimulatory effects in mice.

Wound Healing Activity

The effect of the various extracts of *L. cylindrica* seed is presented in Table 3 and 4 respectively. Significant promotion of wound healing was observed in diethyl ether, methanol, butanol and ethyl acetate while hexane and chloroform extracts had relatively poor healing activity. Diethyl ether extract showed the most prominent wound contraction rate with a mean percentage closure of 16%, 39%, 65%, 86% and 99% on the 3rd, 6th, 9th, 12th and 15th day post-surgery respectively when compared with the standard. Methanol and butanol also showed relatively fast wound closure at 99% and 96.4% respectively 17th day post-surgery.

The phytochemicals (flavonoids, saponins, carbohydrate, terpenes) present in diethyl ether extract may be responsible for the significant wound contraction and epithelization. It has been evident that phytoconstituents such as catechins can significantly improve the quality of wound healing and scar formation [23], flavonoids because of their antioxidant property accelerates the wound healing process and could be potential therapeutic tool in the treatment of SMC- rich vascular lesions. [24-25] Dhanalekshmi *et al.* [26] also reported better wound healing pattern with complete wound closure observed in groups treated with ethanolic extract of *L. cylindrica* leaf and flower within 16 days. This report justifies the use of *L. cylindrica* in folkloric medicine for wound healing.

Table 1: Phytochemical screening of *L. cylindrica* seed extracts

Test	Chloro form extract	Diethyl ether extract	Ethyl acetate extract	Butanol extract	Metha nol extract
Saponins	+	++	-	++	+++
Anthraquinones	-	-	-	-	-
Deoxy-sugar	+++	+++	+	+++	++
Cardiac glycosides	+++	+++	++	++	++
Tannins	-	++	-	+	++
Alkaloids	++	+++	++	+++	+++
Flavonoids	-	++	-	+	+
Phlobatannins	-	-	-	-	+++
Carbohydrate	+	+++	+	++	++
Terpenes	+	++	+	++	++

+++ = Highly present; ++ = Moderately present; + = Presence in trace amount; - = Absent or negligible amount

Table 2: Acute toxicity (LD_{50}) of methanol extract of *L. cylindrica* seeds

Concentration of Extract (mg/kg)	Mortality rate	% mortality
5000	0/3	100
1000	0/3	100
800	0/3	100
600	0/3	100
400	0/3	100
300	0/3	100
200	0/3	100
100	0/3	100
50	0/3	100
40	0/3	100
30	0/3	100
20	3/3	0
10	3/3	0

$LD_{50} = \sqrt{D_o \times D_{100}}$ Where D_o =maximum dose that produced 0% mortality, D_{100} =Minimum dose that produced 100% mortality

$\therefore LD_{50} = \sqrt{20 \times 30} = 24.49$ mg/kg $\simeq$ 24.5 mg/kg

Table 3: Percentage wound healing evaluation of *L. cylindrica* seed extracts

Post Wounding Days	Hexane	Chloroform	Diethyl ether	Ethyl acetate	Butanol	Methanol	Standard (Neobacin)	Control (Water)
0	0%	0%	0%	0%	0%	0%	0%	0%
3	0%	26.5%	15.99%	15.99%	16.7%	15.1%	22.9%	16.9%
6	35.07%	40.9%	39.2%	25.3%	32.3%	39.9%	55.6%	40.6%
9	26.5%	43.3%	64.69%	49.4%	34.1%	53.4%	68.7%	58.7%
12	53.5%	41.3%	86.4%	49.7%	60.9%	64.1%	80.9%	76%
15	57.14%	19.3%	99%	72.2%	87.5%	90.5%	94.2%	84.3%
17	70.9%	31.6%	100%	83.1%	96.4%	99.1%	99.6%	95%

Table 4: Effect of topical application of extracts of *L. cylindrica* seed on excision wound model

Post Wounding Days	Comparative Mean Wound Area in mm²							
	Hexane	Chloroform	Diethyl ether	Ethyl acetate	Butanol	Methanol	Standard (Neobacin)	Control (Water)
0	196 ± 0.00	196 ± 0.00	211 ± 15.00	211 ± 15.00	196 ± 0.00	243 ± 0.00	262 ± 48.12	196 ± 0.00
3	196 ± 0.00*	144 ± 0.00*	177 ± 20.08*	177 ± 20.08*	163 ± 12.40*	206 ± 30.18	202 ± 42.47	163 ± 6.25
6	127 ± 10.57*	116 ± 5.27	128 ± 17.08*	156 ± 15.17*	132 ± 6.69*	146 ± 19.62*	116 ± 0.49	116 ± 10.49
9	144 ± 0.00*	111 ± 11.00*	75 ± 14.72	107 ± 15.19*	129 ± 22.79*	113 ± 19.34*	82 ± 0.39	81 ± 7.35
12	91 ± 9.00*	90 ± 8.71*	28 ± 12.27*	106 ± 25.53*	76 ± 22.77*	75 ± 15.52*	50 ± 8.08	47 ± 18.28
15	84 ± 16.00*	157 ± 81.44*	2 ± 1.15*	58 ± 24.91*	24 ± 13.26*	23 ± 7.89*	15 ± 7.54	12 ± 8.38
17	57 ± 7.00*	134 ± 88.91*	0 ± 0.00	35 ± 16.93*	9 ± 9.00	2 ± 1.15	1 ± 1.00	2 ± 2.00

Data presented as Mean ± S.E.M; Significant at $P<0.05$ where n = 4

Table 5: Zone of inhibition of *L. cylindrica* seed extracts (mm)

		Microorganisms					
		S. aureus	*P. aeruginosa*	*K. pneumonia*	*P. vulgaris*	*E. coli*	*C. albicans*
Hexane (g/ml)	0.1	-	-	-	-	13	-
	0.2	-	-	-	-	15	-
	0.3	-	-	-	-	16	-
	0.4	-	-	14	-	20	-
Chloroform (g/ml)	0.1	-	-	-	-	30	-
	0.2	-	15	-	20	30	-
	0.3	-	18	-	20	30	-
	0.4	-	20	-	20	30	-
Diethyl ether (g/ml)	0.1	15	-	15	12	-	-
	0.2	15	-	15	13	15	-
	0.3	20	-	20	20	17	-
	0.4	25	-	25	25	20	-
Ethyl acetate (g/ml)	0.1	12	16	-	-	11	-
	0.2	15	16	-	-	15	-
	0.3	15	16	-	-	15	-
	0.4	16	20	15	15	20	-
Butanol (g/ml)	0.1	-	25	20	-	16	15
	0.2	12	30	20	20	18	16
	0.3	13	30	22	20	18	18
	0.4	13	30	23	23	20	20
Methanol (g/ml)	0.1	-	12	-	-	12	11
	0.2	-	15	-	-	12	12
	0.3	15	18	-	20	20	15
	0.4	18	23	15	20	20	20
Streptomycin (g/ml)	0.1	20	25	18	20	20	ND
Nystatin (g/ml)	0.1	ND	ND	ND	ND	ND	12

ND = Not detected; (–) = No zone

Table 6: Minimum inhibitory concentrations of *L. cylindrica* seed extracts

Microorganism	Hexane (g/ml)	Chloroform (g/ml)	Diethyl ether (g/ml)	Ethyl acetate (g/ml)	Butanol (g/ml)	Methanol (g/ml)
S. aureus	0.46	0.5	0.08	0.1	0.2	0.24
P. aeruginosa	0.5	0.16	0.5	0.06	0.04	0.08
K. pneumonia	0.4	0.5	0.44	0.38	0.06	0.36
P. vulgaris	0.44	0.14	0.1	0.36	0.15	0.26
E. coli	0.06	0.04	0.16	0.1	0.08	0.1
C. albicans	0.5	0.6	0.44	0.46	0.06	0.1

Antimicrobial Activity

Table 5 reveals that the antimicrobial activity of various extracts showed concentration-dependence against test organisms. The test microorganisms show various sensitivity indices for different seed extracts. Extract of diethyl ether at 0.1 g/ml demonstrated highest antimicrobial activity against *S. aureus* (15 mm) and *P. vulgaris* (12 mm). Also, butanol extract at 0.1 g/ml demonstrated significant antimicrobial activity against *P. aeruginosa* (25 mm), *K. pneumonia* (20 mm) and *C. albicans* (15 mm). The hexane extract (fixed oil) showed no activity against *S. aureus, P. aeruginosa, P. vulgaris*

and *C. albicans.* The result revealed that *C. albicans* was most resistant to the test extracts (hexane, chloroform, diethyl ether and ethyl acetate). Streptomycin (0.1 g/ml) used as standard for the bacterial isolates exhibited the highest inhibitory activity against *P. aeruginosa* (25 mm); followed by *S. aureus, P. vulgaris* and *E. coli* with an inhibition zone of 20 mm. Nystatin tablet (0.1 g/ml) used as a standard for *C. albicans* exhibited 12 mm zone of inhibition.

Minimum inhibitory concentration (M.I.C.) which is the lowest concentration of an extract that inhibits completely the growth of micro-organism in 24 hours ranged from 0.04 g/ml to 0.6 g/ml on tested bacteria and fungus for various extracts (Table 6). Butanol extract had the least MIC against *P. aeruginosa* (0.04 g/ml), *K. pneumonia* (0.06 g/ml) and *C. albicans* (0.06 g/ml); diethyl ether extract had the least MIC against *S. aureus* (0.08 g/ml) and *P. vulgaris* (0.1 g/ml) while chloroform extract had the least MIC for *E. coli* (0.04 g/ml). Diethyl ether and butanol extracts demonstrated potent antimicrobial activities against selected wound pathogens in this study. Phytochemicals which are secondary metabolites present in various extracts may be responsible for the demonstration of antibacterial and antifungal activity against gram negative, gram positive bacteria and fungus. These findings are consistent with the studies of Kumar *et al.* [27] and Velmurugan *et al.* [28] who reported the antimicrobial activity of fruit and whole plant of *L. cylindrica*. This study shows that the seed extracts have significant wound healing promoting activity and prominent antimicrobial activity against some wound pathogens, and therefore, offers scientific support for the use of *L. cylindrica* seeds for treating wounds and as an antiseptic.

REFERENCES

1. Ncube NS, Afolayan AJ, Okoh AI. Assessment Techniques of Antimicrobial Properties of Natural Compounds of Plant Origin: Current Methods and Future Trends. Afr. J. Biotech. 2008; 7 (12): 1797-1806.
2. Ajiwe VIE, Ndukwe GI, Anyadizegwu IE. Vegetable Diesel Fuels from *Luffa cylindrica* oil, its Methylester and Ester-diesel Blends. Chem. Class J. 2005; 2:1-4.
3. Dunnill PM, Fowden L. The Amino Acid of Seeds of the Cucurbitaceae. Phytochem. 1965; 4: 933-944.
4. Gray DO, Fowden L. N-ethyl-L-Asparagine: A New Amino-acid Amide from *Ecballium*. Nature 1961; 189: 401
5. Peters RR, Farias RM, Ribeiro-Do-Valle MR. Anti-inflammatory and Analgesic Effects of Cucurbitacins from *Wilbrandia ebracteata*. Planta Med. 1997; 63:525-528.
6. Pal S, Chakraborthy SK, Banerjee A, Mukorji B. Search for Anti-cancer Drugs from Indian Medicinal Plants (Ayurvedic, Unani). Ind. J. Med. Res. 1968; 56: 445.
7. Lauria P, Sharma VN, Vanjani S, Sangal BC. Effect of Prolonged Stress of Repeated Electric Shock on Rat Myocardium. Ind. J. Physiol. Pharmacol. 1972; 4 (2): 152.
8. Muthumani P, Meera R, Mary S, Mathew J, Devi P, Kameswari B, Eswara BP. Phytochemcial Screening and Anti-inflammatory, Bronchodilator and Antimicrobial Activities of the Seeds of *L. cylindrica.* Res. J. Pharm. Biol. Chem. Sci. 2010; 1(4): 11-18.
9. Polanowski A, Wilusz T, Wienartowicz B, Cleslar E, Stominska A, Noqak, K. Preparation and Characteristics of Trypsin Inhibitors. Acta Biochimica. 1980; 27: 371.
10. Nagao T, Lanaka R, Iwase Y, Hanazone H, Okabe H. Studies on the Constituents of *Luffa acutangula* (Roxb.). Clin. Pharm. Bull.1991; 39: 599-606.
11. Lorke D. A New Approach for Acute Practical Toxicity Test. Arch. Toxicol. 1983; 54: 275-287.
12. Morton JJ, Malone MH. Evaluation of Vulnerary Activity by Open Procedure in Rats. Arch. Int. Pharmacodyn. Ther. 1972; 196: 117-126
13. Adeniyi BA, Odelola HA, Oso BA. Antimicrobial Potentials of *Diospyros mespiliformis* (Ebenaceae). Afr. J. Med. Sci. 1996; 25: 221-224.
14. Shadomy S, Espinel A. Manual of Clinical Microbiology, American Society for Microbiology: 4th Edition, Washington D.C. 1980, pp. 647.
15. Trease GE, Evans CW. Textbook of Pharmacognosy. 13th Edition, University Press, Cambridge, Great Britain, 1989, pp. 480-481.
16. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. John Wiley and Sons Ltd. Chicester, New York, 1993, pp. 6-65.
17. Okuyama T, Yoshikawa K, Arihara S, Wang JD, Narui T. A Review of the Biological and Pharmacological Activities of Saponins. Chem. Pharm. Bull. 1991; 39(5): 185-188.
18. Anamika K, Gupta A, Garai S, Basanti PW. Immunomodulatory Effects of Two Sapogenins 1 and 2 Isolated from *Luffa cylindrica* in Balb/c Mice. Biorg. Med. Chem. Lett. 2007; 17(6): 1608-1612.
19. Hussain MM, Dastagir MG, Billah M, Ismail MD, Quader A. Phytochemical and Antimicrobial Investigation of *Luffa cylindrica.* Bol. Lat. Am. 2010; 9(5): 328.
20. Khan MSY, Bhatia S, Javed K, Khan MH. Evaluation of Anti-Stress (Adaptogenic) Activity of *Luffa cylindrica* Fruit Extract. Ind. J. Pharm. Sci. 1992; 54(2): 75-76.
21. Committee for Veterinary Medicinal Product. The European Agency for the Evaluation of Medicinal Products (1996).
22. Ilango K, Yoganandam GP, Usha K, Priyanga KS, Ilansezhiyan M, Kalaiarasi V. Evaluation of Wound Healing Activity of Hexane and Methanolic Extract of Fruit Plum of *Momordica balsamina* (Linn.) in Albino Rats. Int. J. Pharm. Pharmaceu. Sci. 2010; 2(1): 88-92.
23. Kapoor M, Howard R, Hall I, Appleton I. Effects of Epicatechin Gallate on Wound Healing and Scar Formation in a Full Thickness Incisional Wound Healing in Rats. Am. J. Path. 2004; 3: 299-307.
24. Mensah AY, Sampson J, Houghton PJ. Effects of *Buddleja globosa* leaf and its Constituents Relevant to Wound Healing. J. Ethnopharm. 2001; 77: 219-226.
25. Ruef J, Meshel AS, Hu Z. Flavopiridol Inhibits Smooth Muscle Cell Proliferation *in vitro* and Neointimal Formation *in vivo* after Carotid Injury in Rat. Circulation, 1999; 10 (1): 659-665.
26. Dhanalekshmi UM, Poovi G, Kishore N, Raja MD, Reddy PN. Enhancement of Cutaneous Wound Healing by Ethanolic Extract of *L. cylindrica* Leaf and Flower in Wistar Rat Model. Int. J. Toxicol. Pharmcol. Res. 2010; 2(3): 85-91.
27. Kumar DS, Indumathy R, Pallavi K, Devi GS. Antimicrobial Activity of Whole Plant of *Luffa cylindrica* (Linn.) Against some common Pathogenic Microorganisms. Int. J. Pharm. Sci. Drug Res. 2011; 3(1): 29-31.
28. Velmurugan V, George S, Surekha SP. Phytochemical and Biological Screening of *Luffa cylindrica* (Linn.) fruit. Int. J. Pharmtech Res. 2011; 3 (3): 1582-1585.

Source of Support: Nil, Conflict of Interest: None declared.