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ORIGINAL RESEARCH ARTICLE

Analgesic and Antibacterial Activity of Methanolic Extract of *Smilax lanceifolia*

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ABSTRACT

The methanolic extract of the leaves of *Smilax lanceifolia* Roxb. (Family- Smilacaceae) was subjected to pharmacological investigation to ascertain analgesic and antibacterial activity. The phytochemical screening demonstrated the presence of different types compound like anthraquinone glycoside, alkaloids, terpenoids, tannins, carbohydrate,flavone aglycone, saponin, phenolic compound, reducing sugar, and phlobotannin. The methanolic extract of the plant was tested for analgesic activity using chemical writhing method (0.6% acetic acid) and antibacterial activity against *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi* bacteria using cup diffusion method. The extract significantly($p<0.05$) inhibited in a dose dependent fashion analgesia(nociception) induced by acetic acid as indicated by the reduction in number of writhing movement in mice. Antibacterial activity was observed against *S.aureus, S.typhi, E.faecalis,* and *E.coli* in dose dependent manner.

Keywords: Antibacterial, analgesic, cup diffusion, chemical writhing, smilacaeae, *Smilax lanceifolia*, Nepal.

INTRODUCTION

Herbal medicines which formed the basis of health care throughout the world since the earliest days of mankind are still widely used. Recognition of their clinical, pharmaceutical and economic value is still growing[1]. It has been found that 80% people of the developing countries rely on the traditional medicine which is mainly derived from plant and many pharmacopoeias still contain at least 25% drugs derived from plants [2]

Although, Nepal is a small country, it is rich in biodiversity due to its geographical features and has many plants with medicinal and aromatic values. Some of them are used in traditional medicine and some are still not explored scientifically for their medicinal values. So, the present study of plant *Smilax lanceifolia* was selected for biological scrutiny. Literature survey of this plant revealed not much information about its medicinal uses; however other species of this plant have reported to have antibacterial and analgesic properties [3,4,5]. It is likely that when bioactive compounds are found in one species of the same genus may contain active compounds of the similar nature.

*S.lanceifolia*of the smilacaeae family, locally called as kukurdyano, is deciduous climber with dioecious flower. It is found temperate zones, tropical and sub-tropical Zones. It is in flower from Sep to March, and the seeds ripen from Nov to March.

MATERIALS AND METHODS

The roots and rhizome of the *S.lanceifolia* was collected in the month of March-April, 2013, from Phulchowki Hill of Kathmandu valley, Nepal. It was duly identified as *S.lanceifolia*in National Herbarium and Plant laboratory, Godawari, Nepal.

Extraction of Plant

The roots and rhizome of *S.lanceifolia* was cleaned with distilled water, air-dried, powered and 500 gm of the powered plant material was extracted by maceration using methanol. Extract was evaporated to dryness, leaving dried residue.

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Phytochemical Screening

The phytochemical screening of methanol extract was done to identify the main groups of chemical constituents present in methanol extract of *S.lanceifolia*by their color reaction.[6]

Analgesic Activity

Analgesic activity on intact mice was carried out by Chemical writhing method. Writhing test is the most common test to evaluate peripheral analgesic activity [7,8]. This writhing is also called as 'stretching', 'cramping' & 'squirming' [9].Albino wistar mice of 25-35 gm were divided in to five groups of 3 mice each.The first group of mice were given normal saline at 10ml/kg followed by 0.6% acetic acid at 10ml/kg body weight after 30 minutes as a control group. Writhing produced for 20 minutes were observed.Second group was given 25mg/kg of diclofenac made in normal saline at 10ml/kg followed by 0.6% acetic acid at 10ml/kg body weight after 30 minutes as a positive control group. Writhing produced for 20 minutes were then observed.Third group was given 50mg/kg of diclofenac sodium made in normal saline at 10ml/kg followed by 0.6% acetic acid at 0.01ml/gm body weight after 30 minutes as a positive control group. Writhing produced for 20 minutes were then observed.Fourth group was given 250mg/kgof methanol extract made in normal saline at 10 ml/kg followed by 0.6% acetic acid at10ml/kg body weight after 30 minutes as a test group. Writhing produced for 20 minutes were then observed.Fifth group was given 500mg/kg of methanol extract made in normal saline at 10 ml/kg followed by 0.6% acetic acid at 10ml/kg body weight after 30 minutes as a test group. Writhing produced for 20 minutes were then observed.[10-14]

$$\% \text{ Protection} = \frac{\text{Writhing in control} - \text{writhing in test}}{\text{Writhing in control}} \times 100\%$$

Antibacterial Activity

Cup diffusion method according to Lorian[15] was used in antibiotic assay with its modifications. Muller-Hinton agar plates of 4 mm thickness were prepared and cup of uniform diameter of 6 mm diameter were bored. Antibacterial screening of methanolic extract dissolved in 15% dimethyl sulphoxide (DMSO) was done at concentration of 100mg/ml,200mg/ml and 500mg/mlkeeping standard disk of amikacin (30mcg) for Gram negative organism and Vancomycin (30µg) for Gram positive organism and DMSO 15% as control against two positive bacteria *Staphylococcus aureus* and *Enterococcus faecalis* and three gram negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* by inoculating bacterial broth of 0.5 McFarland standard turbidity in Muller-Hinton agar.[16,17].MRSA was also tested using Cefoxitine and Vancomycin for its antimicrobial activity and DMSO 15% as control so as to check the validity of zone of inhibition.The agar plates were then kept overnight at 2^0C to allow diffusion of extract. The plates were then incubated at 37^0C and zones of inhibition were measured after 24 hours. All the bacteria isolates were collected from patients visiting Tribhuvan University Teaching Hospital, Kathmandu.

Data Analysis

Data obtained from experiments were expressed as Mean ± SE using SPSS version 20.0, MS Excel 2010.

RESULTS

Phytochemical Screening

The extractive value of the methanol extract was 8.40 % on dry basis. The methanol extract of the plant revealed the following phytochemicals (**Table 1**).

Table 1: Different group of phytochemicals constituents present in methanolic extract of *S lanceifolia*

S. No	Test Compound	Result
1	Alkaloids	+
2	Anthraquinone Glycoside	+
3	Terpenoids	+
4	Proteins and amino acid	-
5	Tannins	+
6	Flavone Aglycone	+
7	Flavonoid	+
8	Carbohydrate	+
9	Saponin	+
10	Coumarin	-
11	Phenolic Compound	+
12	Reducing Sugar	+
13	Phlobotannin	+

*- absent, + present

Analgesic Activity

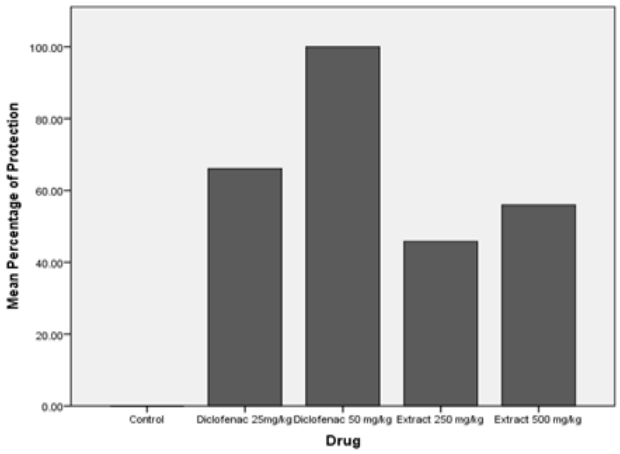

Fig 1: Effect of extracts against pain by chemical writhing

Antimicrobial Activity

The following table shows the result of preliminary antimicrobial activity of plant extracts. The results showed extracts were active against *S. aureus, Enterococcus faecalis*, *E.coli* and *Salmonela typhi* but did not show any activity against *P. aeroginosa*. Also the extract shows active against *Methicillin Resistant Staphylococcus aureus*. Control DMSO had no inhibitory activity against the bacteria used.

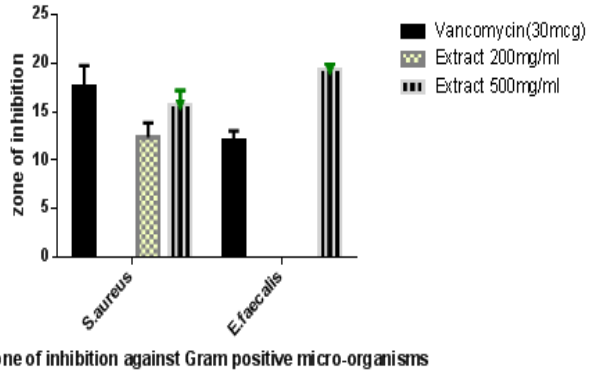

Fig 2: Zone of inhibition of different concentrations of methanolic extracts and standard drugs

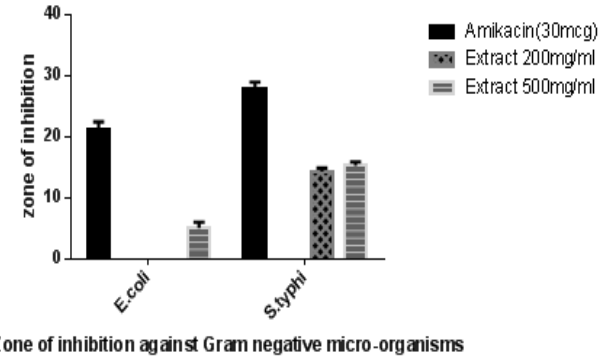

Fig 3: Zone of inhibition of different concentrations of methanolic extracts and standard drugs

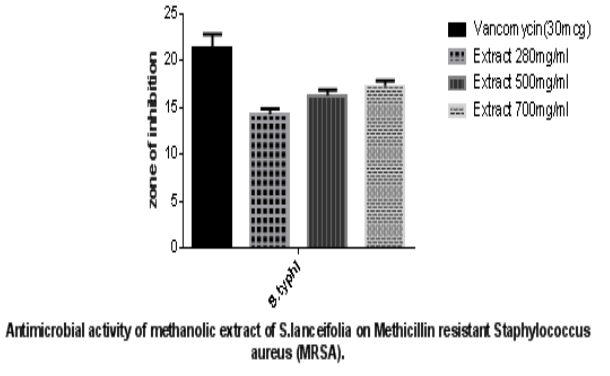

Fig 4: Zone of inhibition of different concentrations of methanolic extracts and standard drugs on Methicillin resistant *Staphylococcus aureus* (MRSA).

DISCUSSION

The methanolic extract of the plant (roots and rhizome) delineated some promising phytochemicals, namely, anthraquinone glycoside, alkaloid, terpenoids, tannins, carbohydrate, flavone aglycone, saponin, phenolic compound, reducing sugar, and phlobotannin.

For assessing in vivo analgesic activity in mice, more purposely peripheral analgesic activity, acetic acid induced writhing model in mice is most extensively used. The extract showed significant analgesic activity in dose dependent manner. The analgesic action of Diclofenac is mainly due to peripheral pain by prevention of prostaglandin mediated sensitization of nerve endings. It acetylates cyclo-oxygenase enzyme and inhibits prostaglandin synthesis. The effect of methanolic extract of *S.lanceifolia (*Smilaceae) root and rhizome was investigated for its analgesic activities in mice. The extract significantly ($p<0.05$) inhibited in a dose dependent fashion analgesia (nociception) induced by acetic acid as indicated by the reduction in number of writhing movement in mice. The exact mechanism of action of extract was not known; however, the suppression of the formation of pain substances in peripheral tissues, particularly prostaglandins and bradykinin cannot rule out.The presence of saponin, tannins, reducing sugars, flavonoids, and alkaloids in the ethanol extract of *S.lanceifolia* may be accountable for the investigated activities, because it is well estabilished that these phytochemicals are responsible for a wide range of bioactivities [18-23].

Antibacterial activity of the methanol extract of *S. lanceifolia* was investigated against all the tested Gram-positive and Gram-negative bacterial strains in cup diffusion assay based on its traditional uses in the insecticide.Methanolic extract showed the activity against *Staphylococcusaureus, Salmonella typhi, Enterococcus faecalis,andEscherichia coli.* . It also showed the activity against *Methicillin ResistantS. aureus*.Literature has shown that terpenoids and phenolic compound shows most of the antibacterial activity. *S. aureus* are specifically susceptible to phenolic compounds [24]. The activity of extract upto 500 mg/ ml is not equal to that of 30mcg antibiotics used, which may be because of the antibacterial compound may be present in very few amounts in the crude extract. The pure compound which on further modification can be safe and effective antibiotics. This plant also could be useful for the future research in antibiotics against Methicillin resistant *S.aureus.*

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REFERENCES

1. Jayasuriya DC., *A review of legislation concerning medicinal plants*. Unpublished Report, (1990).
2. World Health Organization. Legal status of Traditional Medicine and Complementary / Alternative Medicine: *A Worldwide Review. Geneva: World Health Organization*., 137,(2001).
3. S.Raghunadha R., Pobba R., Koteswararao. D., YK Naidu, SarathChandiran I and MP KalyanR.Anti-inflammatory and analgesic activity of Smilax chinensis,*Res J. PharmBiolChemSci.*, 1:1,(2010).
4. Hye-Kyung S., Jong-HawaL.,Hyun-Su Kim,Chang-Kwon L. and Seung-CheolL.,Antioxidant and Antimicrobial Activites of *Smilax chinaI* L. leaf Extracts. *Food Science and Biotechnology*.,21:1723-1727,(2012).
5. Zhang QF.,Zhang ZR., Cheung HY.,Antioxidant activity of *RhizomaSmilacisGlabrae* extracts and its key constituent-astilbin. *J Food Chem.*, 115:297-303,(2009).
6. Evans WC.,Trease& Evans Pharmacognosy.14th edition.,*Harcourt Brace & Company Asia Ltd., India*,(1997).
7. Vogel, H.D. Drug Discovery and Evaluation:Pharmacological Assays. *3rd ed. Germany: Springer*,2008.
8. Lambert-Baird, J. and Jamieson, D.D., Possible mediators of the writhing response induced by acetic acid or phenylbenzoquinone in mice. *Clinical and Experimental Pharmacology & Physiology*.,10:15-20,(1983).
9. Collier, H.O.J., Dinneen, L.C., Johnson, C.A. and Schneider, C., The abdominal constriction response and its suppression by analgesic drugs in the mouse. *Br J Pharmacol*., 32: 295-310, (1968).
10. Ahmed F. Selim M.S.T., Das D.K., Chaudhari M.S.K., Antiinflammatory and antinoceiceptive activities of Lippianodiflora Linn.*Pharmazine*., 59:329-333,(2004).
11. Zulfiker A.H.M., Rahman M.M., Hossain M.K., Hamid K., Mazumader M.E.H. Rana M.S., In vivo analgesic activity of ethanolic extracts of two medicinal plants-Scoploraduolcis L, and Ficusracemosa Linn. *Biol Med.*,2:42-48,(2010).
12. Ndebia E.J Kangang R and Nkehchungag B.N., Analgesic and antiinflamatory properties of aquous extracts from leaves of Solanumtorvum(Solanaceae). *African J Trad Med*., 4:240-244, (2007).
13. Igbe I., Ozolua I.R., Okpo O. Steve, and Obasuyi O., Antipyretic and Analgesic effects of the aquous extracts of the fruit pulp of Hunteria umbellate(Apocynaceae), *Tropical J PharmRes.*,8:331-336,(2009).
14. Jakson C., Mbagwu H., Jackson I., Ekpe G., Etienam F., Analgesic activities of ethanolic extracts of the root of Carpolobialutea, *African J Pharm Pharmacol*., 5:367-370,(2011).
15. LorianV.,Antibiotics in laboratory medicine, 4th edition. *Newyork: William and Wilkins.*,14,(1998)
16. Clutterbuck AL, Cochrane CA, Dolman J & Percival SL, Evaluating antibiotics for use in medicine using a poloxamer bio film model. *Annals of Clinical Microbiology and Antimicrobials.*, 6:2,(2007).
17. DuraipandiyanV,Ayyanar M &Ignacimuthu S., Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. *BMC Complemetary and Alternative Medicine.*, 6:35,(2006).
18. Saha S, Islam MK, Anisuzzman M, Hasan MM, Hossain F, Talukder C., Evaluation of antioxidant, analgesic and antidiarrhoeal activity of Phoenix paludosaRoxb. leaves. *International J Med Sci Pharm*., 2:46-52,(2012).
19. Adedapo AA, Sofidiya MO, Maphosa V, Moyo B, Masika PJ,Afolayan AJ., Anti-inflammatory and analgesic activities ofthe aqueous extract of Cussoniapaniculata stem bark. *RecNat Prod*.,2:46-53,(2008).
20. Parmar NS, Ghosh MMN., Current trends in flavonoid research. *Indian J Pharm.*, 12: 213-228,(1978).
21. Choi J, Jung HJ, Lee KT, Park HJ.,Antinociceptive and antiinflammatory effects of saponin and sapogenin obtained

from the stem of Akebiaquinata. *J Med Food*. 8:78-85,(2005).

22. Narayana KR, Reddy MS, Chaluvadi MR, Krishna DR.,Bioflavanoids classification, pharmacological, biochemical effects and therapeutic potential. *Indian J Pharmacol*., 33:2-16, (2001).
23. Ahmadiani A, Hosseiny J, Semnanian S, Javan M, SaeediF, Kamalinejad M, et al., Antinociceptive and anti-inflammatory effects of Elaeagnusangustifolia fruit extract. *J Ethnopharmacol.*, 72:287-292,(2000).
24. Cowman MM, Plant Products as Antimicrobial Agents. *Clinical microbiology reviews*, 12:564,(1999).