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Biological investigations of different leaf extracts of *Litsea liyuyingi* (Family-Lauraceae)

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Abstract: *In this present study, the leaf extracts of Litsea liyuyingi, were subjected to evaluate the phytochemical screenings, thrombolytic, membrane stabilizing, antioxidant, antimicrobial, cytotoxic, anti-nociceptive, anti-diarrheal and hypoglycemic activity. In phytochemical screening the presence of flavonoids, saponins, alkaloids, phenols and tannins were detected in the plant leaf extract. Methanol (43.55%) extract and its pet-ether fraction (42.11%) showed good thrombolytic effects and good membrane stabilizing effects were observed by carbon tetrachloride and aqueous soluble fractions respectively. Two different assays were performed to evaluate antioxidant activity. The presence of phenolic contents and free radical scavenging activity was remarkable in the methanol extract and its pet-ether fraction. Crude methanol extract and other fractions showed effects against gram positive and gram negative bacteria as well as demonstrated effects to A. Salina with LC_{50} values ranging from 1.04 to 9.41 μg/ml and LC_{90} values ranging from 30.20 to 82.14 μg/ml as compared to contro. Statistically significant (**p<0.01; ***p<0.001) result was found in case of in vivo anti-nociceptive activity test for the 400 mg/kg methanol extract when compared to control. The crude methanol extracts of leaves also possessed significant anti-diarrheal (*p<0.05) and hypoglycemic (**p<0.01) activity at 400 mg/kg.*

Keywords: *Litsea liyuyingi, phytochemical screening, in vitro thrombolytic, membrane stabilizing, antioxidant, antibacterial, cytotoxic, in vivo anti-nociceptive, anti-diarrheal and hypoglycemic activity*

I. Introduction

Herbal products are often perceived as safe because they are "natural" [1]. The term 'crude drugs of natural or biological origin' is used by pharmacists and pharmacologists to describe whole plants or parts of plants which have medicinal properties [2] because of having enormous versatility in synthesizing complex materials. Secondary metabolites or phytochemicals are naturally, occurring and biologically active plant compounds that have potential disease inhibiting capabilities as well as believed phytochemicals may be effective in combating or preventing disease due to their antioxidant effect [3]. Antioxidants are vital substances that protect other molecules (*in vivo)* from oxidation when they are exposed to free radicals and reactive oxygen species which have been implicated in the etiology of many diseases and in food deterioration and spoilage [4-5].

Litsea liyuyingi is an evergreen shrubs or small trees grows up to 3 meter tall having leaves opposite or alternate on same tree with hairy like branchlets; leaf blade elliptic, oblong, lanceolate, oblong-lanceolate, or elliptic-lanceolate, yellow brown or ferruginous tomentose or gray-yellow appressed pubescent abaxially, base cuneate or rotund, apex acute or acuminate and belongs to the family of Lauraceae which is one of 200 species. *Litsea liyuyingi* is one of the medicinal plants grown in Bangladesh mainly in tropical and subtropical Asia, a few species in Australia and from North America to subtropical South America and China. This plant has also been used in traditional medicine for the treatment of erectile dysfunction [6-7], urinary tract infection, sexually transmitted diseases, leucorrhoea, tonic, stimulant [8] and gestational diabetes [9]. Despite the popular use of this species as a medicinal plant; no previous attempts have been made to examine the biological activity of this plant.

As a part of our continuing studies on medicinal plants of Bangladesh the organic soluble materials of the leaf extracts of *Litsea liyuyingi* were evaluated for phytochemical screening, thrombolytic, membrane stabilizing, antibacterial, cytotoxic, antioxidant, anti-nociceptive, hypoglycemic and anti-diarrheal activity for the first time.

II. Materials And Methods

2.1 Collection, identification and processing of plant samples

The leaves of *Litsea liyuyingi* were collected from Bogra District, Bangladesh and then plant sample was submitted to the National Herbarium of Bangladesh, Mirpur-1, Dhaka for its identification and the voucher specimen is DACB- 42740. Leaves were sun dried for seven days in order to remove the moisture contents and

then ground into coarse powder using high capacity grinding machine (Jaipan designer mixer grinder, jaipan, India) which was then stored in air-tight container with necessary markings for identification and kept in cool, dark and dry place for the investigation.

2.2 Extraction procedure

The powdered plant parts (30 gm) were successively extracted in a soxhlet extractor at elevated temperature using 500 ml of distilled methanol (40-60)ºC. After drying all extracts, an aliquot (5 gm) of the concentrated methanol extract was fractionated by the modified Kupchan *et al.* [10] into pet-ether, carbon tetrachloride and aqueous soluble fractions followed by solvent evaporation.

2.3 Preliminary phytochemical screening

Ethanol extract was subjected to preliminary phytochemical screenings for determining nature of phytoconstituents by using standard protocols [11-13].

2.4 Streptokinase (SK)

Commercially available lyophilized alteplase (Streptokinase) vial (Popular pharmaceutical Ltd.) of 15, 00,000 I.U, was collected and 5 ml sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100 µl (30,000 I.U) was used for in vitro thrombolytic activity evaluation.

2.5 Blood sample

Blood (n=6) was drawn from healthy human volunteers without a history of oral contraceptive or anticoagulant therapy and 1ml of blood was transferred to the previously weighed micro centrifuge tubes and was allowed to form clots.

2.6 Thrombolytic activity

The thrombolytic activity of all extracts were evaluated by the method developed by Prasad *et al*. [14] and slightly modified by Sharif *et al*. [15] using streptokinase (SK) as the standard.

2.7 Membrane stabilizing activity

The membrane stabilization by hypotonic solution and heat-induced haemolysis method was used to assess anti-inflammatory activity of the plant extracts by following standard protocol. [16] The erythrocyte membrane resembles to lysosomal membrane and as such, the effect of drugs on the stabilization of erythrocyte could be extrapolated to the stabilization of lysosomal membrane [17-18]. Membrane stabilizing activity of the extractives was assessed by using hypotonic solution-induced and heat-induced human erythrocyte haemolysis. To prepare the erythrocyte suspension, whole blood was obtained from healthy human volunteer and was taken in syringes (containing anticoagulant 3.1% Na citrate). The blood was centrifuged and blood cells were washed three times with solution (154 mM NaCl) in 10 mM sodium phosphate buffer (pH 7.4) through centrifugation for 10 min at 3000 rpm.

2.7.1 Hypotonic solution induced haemolysis

The test sample consisted of stock erythrocyte (RBC) suspension (0.5 ml) mixed with 5mL of hypotonic solution (50 mM NaCl) in 10mM sodium phosphate buffered saline (pH 7.4) containing either the extract (1.0 mg/ml) or acetyl salicylic acid (ASA) (0.1 mg/ml). The control sample consisted of 0.5 ml of RBCs mixed with hypotonic-buffered saline alone. The mixture was incubated for 10min at room temperature, centrifuged for 10 min at 3000g and the absorbance of the supernatant was measured at 540 nm.

The percentage inhibition of either haemolysis or membrane stabilization was calculated using the following equation:

$$\% \text{ inhibition of haemolysis} = 100 \times (OD_1\text{-}OD_2)/OD_1$$

Where, OD_1= optical density of hypotonic-buffered saline solution alone (control)
OD_2= optical density of test sample in hypotonic solution

2.7.2 Heat induced haemolysis

Isotonic buffer containing aliquots (5 ml) of the different extractives were put into two duplicate sets of centrifuge tubes. The vehicle, in the same amount, was added to another tube as control. Erythrocyte suspension was added to each tube and mixed gently by inversion. One pair of the tubes was incubated at 56°C for 30min in

a water bath, while the other pair was maintained at (0-5) °C in an ice bath. The reaction mixture was centrifuged for 5 min at 2500 rpm and the absorbance of the supernatant was measured at 560 nm.

The percentage inhibition or acceleration of hemolysis in tests and was calculated according to the equation:

$$\% \text{ Inhibition of hemolysis} = 100 \times [1- (OD_1-OD_2)/ (OD_3-OD_1)]$$

Where, OD_1= optical density of unheated test sample
OD_2= optical density of heated test sample
OD_3= optical density of heated control sample

2.8 Antioxidant activity

2.8.1 Determination of total phenolic content

Total phenolic content in the methanol and other extracts were determined by using the Folin-Ciocalteu reagent [19-20]. The plant extracts and standard were diluted by serial dilutions as (6.25 µg/ml to 200 µg/ml) then, on each test tube containing 1ml of a diluted solution of sample and standard, following reagent solutions were added 5 ml folin-ciocalteu reagent (10 fold dilution) and 7.5% sodium carbonate (4 ml). Test tube containing the standard solution and test tube containing extracts was incubated for 30 minutes and 60 minutes respectively at room temperature. Absorbance of samples and standard were measured at 765 nm using UV-VIS spectrophotometer against the blank. A typical blank solution contained the solvent used to dissolve the plant extract. The Total content of phenol compounds in plant extract was calculated as gallic acid equivalents (GAE) using the following equation:

$$C = (c \times V)/m$$

Where; C = total content of phenol compounds, mg/gm plant extract, in GAE
c = the concentration of Gallic acid established from the calibration curve (mg/ml)
V = the volume of extract in ml
m = the weight of crude plant extract in gm

2.8.2 DPPH free radical scavenging assay

The free radical scavenging capacities of the extracts were determined by using DPPH [21-22]. 1 ml of plant extract or standard of different diluted (6.25 µg/ml - 800 µg/ml) concentration solutions was taken in test tube and freshly prepared 2 ml of 0.004% DPPH solution was added in each test tube to make the final volume 3 ml. The mixture was incubated at room temperature for 30 minutes; the absorbance was read at 517 nm using a UV-VIS spectrophotometer. Ascorbic acid was used as standard. Control sample was prepared containing the same volume without any extract and standard. The absorbance was read at 517 nm using a UV-VIS spectrophotometer. Methanol was used as blank. Percent of inhibition of the DPPH free radical was measured by using the following equation:

$$\% \text{ inhibition} = (1-A_1/A_0) \times 100\%$$

Here, A_1 = Absorbance of the extract or standard
A_0 = Absorbance of the control.

2.9 Antibacterial activity

The antibacterial screening, which is the first stage of antimicrobial drug discovery, was performed by the disc diffusion method against gram positive and gram negative bacteria (Table 5) collected as pure cultures from the department of microbiology, Dhaka University, Bangladesh. Standard disc of ciprofloxacin (5 µg/disc) and blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control, respectively. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm [23].

2.10 Brine shrimp lethality bioassay

Brine shrimp lethality bioassay technique was applied for the determination of general toxic properties of the plant extracts [24-25]. Dimethylsulfoxide (DMSO) solutions of the samples were applied against *Artemia salina* in 1 day for the assay. For the experiment, 1 mg of each extracts was added with 5 ml of sea water. Concentration was found to be 200 µg/ml. Then 50 µl DMSO was added to these and sample was prepared.

Then the solution was serial diluted to 100, 50, 25, 12.5, 6.25, 3.125, 1.563 µg/ml with sea water. Then 2.5 ml of plant extract solution was added to 2.5 ml seawater containing 10 nauplii. Vincristine Sulphate (VS) was used as standard.

2.11 Experimental animal

For the experiment Swiss albino mice of either sex, 4-5 weeks of age, weighing between 15-30 gm were collected from ICDDR, B, Mohakhali, Dhaka, Bangladesh. Animals were maintained under standard environmental conditions [temperature: (27.0±1.0)ºC, relative humidity: (55-65)% and 12 hour light/12 hour dark cycle] and free access to feed and water. The animals were acclimatized to laboratory condition for one week prior to experiments. All protocols for animal experiment were approved by the institutional animal ethical committee.

2.12 Anti-nociceptive activity

Anti-nociceptive activity was evaluated by acetic acid induced writhing and tail immersion methods.

2.12.1 Acetic acid induced writhing test

The acetic acid writhing test in mice as described by Koster *et al*. [26] was employed with slight modification. Mice were divided into 4 groups containing 5 mice in each group. The first group was given 10 ml/kg of 1% Tween 80 intraperitoneally and served as control. Group 2 was served as standard where diclofenac Na has given to mice as dose of 50 mg/kg of body weight. Groups 3, 4 received methanol extracts 200 mg/kg and 400 mg/kg of body weight. Thirty minutes later each mouse was injected intraperitoneally with 0.7% acetic acid at doses of 10 ml/kg of body weight. Full writhing was not always completed by the mice. Accordingly, two half writhing were considered as one full writhing. The number of writhing responses was recorded for each mouse during a subsequent 5 min period after 15 min intra peritoneal administration of acetic acid and the mean abdominal writhing for the each group was obtained and recorded.

2.12.2 Tail immersion test

The tail immersion method was used to evaluate the central mechanism of analgesic activity. Here the painful reactions in animals were produced by thermal stimulus that is by dipping the tip of the tail in hot water [27]. On the test day, Swiss albino mice were divided into 4 groups of 5 mice each. Here diclofenac Na (50 mg/kg) is used as standard drug as well. Animals were fasted for 16 hours with free access to water. After administration of standard and test drugs, the basal reaction time was measured by immersing the tail tips of mice (last 1-2 cm) in hot water of water bath, where temperature was previously adjusted at 51ºC. The actual flick response of mice that is time taken in second to withdraw it from hot water source was calculated and results were compared with control group. The latent period of the tail-flick response was determined at 30, 60 and 90 minute after the administration of drugs.

2.13 *In vivo* anti-diarrheal activity test

The present study was performed to evaluate the preventive and curative anti-diarrheal effects of the methanol extracts at two different concentrations. All the animals housed under standard laboratory condition at 25°C and 12 hour light such as dark cycle, acclimatized for 10 days before experiment. Standard diet and water were provided constantly. When test started all organized groups served with their respective doses and standard group served with loperamide (5 mg/kg) doses. After 1 hour, all groups received castor oil 2 ml each orally. Then they were placed in cages lined with adsorbent papers and observed for 4 hour for the presence of characteristic diarrheal droppings. 100% was considered as the total number of feces of control group. The activity was expressed as percent inhibition of diarrhea [28-29].

2.14 Hypoglycemic activity

The aim of this hypoglycemic activity study was to evaluate hypoglycemic activity in normal mice [30]. The experimental mice were fasted for 12 hours, and then randomly selected and divided into 4 groups of 5 mice in each group. At zero hour, fasting blood glucose level was measured in each group from tail vein prior to the glucose administration by using glucometer with glucose oxidase-peroxidase reactive strips. Control vehicle (1% Tween-80 solution in saline), standard drug (glibenclamide) at dose of 10 mg/kg of body weight) and extracts (200 and 400 mg/kg of body weight) were administered to Group 1, 2, 3 and 4 respectively using oral feeding needle. After 60, 120 & 180 min, blood samples were collected in the same procedure and blood glucose level is measured to see the hypoglycemic effect in comparison with control and standard groups.

2.15 Statistical analysis

Data was expressed as Mean ± Standard deviation. Results below $*p<0.05$, $**p<0.01$ and $***p<0.001$ are considered statistically significant.

III. Results

3.1 Preliminary phytochemical screening

Preliminary phytochemical screening of the methanol extract of *L. liyuyingi* revealed either presence or absence of various bioactive components. *L. liyuyingi* was found to contain flavonoids, saponins, alkaloids, phenols and tannins (Table 1).

Table 1: Analysis of phytochemicals in the methanol extracts of *L. liyuyingi*

Name of tests	Name of extract
Flavonoids	+
Saponins	+
Alkaloids	+
Phenols	+
Tannins	+

(+) Presence

3.2 Thrombolytic activity

In thrombolytic activity using *in vitro* clot lysis assay method, prominent thrombolysis was demonstrated by the crude methanol extract 43.55% and its pet-ether fraction 42.11% compared to standard 65.16%. The thrombolytic activity of the plant extractives is presented in Table 2.

Table 2: *In vitro* thrombolytic activity of different extractives of *L. liyuyingi*

Samples	% of clot lysis
Crude Methanol Extracts	43.55%
Pet-ether Soluble Fraction	42.11%
Carbon Tetrachloride Soluble Fraction	12.59%
Aqueous Soluble Fraction	21.20%
Control	8.20%
Streptokinase	65.16%

3.3 Membrane stabilizing activity

The crude methanol extract of *L. liyuyingi*, as well as different soluble fractions derived from this extract, were subjected to assay for membrane stabilizing activities following standard protocols and the obtained results were represented in Table 3. For hypotonic solution induced haemolysis, at a concentration of 1.0 mg/ml the highest level of membrane stabilizing activity was exhibited by the carbon tetrachloride fraction 88.78%, whereas acetyl salicylic acid inhibited 71.91%. On the other hand, during heat induced condition aqueous fractions of *L. liyuyingi* demonstrated highest inhibition (94.24%) of haemolysis of RBCs, which was even higher than acetyl salicylic acid and crude methanol extracts.

Table 3: Membrane stabilizing activity of different extractives of *L. liyuyingi*

Samples	% Inhibition of haemolysis	
	Hypotonic solution induced	Heat induced
Crude Methanol Extracts	59.19	46.53
Pet-ether Soluble Fraction	81.33	34.75
Carbon Tetrachloride Soluble Fraction	88.78	49.33
Aqueous Soluble Fraction	79.47	94.24
Acetyl salicylic acid	71.91	77.23

3.4 Antioxidant activity

3.4.1 Determination of total phenolic content

Total phenolic content of the different extracts was determined by using the Folin-Ciocalteu reagent and were expressed as Gallic Acid Equivalents (GAE) per gram of plant extract. Among all extractives of *L. liyuyingi*, the highest concentration of phenols was found in crude methanol extracts (200 mg of GAE/gm of extractives) followed by pet-ether, carbon tetrachloride and aqueous soluble fraction (Table 4).

Table 4: Total phenolic content of different extractives of *L. liyuyingi*

Samples	Total phenolic content (mg of GAE / gm of extractives)
Crude Methanol Extracts	200
Pet-ether Soluble Fraction	16.56
Carbon Tetrachloride Soluble Fraction	132.19
Aqueous Soluble Fraction	135.19

3.4.2 DPPH free radical scavenging assay

The IC_{50} values of different extracts of *L. liyuyingi* are presented in table 5. IC_{50} of ascorbic acid was found 2.57µg/ml. In comparison to standard, pet-ether, carbon tetrachloride, aqueous fractions and crude methanol extracts showed IC_{50} values of 51.70, 11.98, 7.75 and 2.61µg/ml respectively (Table 5).

Table 5: IC_{50} values of different extractives of *L. liyuyingi* and standard of DPPH free radical scavenging assay

Samples	IC_{50} value (µg/ml)
Crude Methanol Extracts	2.61
Pet-ether Soluble Fraction	51.70
Carbon Tetrachloride Soluble Fraction	11.98
Aqueous Soluble Fraction	7.75
Ascorbic Acid	2.57

3.5 Antibacterial activity

The crude methanol extract of *L. liyuyingi*, as well as different soluble fractions derived from this extract, were subjected to assay for antibacterial activity following standard protocols and the obtained results were represented in Table 6. The different fractions (1000 µg/disc) of *L. liyuyingi* were screened against four gram positive and five gram negative bacteria where ciprofloxacin was used as standard and showed zone of inhibition, ranging from (36-42) mm in diameter. Crude methanol extract and its fractions showed more or less inhibition against selected microorganisms (bacteria).

Table 6: Antibacterial activity by disc diffusion assay

Bacterial isolates	Zone of inhibition in diameter (mm)				
	Crude methanol extracts	Pet-ether soluble fraction	Carbon tetrachloride soluble fraction	Aqueous soluble fraction	Ciprofloxacin
Gram positive bacteria					
Bacillus cereus	12	-	7	8	40
Bacillus subtilis	10	8	13	-	40
Staphylococcus aureus	10	9	16	-	37
Sarcina lutea	16	-	14	10	36
Gram negative bacteria					
Escherichia coli	11	7	16	5	40
Salmonella typhi	10	15	16	-	37
Shigella dysenteriae	17	-	17	-	30
Vibrio mimicus	17	8	13	-	42
Vibrio parahemolyticus	13	10	17	8	38

3.6 Brine shrimp lethality bioassay

In the brine shrimp lethality bioassay the LC_{50} and LC_{90} value of the test samples after 24 hours was obtained by a plot of percentage of the shrimps died against the logarithm of the sample concentration. The best-fit line was obtained from the curve data by means of regression analysis. Vincristine sulfate was used as positive control and the LC_{50} and LC_{90} were found to be 0.45 µg/ml and 8.97 µg/ml respectively. The LC_{50} and LC_{90} value of different extractives of *L. liyuyingi* are represented in Table 7.

Table 7: LC_{50} and LC_{90} values of different extractives of *L. liyuyingi*

Samples	LC_{50} value (µg/ml)	LC_{90} value (µg/ml)
Crude Methanol Extracts	9.14	82.14
Pet-ether Soluble Fraction	1.04	32.28
Carbon Tetrachloride Soluble Fraction	0.85	30.20
Aqueous Soluble Fraction	9.41	34.14
Vincristine sulfate	0.45	8.97

3.7 Anti-nociceptive activity test

The anti-nociceptive activity of methanol extract of *L. liyuyingi* was tested by using two models (acetic acid-induced, tail immersion test) so that both the centrally and peripherally mediated effects could be investigated.

3.7.1 Acetic acid induced writhing test

In acetic acid-induced writhing test, methanol extracts of *L. liyuyingi* induced a significant decrease in the number of writhing and produced 45.73% (**$p<0.01$) and 42.83% (**$p<0.01$) writhing inhibition at the doses of 200 and 400 mg/kg body weight respectively, which was comparable to the standard drug diclofenac Na where the inhibition was 65.71% at the dose of 10 mg/kg body weight (Table 8).

Table 8: Effect of the methanol extracts using acetic acid-induced writhing test

Groups	No. of writhing	% Inhibition
Control	11.67 ± 1.2	-
Diclofenac Na 10 mg/kg	4.00 ± 1.5 ***	65.71
Methanol Extract 200 mg/kg	6.33 ± 1.20**	45.74
Methanol Extract 400 mg/kg	6.67 ± 1.45**	42.83

Values are expressed as mean ± SD (n=5), *p<0.05; **p<0.01; ***p<0.001; significant when compared with the corresponding value of control

3.7.2 Tail immersion test

In vivo anti-nociceptive activity test was done on 200 mg/kg and 400 mg/kg doses of methanol extracts of *L. liyuyingi* leaf, which were presented in (Table 9). Both the doses (200 and 400 mg/kg body weight) of methanol significantly (***p<0.001) raised pain threshold from 30 min up to 60 min when compared with control. Both extracts showed decreased pain with dose dependently like the standard diclofenac Na.

Table 9: Tabular representation of anti-nociceptive activity through tail immersion test

Group	Latency time (Sec)		
	30 minute	60 minute	90 minute
Control	2.03± 0.16	2.63 ± 0.22	3.03 ± 0.13
Diclofenac Na 50 mg/kg	3.87 ± 0.27 ***	4.62 ± 0.38 ***	4.73 ± 0.109 ***
Methanol extract 200 mg/kg	3.99 ± 0.37 ***	4.89 ± 0.51 ***	5.39 ± 0.77 **
Methanol extract 400 mg/kg	3.63 ± 0.21 ***	3.79 ± 0.11 ***	5.35 ± 0.44 ***

Values are expressed as mean ± SD (n=5), *p<0.05; **p<0.01; ***p<0.001; significant when compared with the corresponding value of control

3.8 Anti-diarrheal activity

The crude methanol extracts of *L. liyuyingi* were subjected to assay for anti-diarrheal activity following standard protocols and the obtained results were represented in Table 10. The present study revealed that the both doses of methanol extracts and standard drug showed significant activity (**p<0.01) when compared to control (1% Tween-80 solution in saline).

Table 10: Effect of methanol extracts of *L. liyuyingi* using castor oil induced anti-diarrheal activity test

Groups	No. of writhing	% Inhibition
Control	4.67 ± 1.33	-
Loperamide 5 mg/kg	1.67 ± 0.33 **	64.24
Methanol Extract 200 mg/kg	3.00 ± 0.58*	35.76
Methanol Extract 400 mg/kg	2.67 ± 0.33 *	42.83

Values are expressed as mean ± SD (n=5), *p<0.05; **p<0.01; ***p<0.001; significant when compared with the corresponding value of control

3.9 Hypoglycemic activity

The crude methanol extracts of *L. liyuyingi* were subjected to assay for hypoglycemic activity following standard protocols and the obtained results were represented in Table 11. The methanol extracts of *L. liyuyingi* has statistically significant blood glucose lowering activity at dose of 200 & 400 mg/kg.

Table 11: Effect of methanol extracts of *L. liyuyingi* using hypoglycemic activity test in mice

Groups	Plasma level of glucose
Control	6.63 ± 0.27
Glibenclamide	4.65 ± 0.49 **
Methanol Extract 200 mg/kg	5.72 ± 0.53 *
Methanol Extract 400 mg/kg	5.80 ± 0.04 **

Values are expressed as mean ± SD (n=5), *p<0.05; **p<0.01; ***p<0.001; significant when compared with the corresponding value of control

IV. Discussion

A large proportion of drugs in clinical use are produced by the synthesis of natural products and/or their derivatives, and new plant-derived medicines are continually being discovered as the herbal remedies are cost effective, having minimum toxicity with reduced health hazards and easily available in market as compared to synthetic medicines. By recombinant DNA technology several thrombolytic drugs are developed but site specificity with fewer side effects of thrombolytic drugs are desirables in natural thrombolytic product [31]. As a part of discovery of cardio-protective drugs from natural sources the extractives of *L. liyuyingi* were assessed for thrombolytic activity where the comparison of streptokinase with water clearly confirmed that clot dissolution

does not occur when water was added to the clot and a significant thrombolytic activity was observed after treating the clots with *L. liyuyingi.*

Membrane stabilization assay of erythrocytes is a very popular tool to investigate the anti-inflammatory potential of the plant extract. A possible explanation for the stabilizing activity of the present study of the extractives may be due to an increase in the surface area/volume ratio of the cells which could be brought about by an expansion of membrane or shrinkage of the cell and an interaction with membrane proteins [32]. Several flavonoids and triterpenes have been reported earlier to have anti-inflammatory activity [33-34]. As flavonoids are present in *L. liyuyingi*, it might be a reason for its membrane stabilizing anti-inflammatory potential.

Ascorbic acid had been reported to exhibit greater potential antioxidant activity [21, 35]. The phenomenon in this study is acceptable since ascorbic acid has the highest inhibition percentage inhibits free radical activity. The greater the decolorizing action, upon reduction by either the process of hydrogen or electron donation, the higher the antioxidant activity and is reflected by lower IC_{50} value. Substances which are able to perform this reaction can be considered as antioxidants and, therefore, radical scavengers. In the present study, all the plant extracts showed dose dependent scavenging of DPPH free radicals in a way similar to that of the standard antioxidant ascorbic acid. Presence of total phenol content and flavonoid in the plant extracts may be a reason for this DPPH-scavenging activity [35]. According to Odabasoglu *et al.* [36] there is high correlation between antioxidant activity and phenolics content as the phenolics constituents can react with active oxygen radicals such as hydroxyl radical [37], superoxide anion radical [38] and lipid peroxy radical [39]. The presence of phenolic content in *L. liyuyingi* extract may be responsible for such high antioxidant potentials.

Antimicrobial therapy markedly reduces the morbidity and mortality, emergence of resistance to first line antibiotics poses challenge in treatment of several human infections [40-42] and is prompting a revival in research of the antimicrobial role of plants against resistant strains due to comparable safety and efficacy. Crude methanol and carbon tetrachloride soluble fractions of leaf extracts of *L. liyuyingi* showed a moderate range of antibacterial activity against all selected gram positive and gram negative microorganisms at the concentration of 1000 µg/ml, whereas the range of zone of inhibition was within (7-17) mm (Table. 6). Kunle and Egharevba, [43] suggested to consider the presence of flavonoids in a plant as indication of its antioxidant, antiallergic, antiinflammatory, antimicrobial and anticancer properties.

The findings of this study suggest that there may be cytotoxic compounds in *L. liyuyingi* leaf extracts that can induce the cytotoxic action against cancer cells and initiate antiproliferation effect leading to cancer cell death. The cytotoxicity of plant material would indicate the presence of antitumour compounds in plant extract [44]. In the present study, carbon tetrachloride fraction (LC_{50}=0.85 µg/ml, LC_{90}=30.20 µg/ml) revealed most cytotoxicity in comparison with positive standard of vincristine sulfate (LC_{50}=0.45 µg/ml, LC_{90}=8.97 µg/ml). Crude extracts resulting in LC_{50} values less than 250 µg/ml could be considered active and potential for further investigation [45-46].

The analgesic effect of any plant extract reducing the number of writhing will render analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition [47]. In acetic acid induced writhing test and tail immersion test, methanol extracts of both doses of *L. liyuyingi* induced a significant (**$p< 0.01$) decrease in the number of writhing and significantly (***$p<0.001$) raised pain threshold from 30 min up to 60 min when compared with control respectively. According to phytochemical screening, this anti-nociceptive effect of the leaf extract can be due to the presence of alkaloid, quinine and coumarin which is known to give analgesic effects *in-vivo* [48].

Diarrhea is one of the most common causes for thousands of deaths every year. Therefore, identification of new source of anti-diarrheal drugs becomes one of the most prominent focuses in modern research. The present study revealed that the activity of methanol extract was increased in a dose dependent manner.

Any drug that is effective in diabetes will have the ability to control the glucose level by different mechanisms. Methanol extract of *L. liyuyingi* exhibited significant antihyperglycemic activity at a dose level of 200 and 400 mg/kg; whereas the blood glucose lowering activity of glibenclamide was 4.65 plasma level of glucose (Table 11).

V. Conclusion

All the conducted experiments in the present study are based on crude extract and different soluble fractions are considered to be preliminary and more sophisticate research is necessary to reach a concrete conclusion about the findings of the present study. It can be concluded from the above findings, the plant *L. liyuyingi* have moderate to significant thrombolytic, membrane stabilizing, antioxidant, antimicrobial, cytotoxic, analgesic, anti-diarrheal and hypoglycemic activity. So, further scientific studies are necessary to elucidate detailed mechanism of action and isolate the responsible active principles.

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