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Research Article

## Screening of Phytochemicals and Antioxidant Potential of Leaves Extract of *Litsea glutinosa*

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### ABSTRACT

*Litsea glutinosa* (Lour) leaves are deliberated as worthy traditional medicine. The aim of this study was to screen the phytochemicals, to evaluate the total flavonoid contents as well as antioxidant activity of various extract of *Litsea glutinosa* (Lour). The phytochemical analysis of each solvent extract was also carried out. Total flavonoid content was determined by aluminium chloride colorimetric assay. Antioxidant activity was determined using 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Ferric reducing ability of plasma (FRAP) and Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>) free radical scavenger methods. Phytochemical screening of various extract revealed the presence of Alkaloids, Flavonoids, Diterpenes, Proteins, Carbohydrate and Saponins. The total flavonoid content was found 1.89, 5.01 and 3.16 mg/100mg of dry weight of pet ether, ethanol and aqueous extract respectively, expressed as Quercetin equivalents. Antioxidant activity was performed using three methods DPPH, Ferric reducing ability of plasma (FRAP) assay and Hydrogen Peroxide scavenging activity. Our current results emerged that *Litsea glutinosa* (Lour) act as an antioxidant agent due to its free radical scavenging. So, the plant may be further pursued to find out for its pharmacological active natural products.

**Keywords:** *Litsea glutinosa* (Lour), Qualitative, Quantitative phytochemical, Antioxidant activity.

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### INTRODUCTION

Since very old times, herbal medications have been used for relief of symptoms of disease<sup>1</sup>. Despite the great advances observed in modern medicine in recent decades, plants still make an important contribution to health care. Much interest, in medicinal plants however, emanates from their long use in folk medicines as well as their prophylactic properties, especially in developing countries. Large number of medicinal plants has been investigated for their antioxidant properties. Natural antioxidants either in the form of raw extracts or their chemical constituents are very effective to prevent the destructive processes caused by oxidative stress<sup>2</sup>. Although the toxicity profile of most medicinal plants have not been thoroughly evaluated, it is generally accepted that medicines derived from plant products are safer than their synthetic counterparts<sup>3-4</sup>. In recent years, consumption of vegetables and fruits in the average diet has been highlighted for its contribution towards lowering the risks of several life-threatening diseases such as coronary heart disease, stroke, pulmonary disease, and different types of cancer<sup>5-14</sup>. The benefits are due to the presence of polyphenols, Flavonoids and

vitamins<sup>15-17</sup> of these phytochemicals, polyphenols are largely recognized as anti-inflammatory, antiviral, antimicrobial, and antioxidant agents.

*Litsea glutinosa* (Lour.) C. B. Rob., is an aromatic medicinal tree belongs to the family Lauraceae and rarely distributed in the Western Ghats. It is a medicinal plant of immense pharmaceutical value. The species is critically endangered due to its indiscriminate collection as raw material for pharmaceutical industry, where it is used for manufacturing drugs for pain, arousing sexual power and in treatment of diarrhea and dysentery etc<sup>18-19</sup>. The search for novel natural antioxidants of plant origin has ever since increased. It is not known which constituents of plant are associated in reducing the risk of chronic diseases, but antioxidants appear to play a major role in the protective effect of plant medicine. The present study was designed to investigate the total flavonoids Content (TFC) and to evaluate the *in vitro* antioxidant activities of the various extract of *Litsea glutinosa* (Lour).

## MATERIAL AND METHOD

### Chemicals and equipment

Methanol, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), aluminium chloride, ascorbic acid, quercetin, ferric chloride, potassium ferricyanide, sodium hydroxide and sodium nitrite used in this study. All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals and solvent used in this study were of analytical grade. Labindia 3000+ UV/Visible spectrophotometer was used to measure the absorbance.

### Collection of Plant material

Fresh leaves of *Litsea glutinosa* (Lour) were collected and were authenticated by Dr. Jaswinder Mehta, HOD, Department of Botany, Career College, Bhopal (M.P.).

### Preparation of Extracts

120 gram of powdered leaves of *Litsea glutinosa* (Lour) were exhaustively extracted with different solvent (Pet ether, ethanol and aqueous) by maceration method. The extract was evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extracts<sup>20-22</sup>.

### Calculation of percentage yield

The percentage yield of yield of each extract was calculated by using formula:

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}}$$

### Phytochemical analysis

All the test samples were subjected to phytochemical analysis to find out the presence of chemical constituents according to Trease and Evans, Harborne and Sofowora<sup>23-25</sup>.

### Quantitative Determination of Phytochemical (Total flavonoids Content)

The total flavonoid content was determined using the method of Ordonez *et al.*<sup>26</sup>. 1ml of 2% AlCl<sub>3</sub> solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer. The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/100mg).

### In-vitro antioxidant activity of Leaves of *Litsea glutinosa* (Lour) using different methods

#### DPPH method

DPPH scavenging activity was measured by the spectrophotometer<sup>27</sup>. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10- 100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally the mean was taken. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm.

### Ferric reducing ability of plasma (FRAP) assay

The ferric ion reducing power of extracts was determined by measuring the absorbance of chromophore<sup>28</sup>. Different concentrations of extracts or standard, vitamin C (1 mL, 10-100 µg/mL) were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6), potassium ferric cyanide (2.5 mL, 1% w/v) and incubated at 50 ± 2°C for 20 min. Trichloroacetic acid (2.5 mL, 10% w/v) was added to the mixture and centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5mL) was separated and mixed with 2.5 mL of distilled water and ferric chloride (0.5 mL, 0.1% w/v). Then, the absorbance was measured at λ<sub>max</sub> 700 nm using UV-Vis spectrophotometer (Labindia 3000 plus, India).

### Hydrogen Peroxide scavenging activity

*In-vitro* antioxidant activity of the different extracts using hydrogen peroxide was performed as Czochra and Widensk proposed<sup>29</sup>. Added 2ml hydrogen peroxide (43 mol) and 1.0 ml ethanol sample [20-100 µl different extracts (4mg/ml) ethanol] accompanied by 2.4 ml 0.1 M phosphate buffer (pH 7.4). The resulting solution was maintained for 10 minutes and the absorbance at 230 nm was recorded. Three times all measurements were repeated. Without adding hydrogen peroxide, blank was ready and control was prepared without sample. It was used as a conventional compound with ascorbic acid. Free radical hydrogen peroxide scavenging activity (percent) has been calculated.

$$\text{Calculation of \%Reduction} = \frac{\text{Control Absorbance} - \text{Test absorbance}}{\text{Control Absorbance}} \times 100$$

## RESULTS AND DISCUSSION

The percentage yields of various extract obtained from *Litsea glutinosa* (Lour) are depicted in the Table 1. The maximum percentage yield was found in ethanol extract as compared to pet. ether and aqueous extract. Preliminary phytochemical studies of the extract were done according to standard methods. Phytochemical analysis revealed the presence of various bioactive constituents shown in Table 2. Data for total flavonoid content has been summarized in Table 3. Phenolic compounds of plants fall into several categories; chief among these are the flavonoids which have potent antioxidant activities. Flavonoids are naturally occurring in plants and are thought to have positive effects on human health.

Results for the free radical scavenging activity of ethanolic extract of *Litsea glutinosa* (Lour) are shown in Table 4. The extract showed radical scavenging effect in DPPH assay. The half inhibition concentration (IC<sub>50</sub>) for free radicals achieved by the extract was 82.471µg/ml which is statistically significant compared that (IC<sub>50</sub> 17.681 µg/ml) of reference antioxidative agent ascorbic acid. The extract showed significant reducing power activities as compared to ascorbic acid and proportionally increased with the increasing concentration of the extract, which is shown in Table 5. The scavenging ability of ethanolic extract of *Litsea glutinosa* (Lour) on hydrogen peroxide was shown in Table 6 and compared with Ascorbic acid as standards. The *Litsea glutinosa* extract were capable of scavenging hydrogen peroxide in an amount dependent manner.

**Table No. 1: % Yield of Leaves of *Litsea glutinosa* (Lour)**

S. No.	Extracts	% Yield (W/W)
		<i>Litsea glutinosa</i> (Lour)
1.	Pet. ether	1.37
2.	Ethanol	2.40
3.	Aqueous	1.57

Table No. 2: Result of Phytochemical Screening of Leaves of *Litsea glutinosa* (Lour)

S. No.	Constituents	Pet ether extract	Ethanol extract	Aqueous extract
1.	<b>Alkaloids</b> Hager's Test:	+ve	+ve	+ve
2.	<b>Glycosides</b> Legal's Test:	-ve	-ve	-ve
3.	<b>Flavonoids</b> Lead acetate Test:	+ve	+ve	+ve
4.	<b>Diterpenes</b> Copper acetate Test:	+ve	+ve	+ve
5.	<b>Phenol</b> Ferric Chloride Test:	-ve	-ve	-ve
6.	<b>Proteins</b> Xanthoproteic Test:	-ve	+ve	+ve
7.	<b>Carbohydrate</b> Fehling's Test:	-ve	-ve	+ve
8.	<b>Saponins</b> Froth Test:	-ve	-ve	+ve

+ve=present, -ve=absent

Table No. 3: Estimation of total flavonoids content of *Litsea glutinosa* (Lour) extract

S. No.	Extracts	Total flavonoids content (mg/ 100 mg of dried extract)
1	Pet ether	1.89
2	Ethanol	5.01
3	Aqueous	3.16

## Results of antioxidant activity using different model

## Results of antioxidant activity using DPPH method

Table No. 4: % Inhibition of ascorbic acid and ethanolic extract of *Litsea glutinosa* (Lour) using DPPH method

S. No.	Concentration ( $\mu\text{g/ml}$ )	% Inhibition	
		Ascorbic acid	<i>Litsea glutinosa</i> (Lour) extract
1	10	44.65	41.69
2	20	48.62	46.1
3	40	65.34	46.77
4	60	69.65	47.79
5	80	77.41	50.50
6	100	84.13	50.84
IC 50		17.681	82.471

## Results of antioxidant activity using Ferric reducing ability of plasma (FRAP) assay

Table No. 5: Results of antioxidant activity using Ferric reducing ability of plasma (FRAP) assay

S. No.	Concentration ( $\mu\text{g/ml}$ )	Absorbance	
		(Ascorbic acid)	<i>Litsea glutinosa</i> (Lour) extract
1	0	0	0
2	10	0.345	0.089
3	20	0.489	0.125
4	40	0.596	0.225
5	60	0.662	0.365
6	80	0.895	0.389
7	100	0.896	0.425
8	120	0.892	0.521
9	150	0.891	0.532
10	200	0.896	0.533

Results of antioxidant activity using H<sub>2</sub>O<sub>2</sub> methodTable No. 6: % Inhibition of ascorbic acid and ethanolic extract of *Litsea glutinosa* (Lour) using H<sub>2</sub>O<sub>2</sub> method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	<i>Litsea glutinosa</i> (Lour) extract
1	20	42.872	31.88
2	40	52.192	38.24
3	60	60.416	43.55
4	80	65.789	47.63
5	100	68.750	50.89
IC 50		36.613	91.940

## CONCLUSION

The present study indicated that *Litsea glutinosa* (Lour) contains considerable amount of phytoconstituents especially total flavonoids and exhibited good antioxidant activity by effectively scavenging various free radicals. The extract showed radical scavenging effect in DPPH assay. The half inhibition concentration (IC<sub>50</sub>) for free radicals achieved by the extract was 82.471 µg/ml which is statistically significant compared that (IC<sub>50</sub> 17.681 µg/ml) of reference antioxidative agent ascorbic acid. The antioxidant activities might be due to the synergistic actions of bioactive compounds present in them. However, it is still unclear which components are playing vital roles for this activity. Therefore, further studies are still needed to elucidate mechanistic way how the plant contributes to this property.

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