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PROPAGATION, ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL PROFILES OF *LITSEA GLUTINOSA* (LOUR.) C. B. ROBINSON

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Abstract

The propagation, antibacterial activity and phytochemical profiles of *Litsea glutinosa* have been focused. Percentage of viable seed was 70 by tetrazolium-chloride staining technique, while germination rates were 75, 70, 55 and 20% in clay loam soil, clay loam: compost, compost and sand, respectively. Propagation of basal cutting in clay loam soil under the sun was faster than the apical stem cuttings. Both the ethanolic and water soluble extracts of the leaves and bark showed against *Escherichia coli*, *Enterobacter intermedium, Salmonella* sp., *Staphylococcus aureus* and *Staphylococcus epidermis.* Phytochemical profiling of the bark of *L. glutinosa* showed the presence of a number of secondary metabolites including steroids and terpenoids. The isolated compounds from ethanolic bark extract (T-1 and T-3) were identified as stigmasterol and ◎-sitosterol, respectively by comparing the ^{1}H-NMR data of the isolated compounds with that of the published data.

Introduction

Litsea glutinosa (Lour.) C.B. Robinson is very important from economical, medicinal and conservation point of view. The plant has ethno-medicinal uses in diarrhea, dysentery and rheumatism. It is also used as antispasmodic and in wound healing(1). The leaf extract also show antibacterial and cardiovascular activities(2, 3). The bark of this tree also used in the preparation of energy tonic(4). The plant was reported as red listed plant and considered as critically endangered in the Andhra Pradesh, India(5) and as endangered species in Philippines(6). Once the species was abundant in the forests of Bangladesh but due to unsustainable collection of bark for commercial purpose, and also for illegal felling the tree is now very rare in the natural habitats and vulnerable to extinction(7). For the sake of conservation of such threatened species, knowledge of propagation is very much essential.

Recently, *L. glutinosa* received much attention globally, and several studies were carried out to know its chemical composition, antioxidant activity, analgesic activity and

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antibacterial activity[8-11]. Hardly any work has so far been carried out on the antibacterial activities of *L. glutinosa* using water extract, although the aqueous soluble fraction of leaves and barks are usually taken by the local people in Bangladesh. Hence, an attempt has been made to study propagation strategies of *L. glutinosa,* and to determine its antimicrobial activities and phytochemical profiles.

Materials and Methods

Mature seeds were collected from plants growing in Dhaka University Botanical garden. Seeds were subjected to viability test using tetrazoliumchloride staining technique[12]. Germination and vegetative propagation in different soil medium were conducted following appropriate nursery manual[6,13,14]. Seedling growth, height, leaf size and breath, and number of leaf were measured. Collection and preparation of plant materials for antibacterial activity and phytochemical analysis were done following standard methods[8,15]. The antibacterial activities of the plant extracts were carried out by determining the zone of inhibition using Kirby Bauer disc diffusion method[16]. The ethanolic bark extract was subjected to preparative thin layer chromatographic screening technique to isolate the secondary metabolites[17,18]. The structures of the isolated compounds were confirmed by ^{1}H-NMR spectroscopic data[18].

Results and Discussion

The results showed that out of 20 seeds of *Litsea glutinosa* 14 seeds received colour. Coloration of seeds by tetrazoliumchloride indicates the viability of the seeds. Therefore, average percentage of viable seed was found 70 in *Litsea glutinosa*. Initially, the tetrazoliumchloride solution was colorless but changed to red when it was exposed to hydrogen (reduction) which derived from enzymes in the respiration process. Embryos showing active respiration are considered viable and turned red. The darker the color was the respiratory activity in the seeds was more. Light pink color indicates a seed with reduced viability when compared to a seed that stains dark red.

Among the four soil types, maximum germination rate was found in clay loam soil (75%), followed by clay loam + compost (70%), compost (55%) and sand (20%) (Table 1).

Table 1. Germination of seeds in four types of soil.

Soil types	Number of seed sown	Number of seed germinated	Germination rate (%)
Clay loam soil	20	15	75
Compost	20	11	55
Sand	20	4	20
Compost : Clay loam	20	14	70

The germination started on the tenth day after planting in a clay loam soil. Hypogeal type of seed germination was found. Peak of germination was observed on the 30th day after sowing. Seeds were sown after seven months of storage at room temperature but germination failed which indicated the loss of seed viability or some other factors might have inhibited the germination. Growth of buds occurred on the 7th day after planting with one on a clay loam soil on the basal stem cuttings. No buds produced from the compost and sand (Table 2). Less time was taken to propagate the basal cuttings in clay loam soil under the sun than that of apical stem.

Table 2. Propagation of *Litsea glutinosa* by stem cuttings.

Soil types	Number of stem cutting planted	Production of buds	
		Apical stem	Basal stem
Clay loam soil	8	1	3
Compost	8	0	0
Sand	8	0	0
Compost: Clay loam	8	0	2

Measurement of seedling growth, height, leaf size and breadth, showed that highest growth in terms of height was observed in clay loam soil having an average of 14.15 cm while sand gave the minimum growth (Table 3). Clay loam soil type showed the highest number of leaves while sand manifested the lowest count. The length of the young leaves was longest in clay loam. Sand manifested the shortest length. There were significant differences in the height of seedling, length and width of *L. glutinosa* young leaves in different soil types at 5% significant level (Table 3).

Table 3. The growth of *Litsea glutinosa* in different types of soils in eight months.

Growth parameters	Clay loam soil	Compost	Sand	Clay loam : Compost
Average height (cm)	14.15	8.67	6.09	12.92
Average no. of leaves	12.00	10.00	7.00	12.00
Average length (cm)	9.86	7.10	3.96	8.47
Average width (cm)	3.27	3.43	2.30	3.57

Antibacrerial activities of the leaves and bark extract of *L. glutinosa* were tested against bacteria and the results are presented in Table 5. The leaves and bark extracts of *L. glutinosa* showed marked antibacterial activity against *Escherichia coli, Enterobacter intermedium, Salmonella* sp., *Staphylococcus aureus* and *S. epidermis*. The zone of inhibition of bark ethanolic extract against *E. coli, E. intermedium,, Salmonella* sp. and *S. epidermis* were 28, 26, 26 and 22 mm, respectively after 24 hrs.

The zone of inhibition was comparable with that of standard kanamycin. Ethanolic leaf extract showed maximum antibacterial activity against *E. coli* with a zone of inhibition of 30 mm. Ethanol extracts of the leaves and bark showed better results than distilled water extract. However, leaf extract of distilled water showed maximum zone of inhibition (34 mm) against *S. aureus* (Table 4). This might be due to more solubility of the active principles in water. So far, antibacterial activities of aqueous extract of the leaves of *L. glutinosa* against *E.coli, S. aureus,* and *E. intermedium* have been reported for the first time. Previously, Hosamath[8] observed the antibacterial activities of ethanolic and aqueous extract of bark against *E. coli* and *S. aureus.*

Table 4. Antibacterial activities of leaves and bark extracts of *Litsea glutinosa* against pathogenic bacteria.

Bacteria	Average zone of inhibition (mm)										
	Ethanol extract (1000 µg/disc)					Distilled water extract (10 µg/disc)					Kanamycin
	Leaf		Bark		Control	Leaf		Bark		Control	(30 µg/disc)
	12 h	24 h	12 h	24 h	24 h	12 h	24 h	12 h	24 h	24 h	24 h
Escherichia coli	24	30	23	28	-	14	16	11	13	-	32
Enterobacter intermedium	22	24	25	26	-	10	13	15	18	-	30
Salmonella sp.	13	15	24	26	-	-	10	15	17	-	32
Micrococcus luteus	-	-	-	-	-	-	-	-	-	-	28
Bacillus fastidious	-	-	-	-	-	-	-	-	-	-	28
Staphylococcus aureus	13	14.7	12	14.6	-	18	34	25	27	-	32
Staphylococcus epidermis	13	13	21.5	22	-	-	12	-	11	-	30

- = no zone of inhibition.

The result of phytochemical screening was summarized in Table 5. The table showed the presence of alkaloids, carbohydrate, steroids, flavonoid, glycosides and glucosides in the crude leaves and bark ethanolic extract.

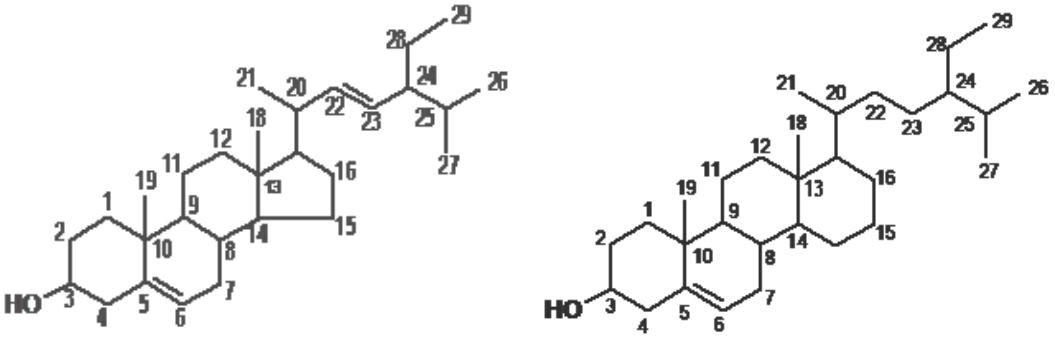
Table 5. Occurrence of secondary metabolites in leaf and bark ethanolic extracts of *Litsea glutinosa*.

Secondary metabolites	Leaf extract	Bark extract
Alkaloids	-	-
Flavonoids	+	+
Steroids	+	+
Carbohydrate	+	+
Glycosides	-	-
Glucosides	-	-

+ = present, – = absent.

The ethanolic bark extract was further subjected to PTLC screening to isolate the secondary metabolites. Two compounds: T-1 and T-3 were isolated from the bark. The isolated compounds T-1 and T-3 were characterized as stigmasterol and ◎-sitosterol, respectively by comparing the ^{1}H-NMR data with those published for the compounds [19].

From the present study, it is cleared that germination rate of *L. glutinosa* is moderately high and no satisfactory result was found from the vegetative propagation. *Ex situ* propagation of *L. glutinosa* is possible from seed. Maximum seedling and highest seedling growth were found by planting in clay loam soil. So clay loam soil is appropriate for the seed propagation of *L. glutinosa*.



T1 T3

Fig. 1. Structure of T1 and T3.

Stigmasterol (T-1), ^{1}H NMR (400 MHz, $CDCl_3$): ◎H 0.66 (3H,s Me-18), 0.81 (3H, t, Me-29), 0.83 (3H, d Me-26), 0.84 (3H, d, Me-27), 0.91 (3H, d, Me-21), 1.00 (3H, s, Me-19), 3.50 (1H, m, H-3), 5.03 (2H, dd, H-23), 5.15 (2H, dd, H-22), 5.35 (1H, m, H-6).

⚜**-Sitosterol** (T-3), ^{1}H NMR (400 MHz, $CDCl_3$): ◎H 0.75 (3H, s, Me-29), 0.82 (3H, s, Me-26), 0.83 (3H, s, Me-27), 0.92 (3H, s, Me-21), 0.95 (3H, s, Me-18), 1.19 (3H, s, Me-19), 3.63 (1H, m, H-3), 5.35 (3H, m, H-6)

The present evaluation confirmed the antibacterial properties of leaves and bark of *L. glutinosa* which supports the traditional uses against diarrhoea and dysentery in Bangladesh. The results clearly indicated that the antibacterial activity vary with the parts of the plant material used. Though leaves and bark both were active against bacteria, present study recommends that leaves should be used because bark collection will destroy the plant. Although, *L. glutinosa* is still found in the natural forests of Bangladesh, its excessive collection is a threat to this species. The results of the current study may help to realize its importance as a medicinal plant and to undertake cultivation program for commercial use and may provide important clue for the isolation of compounds responsible for bacterial growth inhibition.

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