# Screenings of Four Medicinal Plants of Bangladesh for Bioactivities

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**ABSTRACT:** The methanol extracts of leaves of *Nephelium lappaceum* L., *Pandanus foetidus* Roxb., *Ludwigia repens* Rubin. and the whole plant of *Adiantum philippense* L. were subjected to assays of antioxidant, cytotoxic, thrombolytic and membrane stabilizing activities. The antioxidant activity was evaluated by DPPH free radical scavenging activity using butylated hydroxytolune and ascorbic acid as standards. The crude extract of *N. lappaccam* exhibited the highest free radical scavenging activity ($IC_{50}$= 3.93±0.25µg/ml). On the other hand, the crude methanol extracts of *A. philippense* and *P. foetidus* demonstrated significant brine shrimp lethality with $LC_{50}$ values 0.50±0.84 µg/ml and 0.58±0.73 µg/ml, respectively as compared to the standard anti-neoplastic drug, vincristine sulfate ($LC_{50}$= 0.45 µg/ml). Weak thrombolytic activity was observed for the test samples. The crude methanol extracts of *P. foetidus* and *N. lappaceum* showed 17.31±0.82 % and 17.29±0.91 % clot lysis while standard streptokinase and water, used as positive and negative controls, demonstrated 66.77 % and 3.79 % lysis of clot, respectively. The methanol extract of whole plant of *A. philippense* inhibited hypotonic solution and heat induced haemolysis of RBCs by 53.09±1.01 % and 35.36±1.60 % as compared to 71.77 % and 42.20 % by acetyl salicylic acid, respectively.

Key words**:** *Nephelium lappaceum, Pandanus foetidus, Ludwigia repens, Adiantum philippense* free radical scavenging, thrombolysis, haemolysis

## INTRODUCTION

*Nephelium lappaceum* L. (Synonym: *Nephelium lappaceum* Rind.) commonly known as rambutan, is a medium-sized tropical tree belonging to Sapindaceae family. The fruit produced by the tree is also known as rambutan. The plant possesses anti-hyperglycemic activity. Geraniin and an ellagitannin were identified as the major bioactive constituents from *N. lappaceum*.[1]

*Pandanus foresides* Roxb. (Synonym**:** *Fisquetia macrocarpa* Gaudich.; Bengali name: Keya-kanta) is a branched shrub with few prop roots belonging to the family Pandanaceae. The methanol extract of the leaves of *P. foetidus* has neuropharmacological activities. It significantly prolonged the pentobarbitone induced sleeping time in mice and showed mild to moderate central nervous system depressant activity when assessed in mice model.[2]

*Ludwigia repens* Rubin. (Synonyms: *Ludwigia natans* Elliott., *Isnardia intermedia* Small and Alexander; Benali names: Kesardam, Mulcha, Mulsi) commonly known as creeping primrose willow, is a flowering plant belonging to the family Onagraceae. This is a mat forming perennial herb with a creeping stem up to 30 cm long. The methanol extract of leaves possesses CNS depressant analgesic activity. The extract significantly accelerated onset of sleep and maximized the duration of sleeping time when administered with thiopental sodium.[3]

*Adiantum philippense* L. (Synonym: *Adiantum lunulatum* Burm. f., Bengali names: Goyalelata,

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Kalijhant) locally known as walking maiden hair fern, is a small fern belonging to the family Adiantaceae. The plant has demulcent, astringent and emenagogue properties. It is used in the treatment of cough, asthma, fever, leprosy and hair fall.[4]Leaf paste with sugar is given in spermatorrhoea once a day for one month.[5]

As part of our ongoing investigations on medicinal plants of Bangladesh[6,7], the methanol extracts of leaves of *N. lappaceum*, *P. foetidus*, *L. repens* and the whole plant of *A. philippense* growing in Bangladesh were studied for the antioxidant potential in terms of free radical scavenging property; cytotoxic, thrombolytic and membrane stabilizing activities for the first time and we, here in, report the results of our preliminary investigations.

## MATERIALS AND METHODS

**Plant materials.** The leaves of *N. lappaceum*, *P. foetidus*, *L. repens* and the whole plant of *A. philippense* were collected from Kishoregonj in March, 2011. Voucher specimens for these plants have been maintained in Bangladesh National Herbarium, Dhaka, Bangladesh for future references.

The collected plant materials were cleaned, sun dried and pulverized. The powdered materials (500 g each) were separately soaked in 2.0 liters of methanol at room temperature for 7 days. The extracts were filtered through fresh cotton bed and finally with Whatman filter paper number 1 and concentrated with a rotary evaporator at reduced temperature (40-45 ºC) and pressure. The residues were then stored in a refrigerator until further use.

**DPPH free radical scavenging assay.** Following the method developed by Brand-Williams *et al*. (1995)[8] the antioxidant activity of the test samples was assessed by evaluating the scavenging activities of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical by using synthetic antioxidants, butylated hydroxytoluene (BHT) and ascorbic acid as positive controls.

**Brine shrimp lethality bioassay.** This technique was applied for the determination of general toxic properties of the DMSO solutions of plant extractives against *Artemia salina* in a one day *in vivo* assay[9]. Here, vincristine sulphate was used as positive control.

**Thrombolytic activity.** The thrombolytic activity was evaluated by the method developed by Prasad *et al.* (2006)[10] by using streptokinase as positive control.

**Membrane stabilizing activity.** The membrane stabilizing activity of the extractives was assessed by evaluating their ability to inhibit hypotonic solution- and heat- induced haemolysis of human erythrocytes following the method developed by Omale *et al*. (2008).[11]

**Statistical analysis.** For all bioassays, three replicates of each sample were used for statistical analysis and the values are reported as mean ± SD.

## RESULTS AND DISCUSSION

Four medicinal plants *N. lappaccam, P. foctidus, L. repenus* and *A. philippense* growing in Bangladesh and belonging to the family Sapindaceae, Pandanaceae, Onagraceae and Adiantaceae, respectively have been investigated for antioxidant potential in terms of total phenolic content and free radical scavenging property as well as cytotoxic, thrombolytic and membrane stabilizing activities.

The methanolic crude extracts of leaf of *P. foetidus*, *N. lappaceum*, *L. repens* and whole plant of *A. philippense* were subjected to DPPH free radical scavenging activity assay. The highest free radical scavenging activity was demonstrated by the methanol extracts of *N. lappaccam* ($IC_{50}$= 3.93±0.25µg/ml) followed by the *A. philippense* extract ($IC_{50}$=56.50±2.50 µg/ml) (Table 1).

In brine shrimp lethality bioassay, the crude methanol extracts of *A. philippense* ($LC_{50}$= 0.50±0.84 µg/ml) and *P. foetidus* ($LC_{50}$= 0.58±0.73 µg/ml) demonstrated the presence of considerable bioactive principles (Table 1).

**Table 1. The free radical scavenging and cytotoxic activities of the methanol extracts of *N. lappaceum, P. foetidus, L. repens* and *A. philippense* and reference standards.**

| Samples/ Standards | DPPH Free radical scavenging activity ($IC_{50}$ µg/ml) | Cytotoxic activity ($LC_{50}$ µg/ml) |
|---|---|---|
| *N. lappaccam* | 3.93±0.25 | 77.64±0.45 |
| *P. foctidus* | 1093.85±4.01 | 0.58±0.73 |
| *L. repenus* | 707.23±2.54 | 8.85±0.56 |
| *A. philippense* | 56.50±2.50 | 0.50±0.84 |
| Butylated hydroxytolune | 26.65±0.99 | - |
| Ascorbic acid | 5.63±0.58 | - |
| Vincristine sulfate | - | 0.45±0.45 |

All the test samples demonstrated weak thrombolytic activity. *P. foetidus* and *N. lappaceum* extracts showed 17.31±0.82 % and 17.29±0.91 % clot lysis, respectively while standard streptokinase revealed 66.77 % lysis of clot (Table 2).

**Table 2. Thrombolytic activity of methanol extracts of *N. lappaceum, P. foetidus, L. repens* and *A. philippense*.**

| Samples/ Standards | % of lysis of RBC |
|---|---|
| *N. lappaccam* | 17.29±0.91 |
| *P. foctidus* | 17.31±0.82 |
| *L. repenus* | 9.89±0.35 |
| *A. philippense* | 16.56±1.41 |
| Water | 3.79±0.55 |
| Streptokinase | 66.77±0.36 |

The methanol extract of whole plant of *A. philippense*, at concentration 1.0 mg/ml, exhibited significant membrane stabilizing activity as compared to the standard acetyl salicylic acid (0.10 mg/mL). This extract inhibited hypotonic solution- and heat- induced haemolysis of RBCs by 53.09±1.01 % and 35.36±1.60 % as compared to 71.77 % and 42.20 % inhibited by acetyl salicylic acid, respectively (Table 3).

**Table 3. Effect of methanol extracts of *N. lappaceum, P. foetidus, L. repens* and of *A. philippense* on heat- and hypotonic solution-induced haemolysis of erythrocyte membrane.**

| Sample code | % inhibition of haemolysis | |
|---|---|---|
| | Heat induced | Hypotonic solution induced |
| *N. lappaccam* | 33.61±0.71 | 40.75±0.25 |
| *P. foctidus* | 30.19±1.63 | 11.22±0.80 |
| *L. repenus* | 30.16±0.89 | 34.25±0.72 |
| *A. philippense* | 35.36±1.60 | 53.09±1.01 |
| Acetyl salicylic acid | 42.20±1.65 | 71.77±0.95 |

The investigated plants are reported to have many important traditional uses. The smashed leaf of *N. lappaccam* is used by the Kensiu Tribe of Lubuk Ulu Legong, Kedah, Malaysia for the treatment of influenza fever.[12] *P. foctidus* has extensive medicinal uses. The plant has neuropharmacological activity and is used in leprosy, smallpox, syphilis, scarbies and leucoderma. Leaves are alexiteric, tonic and aphrodisiac and are used in diabetes.[13] The methanol extract of leaves of *L. repenus* possesses analgesic activity.[3] The leaf paste of *A. philippense* is used for immediate relief of indigestion.[14] It is clearly evident from the above findings that the crude methanol extracts of *N. lappaccam, P. foctidus, L. repenus* and *A. philippense* possess significant bioactive principles. It is clearly evident from the above findings that the crude methanol extracts of *N. lappaccam, P. foctidus, L. repenus* and *A. philippense* possess significant bioactive principles and these results are consistent with some of the folkloric uses of the plant. Therefore, these medicinal plants are good candidates for further systematic, chemical and biological investigation to isolate the active constituents.

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