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# Phytochemical content, antioxidant and antidiarrhoeal activities of *Limnophila repens*

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**ABSTRACT**

The study was carried out to determine the phytochemical content, antioxidant and antidiarrhoeal activity of methanol extract of whole plant of *Limnophila repens*. The total phenolic, flavonoids, flavonols, tannins, β-carotene, lycopene, chlorophyll-α, and chlorophyll-β contents were found to be 65.21±0.004 mg GAE/g, 44.83±0.003mg QE/g, 17.21±0.002mg QE/g, 56.27±0.002 mg GAE/g, 1.32 ± 0.01μg/g, 0.93±0.012μg/g, 11.85±0.04mg/g and 9.69±0.037 mg/g, respectively. *In vitro* antioxidant potential of *L. repens* was assessed using various methods including 2,2-diphenyl-1 picrylhydrazyl (DPPH), hydroxyl radical scavenging assay, reducing power assay, FRAP assay and thiobarbituric acid assay. $IC_{50}$ values of the aforesaid tests were found 2.33mg/mL, 442.31μg/mL, 374μg/mL, 55.09μg/mL, and 4.30mg/mL, respectively. The total antioxidant capacity was observed 140.7±0.004mg/g AAE. Castor oil induced diarrhoea method was conducted to investigate the antidiarrhoeal activity of *L. repens*. At the dose of 400mg/kg BW, the plant extract (35.62% inhibition of diarrhea) exhibited stronger effect compared to standard drug, loperamide (27.4% inhibition of diarrhea).

**Keywords**: *L repens*, phytochemical contents, antioxidant, antidiarrhoeal.

## INTRODUCTION

*Limnophila repens*, commonly known as ‘creeping meshweed’ is a herb widely distributed in South and South-East Asia, China, Pacific islands and Australia (Rahman, 2006). In Bangladesh, this species is commonly found in Chittagong, Cox's Bazar, Dhaka and Sylhet. The genus ‘*Limnophila*’ belonging to Scrophulariaceae family consists of mostly herbs (Rahman, 2006); most of the species of the family are comparatively small aquatic or semi-aquatic herbs inhabiting in marshes, riversides, forest paths or similar wet places and also as weeds in rice-fields (Dhatchanamoorthy *et al.*, 2016). About forty species of *Limnophila* genus was reported (Roy *et al.*, 2015). Several species of this genus are used as folk medicines in the treatment of various ailments (Brahmachari, 2008) and there are substantial scientific evidences proving their traditional uses. For example, antimicrobial and antioxidant activities of *L. aromatica* (Gorai *et al.*, 2014); antimicrobial activity of *L. indica* (Brahmachari *et al.*, 2013), *L. heterophylla* (Sundararajan and Koduru, 2016), *L. rugosa* (Linh and Thach, 2011), *L. geoffrayi* (Thongdon-a and Inprakhon, 2009), *L. polystachya* (Kalimuthu *et al.*, 2011); anti-inflammatory activity of *L. sessiliflora* (Poquiz *et al.*, 2017); anthelmintic activity of *L. conferta* (Reddy *et al.*, 1991). Diarrhoeal diseases accounts for around 5,00,000 death of children under five years of age each year and this disease claims the second largest number of death of children even more than AID, malaria, and measles combined. There are nearly 1.7 billion cases of diarrhoeal disease every year globally (Mokdad *et al.*, 2016). The disease is defined as the passage of three or more loose or liquid stools per day. Diarrhoea is in fact a symptom of an infection in the intestinal tract caused by a number of bacterial, viral and parasitic organisms. The common bacterial cause of diarrhoeal disease include *Campylobacter*, *E. coli*, non-typhoidal *Salmonella* and *Shigella*. Rotavirus accounts for around 40% hospitalizations due to diarrhoeal case while parasites such as *Cryptosporidium, Entamoeba-histolytica* and *G lamblia* are also found responsible (Chowdhury *et al.*, 2015; Mokdad *et al.*, 2016).


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Herbal medicines have been used effectively against various diseases and it is observed that around 80% of the population in developing countries rely on folk medicines for primary healthcare (Balemba *et al.*, 2010). There are a good number of research works on indigenous medicinal plants reporting therapeutic value against diarrhoea and bacterial infections around the world (Calzada *et al.*, 2017; Derebe *et al.*, 2018; Konaté *et al.*, 2015; Saheed and Tom, 2016; Sharma *et al.*, 2019; Sharma, *et al.*, 2015). The herb *Limnophila repens* mainly grows in marshy places particularly in low lying areas. It is traditionally used in the treatment of leukoderma. To cure leukoderma, a paste of the whole plant of *Mimosa pudica* and *Limnophila repens* is applied to affected areas (Rahman, 2006). Recently, antipyretic effect (Gunji, 2018a) and anthelmintic activity (Gunji 2018b) of methanol extract of whole plant of *L. repens* were reported. Phytochemical screening of the whole plant showed the presence of flavonoids, alkaloids, phenols, tannins, steroids, carbohydrates, glycosides, amino acids, proteins while volatile oil, saponin, acid compounds were reported absent (Gunji, 2018b). Despite its use as folk medicine and other putative therapeutic potentials, no quantitative phytochemical analysis and examination of therapeutic effects of *L. repens* have been performed against important health concerns of Bangladesh– in diarrhoeal disease. To confront this disease, in this present study, we performed experiments on phytochemical analysis, *in vitro* antioxidant activity, and *in vivo* antidiarrhoeal activity of methanol extract of whole plant of *L. repens.*

## MATERIALS AND METHODS

Folin-Ciocalteau reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric chloride, ascorbic acid, tannic acid and catechin were purchased from Sigma Co. (St. Louis, Missouri, USA). Methanol, hydrochloric acid, sodium hydroxide, aluminium chloride, sodium carbonate, sodium hydroxide and potassium ferricyanide were purchased from Merck, Darmstadt, Germany. All the chemicals and reagents were of analytical grade.

### Preparation of plant extract

The whole plant was collected from rural region of Mowlavibazar district, Sylhet, Bangladesh on October 2018 and was identified. A voucher Specimen (No.: DACB 46008) were deposited in the Bangladesh national herbarium, Dhaka. The collected plant mass was washed with water, sun/air-dried for ten days and then ground into coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced. About 500g of the powdered materials of plant was taken separately in a clean, flat bottomed glass container and soaked in 1500 ml of 80% methanol at room temperature for three weeks accompanying occasional shaking and stirring. Then the solution was filtered using filter cloth and Whatman filter paper No. 1. The filtrate (methanol extract) obtained was evaporated with a rotary evaporator (RE-EV311-V, LabTech Italy). It rendered a gummy concentrate; this gummy concentrate was designated as crude methanol extract. This extract was then stored in a beaker covered with aluminium foil in a refrigerator.

### Determination of Phytochemical contents

### Determination of total phenolic content (TPC)

Total phenolic content of extract was determined using Folin-Ciocalteu (FC) reagent described by Ebrahimzadeh *et al* with minor modifications (Ebrahimzadeh *et al.*, 2008). The calibration curve was formed using gallic acid (0-300μg/mL). 0.5mL of extract (1mg/mL) was added to a 2.5mL FC reagent and mixed thoroughly. Then 2mL of sodium carbonate was added to the mixture and the mixture was incubated for 20min at room temperature. Absorbance of the samples was then measured at 760nm and result was expressed as mg gallic acid equivalent per gram of extract.

### Determination of total flavonoid content

The total flavonoid content was determined using the method of (Rezaeizadeh *et al.*, 2011). 0.5 ml of extract was mixed with 1.5mL methanol and then 0.1mL of 10% aluminium chloride was added. After that, 0.1mL of potassium acetate was added followed by 2.8mL of distilled water. The mixture was incubated for 40min at room temperature. The absorbance was measured at 415nm. The total flavonoid content was determined using a standard curve with quercetin (1-100μg/mL) as the standard. Total flavonoid content is expressed as μg of quercetin equivalents (QE)/mg of extract.

### Determination of total flavonol content

The flavonol content was determined by the method of (Kumaran and Karunakaran, 2007) with slight modifications. Twoo mili liter of plant extract (1mg/mL) was mixed with 2mL of 2% aluminium chloride and 6mL of 5% sodium acetate.

Then the mixture was incubated for 2.5h at room temperature and the absorbance was measured at 440nm. Quercetin, treated in the same manner as the sample, was used to produce a standard calibration curve in the range of 0 to 250µg/mL. The results were expressed as mg of quercetin equivalent (QE) per gram of dry of extract.

**Determination of tannin content**

Tannin content of the extract was determined by Folin-Ciocalteu reagent method described by (Mohammed and Manan, 2015). Here, 0.5mL of extract (as 1:200 dilution) and 0.1mL of Folin-Ciocalteu reagent (0.5N) was mixed and incubated at room temperature for 15min. 2.5mL of sodium carbonate (20%) was added, incubated at room temperature for 30min and absorbance was measured at 760nm. Tannin content was expressed as gallic acid equivalent (mg/g).

**Determination of chlorophyll-α and chlorophyll-β**

Here, plant extract of 1mg/mL were taken in three separate test tubes and absorbances were measured in UV-VIS spectrophotometer at 653 and 666nm. Concentrations of chlorophyll-α and -β were determined according to the equations as follows (Lichtenthaler and Wellburn, 1983):

Chlorophyll-α (mg/mL) = $15.65A_{666} - 7.340A_{653}$

Chlorophyll-β (mg/mL) = $27.0A_{653} - 11.21A_{666}$

**Determination of β-carotene and Lycopene**

One hundred (100mg) of dried methanol extract was mixed with 10mL of acetone-hexane mixture (4:6) for 1min and the filtered. The absorbance was reported at three different wavelengths of 453, 505 and 663nm (Kumari *et al.*, 2011). The β-carotene content was calculated by:

β-carotene (mg/100mL) = $0.216A_{663} - 0.304A_{505} + 0.452A_{453}$. Lycopene (mg/100mL) = $-0.0458A_{663} + 0.372A_{505} - 0.805A_{453}$

The results were presented as µg of β-carotene and lycopene/g of extract.

***In vitro* antioxidant assays**

**Determination of total antioxidant capacity**

Total antioxidant activity was determined by phophomolybdenum method with slight modifications (Bag *et al.*, 2015). The method is based on the reduction of Mo (VI) to Mo (V) by the extract and formation of green phosphate Mo (V) complex at acidic pH. 1mL of plant extract was mixed with a mixture of 3mL of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The test tubes were then capped and incubated at 90°C for 90min. After that, samples were cooled to room temperature and absorbance was measured at 695nm against blank. Ascorbic acid was used as standard.

**Free radical scavenging power (DPPH)**

Free radical scavenging power was estimated by using 2,2-diphenyl-picryl-hydrazyl radical scavenging (DPPH) assay (Ahmed *et al.*, 2015). Three (3mL) of DPPH working solution was mixed with 300µL plant extract (1mg/mL). The mixture was then incubated for 30min at room temperature. The absorbance was measured at 517nm. Ascorbic acid and BHA (Butylated Hydroxy Anisole) were used as standard. Percentage of inhibition was calculated using the formula:

$$\text{Inhibition (\%)} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100\%$$

Here, $A_0$ is the absorbance of control and $A_1$ is the absorbance of sample.

**Reducing power assay (RPA)**

The reducing power was determined by the method of Jayanthi *et al* (Jayanthi and Lalitha, 2011). One (1mL) extract was mixed with 2.5mL of phosphate buffer (200mM, pH 6.6) and 2.5mL of potassium ferricyanide (30mM) and incubated at 50°C for 20min. Thereafter, 2.5mL of trichloroacetic acid was added to the reaction mixture, centrifuged for 10min at 3000 rpm. The upper layer of solution (2.5mL) was mixed with 2.5mL of distilled water and 0.5mL of $FeCl_3$ (6mM) and absorbance was measured at 700nm. Ascorbic acid was used as positive control. Increased absorbance of the reaction mixture indicates an increase in reducing power.

**Ferric reducing antioxidant activity (FRAP)**

FRAP reagent was prepared from acetate buffer (pH 3.6), 10mM TPTZ solution in 40mM HCl and 20mM iron (III) chloride solution (Benzie and Strain, 1999). The reagent was then warmed at 37°C in oven before use. 300µL of extract was added to 3mL of FRAP reagent and stirred well. Absorbance was measured at 593nm. Ascorbic acid was used as standard and the curve of ascorbic acid was prepared using the similar procedure. The results were expressed as µg ascorbic acid/gm of sample.

**Hydroxyl radical scavenging assay (HRSA)**

Salicylic acid was used to measure the hydroxyl radical formation according to the modified method of Al-Trad *et al* (2018)(Al-Trad *et al.*, 2018). One (1mL) of the extract solution of different concentrations was added to 250µL of 6mM $FeSO_4$, followed by addition of 0.5mL of 6mM hydrogen peroxide. The reaction mixtures were subjected to shaking followed by standing for 10min. Then 1mL of 6mM salicylic acid was added and incubated for 30min at room temperature. Ascorbic acid was used as a positive control. The absorbance was measured at 510nm against the blank.

**Thiobarbituric acid (TBA) method**

Extracts (2mL) and standard solutions (2mL) were added to 1mL of 20% aqueous trichloroacetic acid and 2mL of 0.67% aqueous thiobarbituric acid. The samples were boiled for 10min and then cooled. The test tubes were centrifuged at 3000 rpm for 30min. Absorbance of the supernatant was evaluated at 532nm (Mackeen *et al.*, 2000).

**Determination of $IC_{50}$ and $EC_{50}$ value**

$IC_{50}$ (Inhibition concentration) value indicates the amount of antioxidant necessary to reduce the initial concentration by 50%. Lower the value higher the antioxidant effects. $EC_{50}$ (Effective concentration) represents the concentration of a compound where 50% of its maximal effect is observed. $IC_{50}$ and $EC_{50}$ values were determined from extrapolating graph of activity versus the concentration of extract based on screening results of the triplicate measurement of the extract.

**Antidiarrhoeal activity in mice model**

Antidiarrhoeal activity in mice model was determined by the method of (Awouters *et al.*, 1978) with slight modifications. All animal procedures and experimental protocols were approved by the Departmental Research Committee, Noakhali science and Technology University (Reference No.: 2018/BKH1403064F) and were carried out in accordance with the Guide for the Care and Use of Laboratory Animals. Six-seven weeks old Swiss albino mice of both sexes with mean body weight 25±5.0g were procured from Jahangir Nagar University, Dhaka, Bangladesh. The animals were housed as 4 in 1 polycarbonate cage in a temperature (23±1)°C and humidity (55-60%)-controlled room with a 12h light-dark cycle. The mice were divided into four groups (n=5). First group was treated with saline (10mL/kg.bw) and second group was treated with loperamide (5mg/kg.bw). Third and fourth group were treated with methanol extract of *L. repens* at 200 and 400mg/kg respectively. Diarrhea was induced by administering castor oil (0.2mL/animal) 30min prior to sample or standard treatment. Immediately after administering castor oil, each animal was kept in an individual cage with a floor lined with blotting paper and observed for 5h. The following parameters were monitored: time to initial evacuation, evacuation classification: 1 (normal stool), 2 (semi-solid stool), and 3 (watery stool) and evacuation index (EI). EI value was calculated according to the following formula: EI=1 x (No 1. stool) +2 x (No 2. stool) +3 x (No 3. stool)(Mbagwu & Adeyemi, 2008). Percentage inhibition of diarrhea was calculated as (EI of vehicle -EI of sample) x 100/(EI of vehicle) (Mbagwu and Adeyemi, 2008).

## RESULT AND DISCUSSION

**Phytochemical contents**

The contents of phenolics, flavonoids, flavonols, tannins, chlorophyll-α, chlorophyll-β, β-carotene and lycopene are 65.22±0.004mg gallic acid equivalent, 44.83±0.003mg quercetin equivalent, 17.21±0.002mg quercetin equivalent, 56.28±0.002mg gallic acid equivalent, 11.85±0.04mg, 9.66±0.04mg, 1.32±0.01µg and 0.93±0.013µg respectively in 1g of methanol extract (Figure 1).

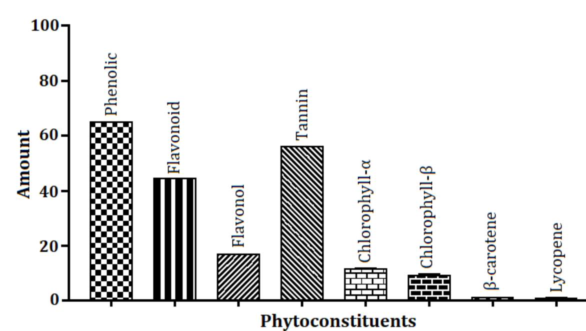


Figure 1: Amount of phytoconstituents in methanol extract of *L. repens*. Here, phenolic and tannin were expressed as 'mg gallic acid equivalent'; flavonoid and flavonol were expressed as 'mg quercetin equivalent'; β-carotene and lycopene in µg in 1g of methanol extract; chlorophyll α and chlorophyll β in mg in 1g of methanol extract. Each sample was assayed in triplicate.

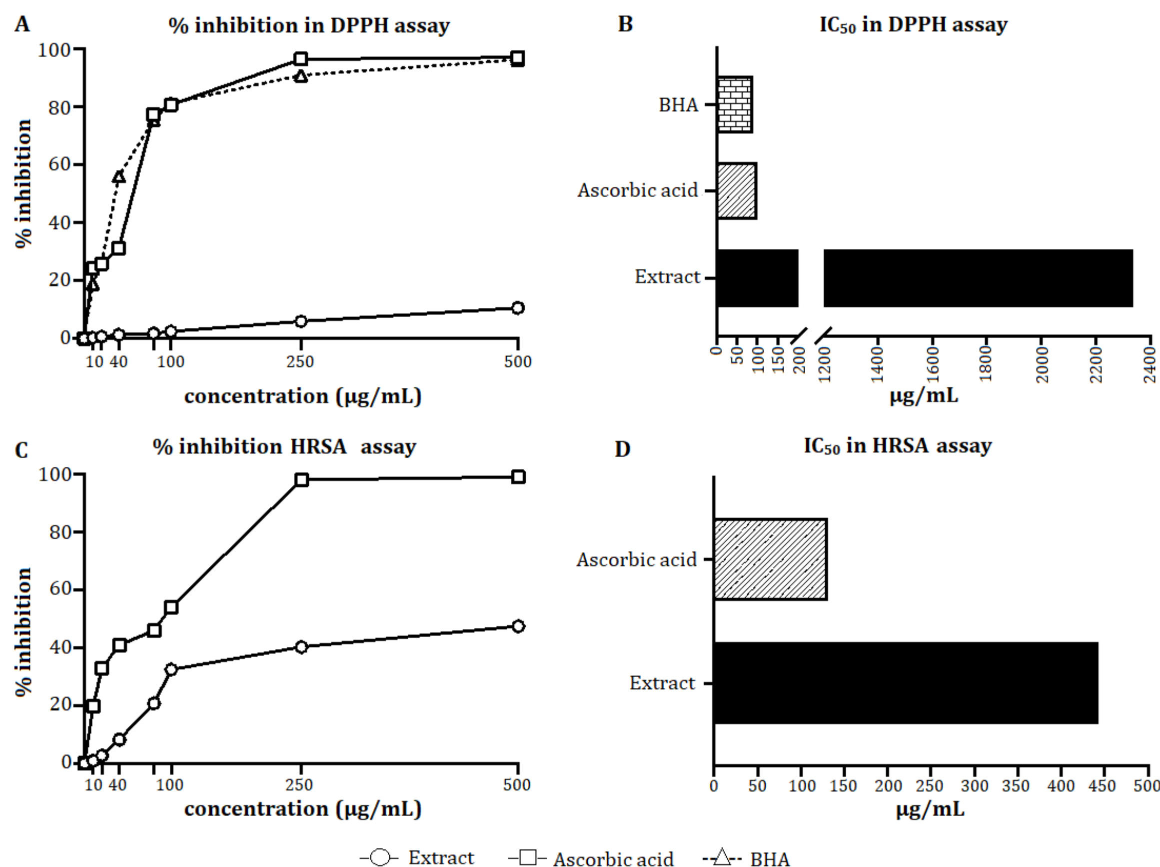


Figure 2. % inhibition and $IC_{50}$ values in DPPH assay (A and B) and in HRSA assay (C and D). All the samples were performed in triplicate. In case of percentage inhibition, standard error of mean and standard deviation were determined but these statistical parameters were so small that eventually merged into the sample data point.

## Antioxidant activity of plant extract

### Total antioxidant capacity

Total antioxidant capacity of any extract is contributed by the phytochemical components present in the extract. Total antioxidant capacity of the methanol extract was determined using the linear regression equation (y = 0.007x –0.304$A_{505}$ +0.452$A_{453}$. Lycopene (mg/100mL) = –0.0458$A_{663}$ 0.0394, $R^2$=0.9982), where x is absorbance and y is ascorbic acid concentration in µg) of the calibration curve and was expressed as ascorbic acid equivalent. The total antioxidant capacity of the extract was found 140.7±0.004mg ascorbic acid equivalent/g extract.

### DPPH free radical scavenging power

DPPH assay is one of the most widely used method for screening antioxidant activities of plants. Scavenging of free radicals in the presence of varying concentrations of extract and standard was monitored at 517nm and the absorbance, corresponding percentage of inhibition and $IC_{50}$ values (Figure 2) (A and B). Extracts and standard scavenged DPPH radicals in a dose-dependent manner. Data indicate that the methanol extract of *L. repens* have the differential capacities to scavenge the DPPH free radicals. However, the plant extract of *L. repens* ($IC_{50}$ =2337.27µg/mL) exhibited very weak DPPH free radical scavenging activity as compared with ascorbic acid ($IC_{50}$ = 100.49µg/mL) and BHA ($IC_{50}$ = 89.12µg/mL) as evidenced by their $IC_{50}$. However, in contrast to present study, different solvents (Water, methanol, ethanol and acetone) extracts of *L. aromatica* gave strong DPPH free radical scavenging capacity.

### Hydroxyl radical scavenging assay

Hydroxyl radical is one of the potent reactive oxygen species that can affect the biomolecules such as proteins, polypeptides, nucleic acids and

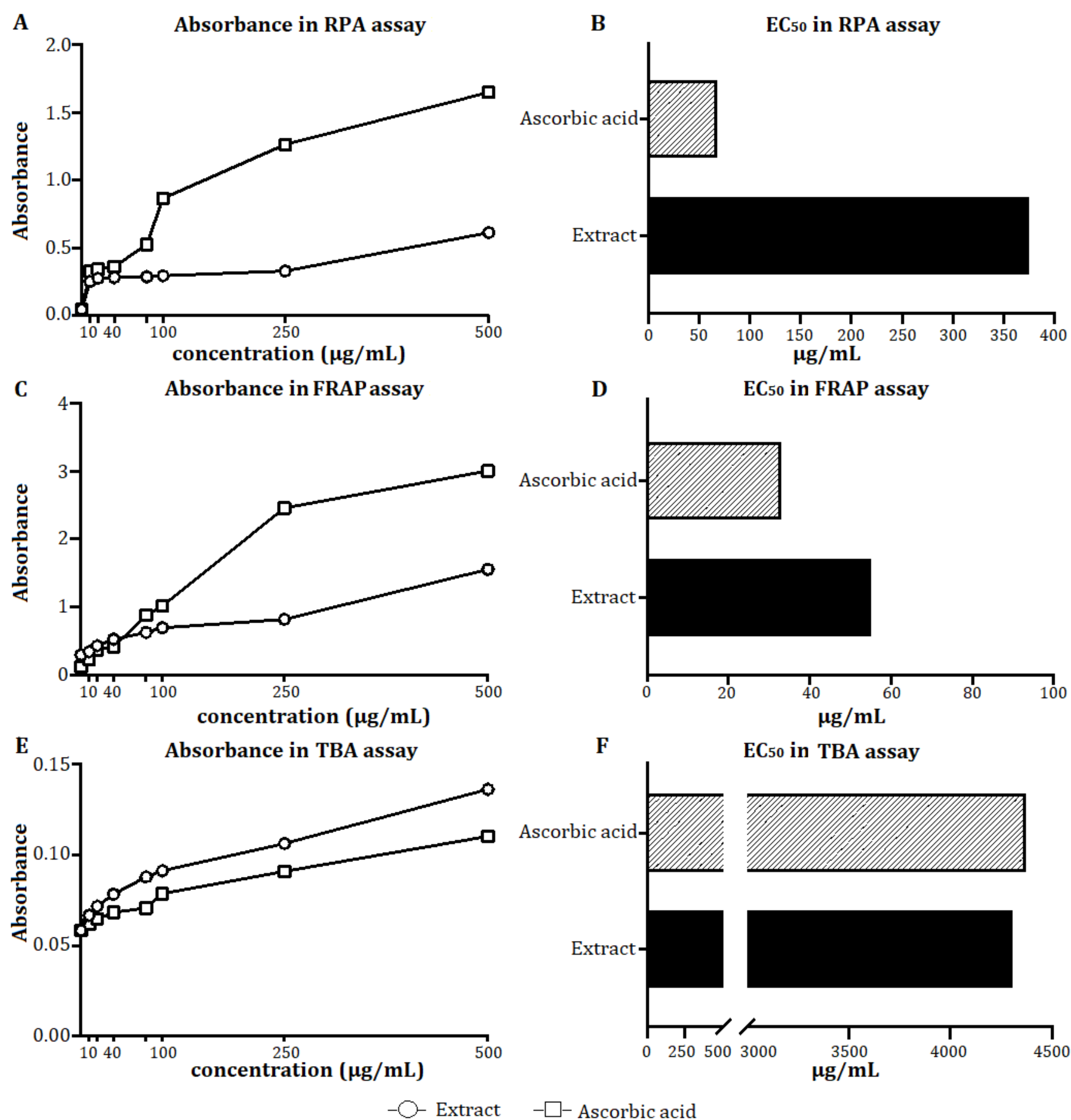


Figure 4. Absorbance and $EC_{50}$ values in RPA assay (A and B), FRAP assay (C and D) and TBA assay (E and F). In case of percentage inhibition, standard error of mean and standard deviation were determined but these statistical parameters were so small that eventually merged into the sample data point.

lipids (Catala, 2009). The extracts methanol of *L. repens* and standards (AA and BHA) exponentially reduced $ABTS^{\bullet+}$ radicals with increasing concentration (Figure 2C and 2D). The plant extracts scavenged hydroxyl radical in dose dependent manner similar to that of DPPH assay. The $IC_{50}$ value of 443.31μg/mL indicate that of the plant extract has weak hydroxyl radical scavenging activity than the reference standard, ascorbic acid ($IC_{50}$ = 131.42μg/mL).

**Reducing power assay**

Reducing power activity assay is based on the reduction of $Fe^{3+}$ manifested by the change of solution colour from yellow to different shades of blue and green. The dose-dependent response of

the reducing power of methanol extract of *L. repens* and reference standard (Figure 3A and B). The plant extracts and standard were able to reduce the ferric ions ($Fe^{3+}$) to ferrous ions ($Fe^{2+}$) and the activities of the extracts varied significantly. Reducing power of the plant extracts increased with increasing concentration which was evident from the absorbance of the reaction mixture at 700nm. At highest concentration (500µg/mL), the extract showed maximum absorbance (0.613) demonstrating moderate to low reducing power activity. The $EC_{50}$ of ascorbic acid and extract were 67.8µg/mL and 374µg/mL respectively. Plant extract showed some activity but lower than that of ascorbic acid.

**Ferric reducing antioxidant activity (FRAP)**

FRAP assay is considered to be rapid and sensitive and is evaluated based on the capability of the sample extracts to reduce ferric tripyridyltriazine (Fe (III)-TPTZ) complexes to ferrous tripyridyltriazine (Fe (II)-TPTZ) (Benzie and Strain, 1996). The higher the FRAP value, the greater will be the antioxidant activity of plant extracts. An increase in the absorbance of reaction mixtures was observed on increasing the concentration (Figure 3 C and D). This indicated reducing power of the extracts. Although it was observed that the reducing potential of the extract was low when compared to standard, the extract may possess reductive ability which could serve as electron donors, terminating the radical chain reactions.

**Thiobarbituric acid (TBA) method**

Thiobarbituric acid assay (TBA test) is used as an index for lipid peroxidation products based on the reactivity of malondialdehyde, the product of lipid peroxidation with thiobarbituric acid to produce a red adduct (Garcia *et al.*, 2005). The absorbance and $EC_{50}$ values of plant extract and ascorbic acid in thiobarbituric acid assay (Figure 3E and F). Based on the results of the thiobarbituric acid test, methanol extract had higher antioxidant activity when compared with ascorbic acid.

**Antidiarrhoeal activity in mice model**

Effect of methanol extract of *L. repens* on castor oil induced diarrhoea (Table I). All mice from the control group (treated with vehicle) produced diarrhoea after castor oil administration. The decrease in the severity of the diarrhoea was measured by the evacuation index (EI). Negative control showed the evacuation index of 14.6 which was greatly reduced in case of positive control- loperamide (10.6). Reduction in evacuation index was also observed at 200mg/kg and 400mg/kg of plant sample producing EI of 12.2 and 9.4 respectively. Loperamide showed 27.4% inhibition of induction of diarrhea while plant extract at 200mg/kg demonstrated 16.44% inhibition. As the dose of the extract was increased to 400mg/kg, greater effect compared to standard was observed showing 35.62% inhibition of diarrhea.

Compounds of diverse classes are responsible to impart different pharmacological effects. Identification of these phytoconstituents reveals possible mechanism of pharmacological effect of the crude extract. Phenolic compounds are important natural compounds which have been shown to have a range of bioactivities including antioxidant activity, anticarcinogenic, antiatherosclerotic, antibacterial, antiviral, and anti-inflammatory activities (Cicerale *et al.*, 2010). They also play an important role in the prevention of diabetes, osteoporosis, neurodegenerative diseases and major cardiovascular diseases such as hypertension (Cook and Samman, 1996). Chlorophylls are pigments that have also been reported such as to stimulate immune system, to help combat anemia, to purify the blood and the organism (Solymosi and Mysliwa-Kurdziel, 2017). β-Carotene is the precursor for vitamin A and is designated an antioxidant. Lycopene is one of the major carotenoids and possesses specific biological properties such as prevention of cardiovascular disease, cancers of the prostate or gastrointestinal tract (Stahl and Sies, 1996); the compound also possesses antioxidant property (Di Mascio *et al.*, 1989). It is therefore justifiable to determine content of the aforesaid phytoconstituents in extracts of *L. repens.* Moreover, ethanol extract of a plant in the same genus, *L. aromatic*, was reported to have similar phytoconstituents as *L. repens* (Do *et al.*, 2014).

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation can produce free radicals, which often initiate chain reactions that damage cellular components. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are generally reducing agents such as thiols, ascorbic acid or polyphenols.

Table I. Effect of methanol extract of *Limnophila repens* on castor oil induced diarrhoea in mice

| Sample (Dose) | Initial evacuation (min) (mean ± SD) | Evacuation classification Normal | Semi-solid | Solid | Evacuation index | % inhibition |
|---|---|---|---|---|---|---|
| Vehicle (10mg/kg) | 148±28 | 3.60±2.1 | 3.40±1.1 | 1.40±0.9 | 14.6 | - |
| Loperamide (5mg/kg) | 193.2±29.8 | 1.40±1.1* | 2.80±2.6 | 1.20±0.8 | 10.6 | 27.40 |
| LR200 (200mg/kg) | 99.2±9.85 | 0.60±0.6** | 1.60±1.1 | 2.80±1.8 | 12.2 | 16.44 |
| LR400 (400mg/kg) | 119.2±15.3 | 1.80±1.1 | 1.40±0.9 | 1.60±0.9 | 9.4 | 35.62 |

Here, 'LR' stands for methanol extract of *Limnophila repens* and data are presented as mean ± SEM. ANOVA was performed followed by Dunnett's test and significant differences were represented by *p<0.05, **p<0.01 vs control group treated with vehicle.

In DPPH assay, stable radical DPPH is reduced to yellow coloured diphenyl-picryl-hydrazine in the presence of a hydrogen donor. Hydroxyl radical- an important active oxygen species, is responsible for lipid peroxidation and causes significant biological damage. Dose-dependent hydroxyl radical scavenging activity was observed by extract of *L. repens*. Reducing power assay corresponds to antioxidant activity and serves as a significant reflection of antioxidant activity. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants. Phenolic compounds found in plants are generally effective to scavenge free radicals, thus can act as antioxidant (Martins *et al.*, 2016; Pang *et al.*, 2018; Rice-Evans *et al.*, 1997; Zheng and Wang, 2001). Phenolic compounds can act as hydrogen donors and also form chelate with metal ions such as iron and copper. Phenolic compounds reduce or inhibit free radicals by transfer of a hydrogen atom from its hydroxyl group. Transfer of a hydrogen cation from phenolic compound to peroxyl radical (ROO•) forms a transition state of an H-O bond with one electron. Antioxidant capacity of phenolic compounds is strongly affected by reaction medium. Alcohols facilitate ionization of the phenols to anion phenoxides, which can react rapidly with peroxyl radicals, through an electron transfer. The overall effect of the solvent on the antioxidant activity of the phenolic compounds depends to a great extent on the degree of ionization of these compounds (Foti, 2007). Flavonoids are hydroxylated phenolic substances and are ubiquitous in plant kingdom. Flavonoids are generally found in the nucleus of mesophyll cells and within centres of reactive oxygen species generation (Dixon *et al.*, 1983). Chemically flavonoids are composed of a fifteen-carbon skeleton consisting of two benzene rings linked via a heterocyclic pyrane ring. Presence of hydroxyl groups in flavonoids renders them beneficial for plant tissues against various abiotic and biotic stresses. The configuration, substitution, and total number of hydroxyl groups in the flavonoids significantly influence their antioxidant activity such as radical scavenging and metal ion chelation. Flavonoids having an unsaturated 2-3 bond in conjugation with a 4-oxo function are more potent antioxidants than the flavonoids lacking one or both features (Heim *et al.*, 2002). Flavonoids inhibit the enzymes involved in generation of reactive oxygen species such as microsomal monooxygenase, glutathione-S-transferase, mitochondrial succinoxidase, NADH oxidase, etc (Brown *et al.*, 1998). They can also chelate with free metal ions responsible for production of highly reactive hydroxyl, superoxide, peroxyl and alkoxyl radicals (Kumar *et al.*, 2013; Kumar and Pandey, 2013). Phenolic and flavonol contents of plant extracts is considered directly proportional to the antioxidant activity of extracts. Thus, lower amount of total polyphenols in methanol extract of *L. repens* supports observed weak antioxidant potential of the plant extract. Lycopene among all carotenoids is the most effective antioxidant owing to its unique molecular shape that is believed to impart in neutralization of free radicals (Di Mascio *et al.*, 1989). Lycopene can suppress singlet oxygen as well as detoxify reactive oxygen species. Due to its highly lipophilic nature, lycopene exerts maximum antioxidant effects at cellular membranes by interacting with the lipid components and thus protect the membranes from lipid peroxidation and intervenes with the tumor initiation process (Sahin *et al.*, 2016). Another carotenoid, β-carotene can also scavenge peroxyl radicals via the formation of an unstable β-carotene radical adduct at low oxygen stress (Burton and

Ingold, 1984; Rice-Evans *et al.*, 1997). This radical adduct is highly resonance stabilized and thus unreactive. Generation of nonradical products from this radical adduct is also observed which finally terminates radical reactions by attacking another free radical (Rice-Evans *et al.*, 1997). Presence of minute amount of β-carotene and lycopene in extract of *L. repens* corroborates weak antioxidant potential of the plant.

Plant extracts containing tannins, flavonoids and alkaloids were reported to possess antidiarrhoeal activity (Rahman *et al.*, 2013; Umer *et al.*, 2013). Tannic acid at clinically relevant concentrations can significantly increase the transepithelial resistance of human gut epithelial cells, thus improving the intestinal epithelial barrier properties (Ren *et al.*, 2012). Tight junction is a major component of the epithelial barrier; disruption of these tight junctions increases intestinal paracellular permeability leading to diarrhea (Madara *et al.*, 1986; Powell, 1981). Improvement of the epithelial barrier function by tannins can thus significantly reduce fluid secretion and ameliorate diarrheal conditions. As methanol extract of our plant was found to contain good amount of tannins and minute amount of other constituents, that's why, *in vivo* antidiarrhoeal test in mice model was conducted. There was a statistically significant reduction in the incidence and severity of diarrhoea produced in experimental animal models and greater effect against diarrhoea.

## CONCLUSION

In the present study, the majority of biological active constituents were present in methanol extracts of *L. repens* in considerable amount. The plant extract revealed a concentration-dependent free radical scavenging and reducing power property. However, in comparison to standard, the extract showed very weak antioxidant activity. The plant extract demonstrated strong antidiarrhoeal activity when analyzed by castor oil induced method. According to the obtained results, these species might be considered as a potential source of natural antidiarrhoeal agent. However, detailed pharmacological studies are required to justify the clinical use of the plant for treating diarrhoea.

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