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Research Article

Estimation of total Phenol and flavonoid contents of *Citrus limon* L Burmf leaves from North Eastern region of India.

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ABSTRACT

Citrus plants belonging to the family Rutaceae, are traditionally used by the local people India to treat scurvy, rheumatism, stomachic, diarrhea, liver disorders and used as an antioxidant. They are valuable source of phenolic and other biologically active compounds. Phenolic compounds have been reported to have multiple biological effects. In this paper quantitative determination of phenols and flavonoids of *Citrus limon* L Burmf leaves extracts was carried out using spectrophotometric methods. The plant material was collected from Assam, north eastern region of India. Total phenolic content was determined by Folin-Ciocalteu method and aluminium chloride method was used for flavonoid determination. Acetone and methanol extracts of leaves showed quite high amount of phenol as well as flavonoid contents compared to ethanol and ethyl acetate extracts. However, all the extracts exhibited significant results.

Keywords: *Citrus limon*, Phenol, Flavonoid, Catechol and Quercetin.

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INTRODUCTION

Phenolic compounds, as secondary metabolites, are widely distributed in plants. Plant phenolics are generally involved in defense against ultraviolet radiation or aggression by pathogens, parasites, as well as contributing to plant's colors. They are widespread constituents of plant foods and beverages and partially responsible for the overall organoleptic properties of plant foods¹. Flavonoids, the most common group of polyphenolic compounds found ubiquitously in plants. These plant secondary metabolites show anti-allergic, anti-inflammatory and anti-cancer activity. Researchers have become interested in flavonoids and other phenolics for their medicinal properties, especially their potential role in the prevention of cancer and heart diseases¹. Citrus is the general term for plants belonging to the family Rutaceae². Citrus fruits and derived products have beneficial impacts on the human health³. Hence, citrus fruits have attracted much attention because of its nutritional and antioxidant properties. They are important source of many bioactive compounds and widely known for its medicinal impact due to presence of flavonoids, flavonones and other compounds which are rare in other plants.

Citrus limon L Burmf belongs to family rutaceae is also known as Assam lemon and one of the most important crops of Assam and other parts of north eastern region. Fruit is widely used for culinary, beverages, industrial and medicinal uses. The fruits are sour, rich in vitamin C which helps the body to fight off infections and also to prevent or treat scurvy⁴. Fruits are thermogenic, digestive, carminative, stomachic laxative, stimulant, antiseptic and have mosquito repellent power⁵. Anthelmintic activity Citrus limon fruit empty juice sacs was investigated from Nagpur region of Maharashtra⁶. Comparative study of antioxidants changes including total flavonoid, total phenol and total antioxidant capacity of *Citrus limon* fruits grown at the north of Iran were examined³. Determination of phenolic contents and total antioxidant activity of *citrus lemon* juice and peel extracts was reported from Bosnia and Herzegovina¹. Yield, fruit quality, economic feasibility and water productivity of *citrus limon* plants were evaluated⁸. Work about genetic resources of Citrus of north-eastern India and their potential use was available. Recovery of pectin from *Citrus limon* was also reported⁸. Antimicrobial activity of juice in ripens and unripe forms of *citrus limon* were studied from Assam⁹. Pharmacognostic, phytochemical, physicochemical property and antimicrobial activity of lemon peel oil were reported¹⁰.

Phenol and flavonoid contents of Ethanol: water extract of *Citrus limon* were reported from Tamilnadu, India¹¹.

Preliminary qualitative phytochemical screening of *Citrus limon* L Burmf leaves revealed the presence of phenolic compounds, terpenoids, flavonoids and steroids. Some of them have antioxidant and antimicrobial activities. This study presents the quantitative estimation of total phenolic and flavonoid contents of *citrus limon* L Burmf leaves available from Biswanath district, Assam. Determination of phenolic and flavonoid contents from peel of *citrus limon* L Burmf was mentioned in authors previous article¹². As per the available reports this is the first study of determining the phenol and flavonoid contents of ethyl acetate, acetone, ethanol and methanol extracts of *citrus limon* L Burmf leaves collected from Biswanath district, Assam, India.

EXPERIMENTAL

The plant material was collected from Naduar area, Biswanath district, Assam, India.

The plant was authenticated at Department of Life Sciences, Dibrugarh University, Assam, India. Folin-Ciocalteu reagent and all other chemicals used were Merck products. UV Vis S1700 Pharma spectrophotometer, Shimadzu was used for absorbance measurements.

Preparation of plant extract: Air shade dried and powdered leaves material of *Citrus limon* (50 mg) was treated separately with ethyl acetate, acetone, ethanol and methanol (250ml) by refluxing for 18 hours. Solvents were recovered under reduced pressure to obtain the crude extracts. Known amounts of dried extracts were dissolved in known volume of methanol and various concentrations of the extract solutions were prepared.

Determination of total phenolics: The total phenolic contents of *Citrus limon* L Burmf leaves extracts were determined according to the method described by Malik and Singh¹³. Aliquots of the extracts were taken in a 10 ml glass tube and made up to a volume of 3 ml with distilled water. Folin-Ciocalteu reagent: water (1:1, 0.5ml) and Na₂CO₃ (20%, 2ml) were added sequentially in each tube. A blue color was developed in each tube because the phenols undergo a complex redox reaction with phosphomolybdic acid in folin-ciocalteu reagent in alkaline medium which resulted in a blue colored complex, molybdenum blue. The test solutions were warmed for 1 minute, cooled and absorbance was measured at 650 nm against the reagent used as a blank. A standard calibration plot was generated (Figure-1) at 650 nm using known concentrations of catechol. The concentrations of phenols in the test samples were calculated from the calibration plot and expressed as mg catechol equivalent of phenol/g of sample.

Determination of total flavonoids: The aluminum chloride method was used for the determination of the total flavonoid contents of the sample extracts¹⁴. Aliquots of extract solutions were taken and made up the volume to 3ml with methanol. Then AlCl₃ (10%, 0.1ml), Na-K tartarate (0.1ml) and 2.8 ml distilled water were added sequentially. The test solution was vigorously shaken. Absorbance at 415 nm was recorded after 30 minutes of incubation. A standard calibration plot was generated (Figure-2) at 415 nm using known concentrations of quercetin. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent /g of sample.

RESULT AND DISCUSSION

Ethyl acetate, acetone, ethanol and methanol extracts of *Citrus limon* L Burmf leaves were subjected to screening for their total phenolic contents and total flavonoid contents. Table-1 presents the total phenolic contents of *Citrus limon* leaves extracts. Table 2 presents total flavonoid contents of various extracts of *Citrus limon* leaves.

The present study revealed the phenolic contents of ethyl acetate, acetone, ethanol and methanol extract of *Citrus limon* leaves in terms of mg catechol equivalent/g of sample (standard plot: $y = 0.0966x$, $R^2 = 0.9878$). The values were found between 210 mg to 485 mg catechol equivalent /g of leaves extracts. The phenolic contents of acetone and methanol extracts were quite high compared to ethyl acetate and ethanol extracts. Acetone extract showed the maximum and ethyl acetate extract exhibited minimum amount of phenolic contents. Phenolics present in the plant have received considerable attention because of their potential biological activities. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers.

Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phenols. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties. Using the standard plot of quercetin ($y = 0.0148x$, $R^2 = 0.975$), the flavonoid contents of ethyl acetate, acetone, ethanol and methanol extracts of *Citrus limon* L Burmf leaves were found ranging from 239 to 582 mg quercetin equivalent/g of sample extracts. Similar to phenolic contents acetone and methanol extracts showed quite high amount of flavonoid contents. Acetone extract showed the maximum and ethyl acetate extract exhibited minimum amount of flavonoid contents. Ethanol extract also showed good amount of flavonoid contents.

Table 1: Phenol contents

Extracts	Phenol contents (mg catechol equivalent / g material)
Ethyl acetate	210
Acetone	485
Ethanol	375
Methanol	427

Table 2: Flavonoid contents

Extracts	Flavonoid content (mg quercetin equivalent/ g material)
Ethyl acetate	239
Acetone	582
Ethanol	402
Methanol	556

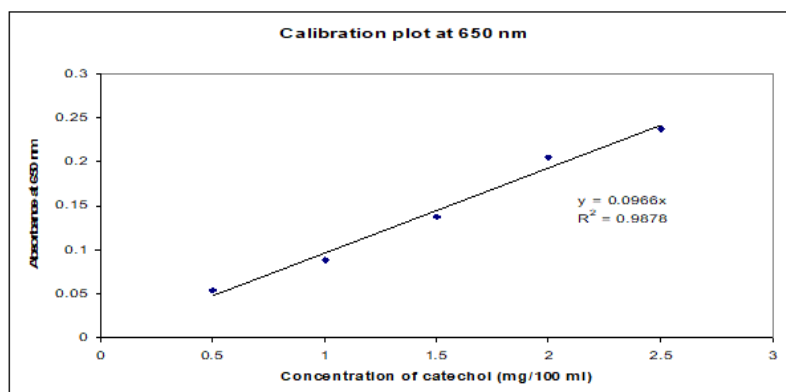


Figure 1: Calibration plot for phenolic determination

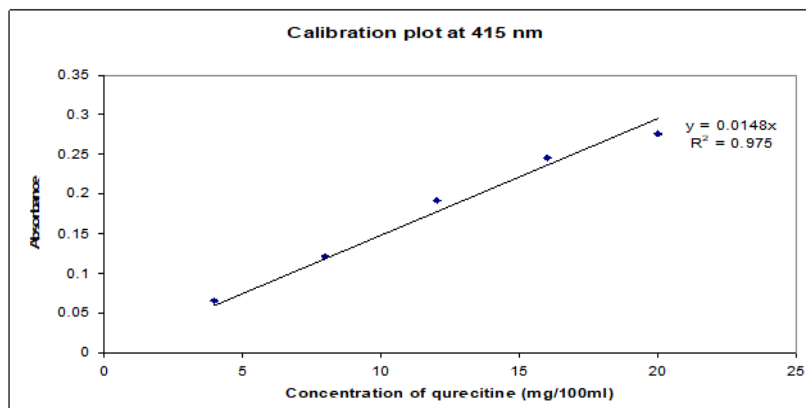


Figure 2: Calibration plot for flavonoid determination

CONCLUSION

The present investigation revealed that *citrus limon* L Burmf leaves extracts contain significant amount of phenols and flavonoids. The objective of this study was to get information of the amount of phenolics and flavonoids from leaves of *citrus limon* L. Burmf available from selected region of north eastern India. Further intention of this study is to correlate relationship of these secondary metabolites to possible biological activities and evaluate *citrus limon* L Burmf as a potential source of natural bioactive chemicals.

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