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RESEARCH ARTICLE

PRELIMINARY PHYTOCHEMICAL SCREENING AND FLUORESCENCE ANALYSIS OF DRY LEAF POWDER EXTRACTS OF *CITRUS LIMONUM*

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ABSTRACT

The plant is found throughout India, entire North-East region. It has a long history of cultivation in Southeast Asia and China. Plants possess some specific chemicals called phytochemicals in them which may not have nutritional property as such, but they can work towards the deterrence of diseases. The present study was carried out to evaluate the phytochemicals present in the leaf powder and also to study the fluorescent characters of the leaf powder. The phytochemical study showed the presence of terpenoids, oil and fat, starch, carbohydrate and cellulose in all the four extracts of the leaf powder. Flavonoid was present in water and chloroform extracts. The fluorescence analysis of leaf powder of *C. limonum* showed varied colour with the treatment of different chemicals.

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INTRODUCTION

Medicine from plant sources have been in use in Homeopathy, Ayurveda, Allopathy and in traditional medicine since time immemorial (Gogoi and Islam, 2012). Knowledge of the chemical constituents of the plant is desirable, not only for the discovery of therapeutic drugs but also to discover the actual value of folklore remedies (Mojab *et al.*, 2003). Usually herbal medicines are widely perceived by the public as being natural, healthful and free from side effects (Mordi and Akanji, 2012). In rural areas, people suffer from common digestive disorders such as diarrhea, dysentery and food poisoning. The extract of medicinal plants in these cases raises the body's immune system and lowers the allergies (Sidhu *et al.*, 2007).

Citrus limonum

The common name of *C. limonum* is lemon. The plant is widely cultivated in tropical and sub tropical regions around the world. The fruits of this plant are used as herbal home remedy. Lemon peel is a wonderful, natural and inexpensive digestive aid. It can be used for abdominal bloating, nausea and poor appetite. Lemon tickles our palate and lemon juice sure knows how to refresh and hydrate the body on a hot summer day. Lemon and its juice have immense benefits that enhance the overall functioning of the body. It acts as a cleanser. With addition of salt, it acts as an excellent purgative.

Apart from abundant proportions of vitamin C that lemon contains, it also possesses special compounds such as flavonoids that have anti-oxidant, anti-carcinogenic, anti-biotic and detoxifying properties. These in turn help in the healing of peptic and oral ulcers. It also helps in respiratory disorders. Flavonoids and other oils are extensively used as anti-congestive medicines such as balms, vaporizers and inhalers (Hedley and Shaw, 2002).

Medicinal Uses

- Fruit juice is taken for kidney and urinary troubles.
- Fresh lemon juice is recommended to be taken in evening for the relief of dyspepsia with vomiting and bilious headaches.
- The juice of baked lemon is an excellent remedy for cough. Fruit juice is used as remedy for indigestion.
- Leaves and fruits are used for prevention and cure of diarrhea and dysentery.

Phytochemicals are responsible for medicinal activity of plants and they have protected human from various diseases (Savithamma *et al.*, 2011). Phytochemicals are defined as bioactive non-nutrient plant compounds found in fruits that have been attributed to reduce the risk of major chronic diseases (Blessy *et al.*, 2012). The major constituents of phytochemical consist of carbohydrates, aminoacids, proteins, and chlorophylls, while, secondary metabolites consist of alkaloids, saponins, steroids, flavonoids, tannins, etc.

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(Kumar *et al.*, 2009, 2013). The phytochemicals are also known as secondary metabolites that are derived from primary metabolites and are used as drugs (Akerle *et al.*, 1991). They also contribute to the flavor, colour and other characteristics of plant parts (Iniaghe *et al.*, 2009). Medically, the presence of these phytochemicals explains the use of the vegetable in ethnomedicine for the management of various ailments (Aja *et al.*, 2010). Phytochemical analysis is very useful in the evaluation of some active biological components of some vegetables and medicinal plants (Oloyed, 2005). These secondary plant metabolites are extractable by various solvents that exhibit varied biochemical and pharmacological actions in animals (Trease and Evans, 1996). There are many families of phytochemicals that help the human body in a variety of ways. Phytochemicals may protect human beings from various diseases.

The phytochemical constituents play a significant role in the identification of crude drugs and also to evaluate drugs derived from the plant sources (Moses *et al.*, 2013). Blessy *et al.* (2012) studied the aqueous extracts of *Citrus limonum* pulp which revealed the presence of carbohydrates, alkaloids, tannins, fixed oils, reducing sugars, proteins, cardiac glycosides, steroids, phytosterols, phenols and flavonoids. Fluorescence is the phenomenon exhibited both in visible and UV- light by various chemical constituents present in the plant material. Some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation (Gupta *et al.*, 2006).

MATERIALS AND METHODS

In the present study, dry sample of *C. limonum* leaves were used. A study was carried out on the phytochemical aspects using different solvent extracts. Fluorescence analysis of the leaf powder of *C. limonum* was also carried out.

Collection of plant samples

The fresh leaves of *Citrus limonum* were obtained from Kengarai village in Nilgiri District of Tamil Nadu, India.

Preparation of Leaf Powder

The leaves of *C. limonum* taken for the present study were collected, cleaned and air dried, under shade for about three weeks. After drying, the leaves were then blended using a household electric blender. This fine powder was used for phytochemical screening and fluorescent analysis.

Preliminary Phytochemical Analysis

The leaf powder was dissolved in various solvents and the preliminary phytochemical tests such as alkaloids, flavonoids, steroids, terpenoids, quinone, phenol, starch, proteins, carbohydrates, cellulose, fixed oil and fats were carried out using the standard method of Harborne (1984).

Fluorescence Analysis

The behaviour of the leaf sample with different chemical reagents and fluorescence characters of *Citrus limonum* was

observed under ordinary and long ultra violet light at 245nm according to Chase and Pratt (1949) and Gupta *et al.* (2006).

RESULTS AND DISCUSSION

Fluorescence Analysis

The experiments conducted in *Citrus limonum* for phytochemical constituents and fluorescence analysis showed the following results.

PRELIMINARY PHYTOCHEMICAL ANALYSIS OF LEAVES OF *C. limonum*

In the present study, a preliminary phytochemical analysis was carried out to identify the active constituents such as alkaloids, flavonoids, sterols, terpenoids, quinone, oil and fat, phenol, starch, anthocyanin, protein, carbohydrate and cellulose present in the leaves of *Citrus limonum*. Preliminary phytochemical analysis of different extract (ethanol, chloroform, benzene and water) of *C. limonum* showed the following results. The dried and powdered leaves of *C. limonum* were dissolved in different solvents viz., water, ethanol, chloroform and benzene and the extracts thus obtained were analyzed for the presence or absence of secondary metabolites. In *C. limonum*, all the four solvent extracts viz., water, ethanol, chloroform and benzene showed the presence of terpenoids, oil and fat, starch, carbohydrate and cellulose. Anthocyanin pigment was completely absent in the leaf extracts. The presence of alkaloids was observed in chloroform and benzene leaf extracts. Flavonoids were present in water and chloroform extracts of the leaves. Among the four solvents used, quinones and steroids were absent only in ethanol extract. The presence of phenol was observed in water and ethanol leaf extracts, whereas, protein was present in water and chloroform leaf extracts of *C. limonum* (Table 1).

Table 1. Preliminary Phytochemical Screening of *Citrus limonum*

| Tests | H ₂ O | Ethanol | Chloroform | Benzene |
|--------------|------------------|---------|------------|---------|
| Alkaloids | - | - | + | + |
| Flavonoids | + | - | + | - |
| Terpenoids | + | + | + | + |
| Quinones | + | - | + | + |
| Oil&fat | + | + | + | + |
| Steroids | + | - | + | + |
| Phenol | + | + | - | - |
| Starch | + | + | + | + |
| Anthocyanin | - | - | - | - |
| Protein | + | - | + | - |
| Carbohydrate | + | + | + | + |
| Cellulose | + | + | + | + |

'+' - Present '-' - Absent

Earlier study carried out by Blessy *et al.* (2012) have revealed the presence of carbohydrate, alkaloids, tannins, fixed oils, reducing sugars, proteins, cardiac glycosides, sterols, phytosterols, flavonoids and phenols in the aqueous extracts of the leaves of lemon. The study carried out by Savithramma *et al.* (2011) on the preliminary phytochemical analysis of 54 Indian medicinal plants showed positive indication for alkaloids, flavonoids, steroids and cellulose. Phytochemical screening of crude extracts of *Plumaria rubra* (flower and leaf) and *Eucalyptus globulus* (leaf) showed the presence of

tannins, phlobatannins, saponins, flavonoids, steroids, terpenoids, cardiac glycosides and reducing sugar (Egwaikhide *et al.*, 2007). The phytochemical screening and qualitative estimation of the medicinal plant studied, showed that the leaves were rich in carbohydrates, starch, cellulose, quinones, terpenoids and oil and fat in almost all the extracts. Some leaf extracts showed the presence of alkaloids, flavonoids and phenols. Steroids were present in *C. limonum*.

responsible for the folklore and scientifically documented medicinally beneficial effects of the plants. Further studies are required to isolate these components with a view to investigate their effects on specific diseases.

Table 2. Fluorescence analysis of *Citrus limonum*

| Treatment | Leaf Powder | Treatment with chemical reagent | Observation | |
|----------------|-------------|---------------------------------|-----------------|------------------|
| | | | Visible light | UV light (245nm) |
| T ₁ | Leaf Powder | Acetic acid | Pale Green | White |
| T ₂ | Leaf Powder | Picric acid | Yellow | Pale Yellow |
| T ₃ | Leaf Powder | H ₂ SO ₄ | Dark brown | Pale brown |
| T ₄ | Leaf Powder | FeCl ₃ | Brown | Dark Green |
| T ₅ | Leaf Powder | 1N HCl | Pale Green | Pale Green |
| T ₆ | Leaf Powder | As such powder | Green | Pale Green |
| T ₇ | Leaf Powder | HNO ₃ | Pale Orange | Green |
| T ₈ | Leaf Powder | NaOH | Yellowish green | Green |
| T ₉ | Leaf Powder | H ₂ O | Pale Green | Pale Green |

It should be noted that steroidal compounds are of importance and of interest in pharmacy due to their relationship with hormones. Steroidal structure could serve as potent starting material in the synthesis of hormones (Santhi *et al.*, 2011). Plants generally have varied chemical compositions depending upon species. A good number of plants are known to be of economic and medicinal value. Those that are of medicinal value are often used as herbal remedy for the restoration and maintenance of good health. Some herbs have been considered as drugs and therefore generally safe and effective (Treasure, 2000; Uboh *et al.*, 2010). Alkaloids and their synthetic derivatives are used as basic medicinal agents for their antispasmodic and bactericidal effect (Okwu, 2004). Phytochemical analysis is very useful in the evaluation of some active biological compound of medicinal plants (Aja *et al.*, 2010).

Fluorescence analysis

The leaf powder of *C. limonum* was extracted in acetic acid, picric acid, H₂SO₄, FeCl₃, 1N HCl, HNO₃, NaOH and H₂O. The fluorescence analysis of these leaf extracts were observed under ordinary visible light and also under UV light (245 nm) and recorded in Table 2. The fluorescence analysis of leaf powder of *C. limonum* showed pale green colour under both visible and UV light in treatment with 1N HCl and water. Yellowish green colour was observed under visible light when the leaf powder was treated with NaOH. The leaf powder in treatment with HNO₃ showed pale orange colour under visible light. Similarly, different colours viz., white, pale yellow, pale brown, green and dark green were observed under UV and visible light when the leaf powder was treated with different chemical reagents (Table 2).

The results of fluorescent analysis of leaf powder of the medicinal plant *Citrus limonum* showed characteristic colouration in treatment with various chemical reagents. These results are supportive with *Cajanus cajan* leaf extracts (Ramaswamy *et al.*, 2013). In conclusion, the leaves of *Citrus limonum* contain biologically active components that may be

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