CHAPTER 2

PHYTOCHEMICAL PROFILING OF
HELICIA NILAGIRICA (BEDD.)
Chapter 2

Abstract

The mature non-infected stem bark of Helicia nilagirica was collected, dried and powdered and subjected to sequential extraction with increasing polarity using petroleum ether, chloroform, ethanol and distilled water. The different extracts were cooled and evaporated with rotary evaporator and kept at -80°C for further use. Phytochemical analysis was done on all the three extracts in which the presence of flavonoids, tannins, terpenoids, cardiac glycosides were found in the chloroform and ethanol extracts whereas in the aqueous extract saponin, tannins and cardiac glycosides were present. The TLC profile also showed the presence of different phytochemicals as indicated by different Rf values revealed by the different extracts for the various solvent systems used.

1. INTRODUCTION

Medicinal plants have been used as the main traditional herbal medicine amongst rural dwellers worldwide since antiquity to date (Doughari, 2012). The earliest written evidence of use of plants as medicine has been found around 5000 years before on a Sumerian clay slab from Nagpur which comprised of 12 recipes for drug preparation comprising over 125 plants including poppy, henbane and mandrake. In 2500 BC the Chinese emperor has written a book for 365 drugs some of which have been used nowadays such as Rhei rhisoma, camphor, Theae folium, Podophyllum, the great yellow gentian, ginseng, jimson weed, cinnamon bark, and ephedra (Bottcher 1965; Wiart 2006). Over the years plants have been used as the main source of medicine especially in the developing countries and more than 80% of the world’s population relies on traditional medicine for their primary healthcare needs (WHO, 2010).

Phytochemicals are natural bioactive chemical compounds found in plants, protect plants from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack (Ali et al, 2006). These compounds known as secondary plant metabolites include, organic substances like alkaloids, carotenoids, glycosides, terpenoids, steroids, tannins, flavonoids,
vitamins, mucilages, minerals, organic acids etc (Bravo, 1998; Brown et al., 1999; Gossau and Chen, 2004; Heber, 2004). These secondary metabolites are of great health benefits to humans. Some of the beneficial roles of phytochemicals are low toxicity, low cost, easy availability and they have an extensive range of therapeutic activities such as antioxidant, antimicrobial, hypoglycemic, antidiabetic, antimalarial, anticholinergic, antileprosy, and antineoplastic. These phytochemicals also help in the modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism (Andre et al, 2010; Negi et al, 2011). Even with a remarkable progress in synthetic drugs, therapies using medicinal plants make a major contribution to the pharmaceutical industry because they are safe, easily available, cost effective, and there are synergistic effects of other biologically active ingredients and the presence of beneficial minerals. (Farnsworth and Soejarto, 1991, Jagetia, 2017).

_Helicia nilagirica_ Bedd. (Family: Proteaceae) is locally known as Pasaltakaza, is a medium-sized tree, which grows up to a height of 12 meters. It is widely distributed in Sri Lanka, southern India, Burma (Myanmar), Indochina, Japan, Taiwan, and Thailand. It is also found scattered in lowland to montane rain forests, up to 2,000 -3,350 m altitude. Some species are found in habitats along the streams whereas other species are found on hilltops or ridges (Khamyong et al., 2004). This tree has been used as folk medicine since time immemorial in Mizoram, India by the Mizo people. Its decoction prepared by boiling the leaves or bark is used to treat various stomach ailments including peptic ulcers, indigestion, mouth ulcer, urinary tract infection and gynaecological disorders, It is also used in scabies and other skin diseases (Sawmliana 2003). The fruits of _H. nilagirica_ have been used as a medicine to cure cough and
cold in Sikkim (Chauhan 2001). The ethnomedicinal use of *Helicia nilagirica* stimulated us to investigate its phytochemical composition.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Potassium iodide, bismuth nitrate, sulphuric acid, ferric chloride, hydrochloric acid, aluminium chloride, ammonium hydroxide, glacial acetic acid, chloroform, ethanol, methanol, n-butanol, ethyl acetate, sodium chloride, sulphuric acid, olive oil, and Whatman filter paper were procured from Sd fine Chemical Ltd., Mumbai, India. The TLC plates were commercially procured from Merck India, Mumbai.

2.2. Collection and extraction

The mature non-infected stem bark of *Helicia nilagirica* Bedd. (Family: Proteaceae) was collected from Sialsuk, Aizawl District of Mizoram during the dry season and the plant was authenticated by the Botanical Survey of India, Shillong. The cleaned and non-infected bark was spread into stainless steel trays and allowed to dry in the shade at room temperature in dark in clean and hygienic conditions to avoid entry of insects, animals, fungus, and extraneous terrestrial materials. The exhaust and free air circulation was allowed. The dried bark was powdered in a grinder at room temperature. A sample of 100 g of the powder was extracted sequentially with chloroform, ethanol and water in a Soxhlet apparatus (Suffness and Dorous, 1979). The extract was then concentrated to dryness under reduced pressure and stored at -80°C until further use.

2.3. Phytochemical screening

The different extracts of *Helicia nilagirica* were analyzed for the presence of various phytochemicals using standard procedures as described below.
2.3.1. Alkaloids

The presence of alkaloids was determined by mixing 0.1g of the extract with 0.5 ml of Mayer’s reagent and Dragendorff’s reagent. The formation of a creamy (Mayer’s reagent) or reddish brown precipitate (Dragendorff’s reagent) indicated the presence of alkaloids (Harborne, 1998; Doughari, 2012).

2.3.2. Tannins

About 0.5 g of dried powdered samples was boiled in 20 ml of water in a test tube and filtered. A few drops of 0.1% ferric chloride was added to the filtrate. The formation of brownish green or a blue-black colour indicated the presence of tannins (Harborne, 1998; Doughari, 2012).

2.3.3. Phlobatannins

The aqueous extract of Helicia nilagirica was boiled with 1% aqueous hydrochloric acid and deposition of a red precipitate indicated the presence of phlobatannins (Harborne, 1998; Doughari, 2012).

2.3.4. Saponins

About 2 g of the powdered sample was boiled with 20 ml of distilled water in a water bath for 10 minutes and filtered while hot and cooled before conducting the following tests:

**Frothing:** 3 ml of filtrate was diluted up to 10 ml with distilled water and shaken vigorously for 2 minutes. The formation of a fairly stable froth indicated the presence of saponins in the filtrate.

**Emulsification:** 3 drops of olive oil was added to the solution obtained by diluting 3 ml filtrate to 10 ml distilled water and shaken vigorously for a few minutes. The formation of a fairly stable emulsion indicated the presence of saponins (Trease and Evans 1989; Harborne, 1998; Doughari, 2012).
2.3.5. Flavonoids

Three different methods were used to test the presence of flavonoids in all the extracts (Sofowara, 1993; Harborne, 1998; Doughari, 2012). Five ml of dilute ammonia solution was added to a portion of the aqueous filtrate of each plant extract followed by the addition of a concentrated H₂SO₄. Appearance of a yellow colour (disappeared on standing) in each extract indicated the presence of flavonoids.

A few drops of 1% aluminum solution was added to a portion of each filtrate. A yellow colour indicated the presence of flavonoids.

A portion of the plant powder was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colour indicated the presence of flavonoids.

2.3.6. Terpenoids

Salkowski test: Five ml of each extract was mixed with 2 ml of chloroform, with a careful overlaying of 3 ml concentrated sulphuric acid. The formation of a reddish brown precipitate at the interface indicated the presence of terpenoids (Harborne, 1998).

2.3.7. Cardiac glycosides (Keller-Killani test)

To determine the presence of cardiac glycosides, 5 ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution and underlay with 1 ml of concentrated sulphuric acid. The appearance of brown ring at the interface indicated the presence of deoxysugar, which is a characteristic of cardenolides (Harborne, 1998; Doughari, 2012).
2.3.8. Carbohydrates

**Benedict’s test:** The filtrates were treated with Benedict’s reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

2.4. Quantitative determination of the phytochemicals

2.4.1. Determination of Saponins

20 g of *Helicia nilagirica* powder was weighed in a conical flask and 100 ml of 20% aqueous ethanol was added. The sample was heated over a hot water bath for 4 h with continuous stirring at 55 °C. The mixture was filtered and the residue was re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at 90 °C. The concentrate was transferred into a 250 ml separating funnel with the addition of 20 ml of diethyl ether and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight and saponin contents were calculated as percentage (Brinda et al., 1981).

2.4.2. Determination of Flavonoids

Ten g of the bark powder of *Helicia nilagirica* was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed (Bohm and Kocipai-Abyazan, 1994).

2.5. Determination of moisture content

Determination of the amount of volatile matter (i.e., water drying off from the drug) in the *Helicia nilagirica* is a measure of loss after drying of substances appearing to contain water.
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as the only volatile constituent. The powdered bark of *Helicia nilagirica* was accurately weighed, placed (without preliminary drying) in a tared evaporating dish, dried at 105˚C for 5 hours, and weighed again. The percentage moisture content was calculated with reference to the initial weight. The moisture content was calculated using the following formula:-

\[
\text{Moisture content} = \frac{P_w - F_w}{W} \times 100
\]

Where \( P_w = \) Pre weighed sample
\( F_w = \) Final weight of the dried sample
\( W = \) Total weight of the sample

2.6. Ash values

The ash values including total and acid insoluble ash were determined to estimate the total amount of the inorganic salts present in the drug. The ash contents remained after ignition of plant material was determined by two different methods to measure total and acid insoluble ash contents.

2.6.1. Total ash

The method measures the total amount of material remaining after ignition including both ‘physiological ash’, derived from the plant tissue itself, and ‘non-physiological ash’ which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface.

**Procedure:** Two grams of ground air-dried material of *Helicia nilagirica* was accurately weighed in a previously ignited and tared crucible. The material was spread as an even layer and ignited by gradually increasing the temperature up to 500-600˚C until it became white, indicating the absence of carbon. The crucible was cooled and weighed. The percentage of total ash content was calculated according to the following formula. Total ash content = \( \frac{P_w - F_w}{W} \times 100 \)

Where \( P_w = \) Pre weighed crucible
\( F_w = \) Final weight of the crucible containing ash
\( W = \) Total weight of powdered plant material
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2.7. Extractive values

These are used to determine the amount of the matter which is soluble in the solvents used including alcohol and water. The percentage of alcohol and water-soluble extractives were calculated and used as standards.

2.7.1. Determination of alcohol-soluble extractive

Five grams of air dried coarsely powdered material was macerated in 100 ml of alcohol in a closed conical flask for twenty four hours, with frequent shaking during first six hours and allowed to stand for next eighteen hours thereafter it was filtered rapidly with caution to avoid loss of solvent. The 25 ml of the filtrate was evaporated to dryness at 105°C in a tared flat-bottomed shallow dish and weighed. The percentage of alcohol-soluble extractive was calculated with reference to the air-dried material.

2.7.2. Determination of water-soluble extractive

Five grams of coarsely powdered air dried material was macerated in 100 ml of chloroform-water (0.1%) in a closed flask for 24 h, shaken frequently until six hours and allowed to stand for another eighteen hours. Thereafter it was filtered rapidly, with precautions to avoid loss of solvent by evaporation. The 25 ml of the filtrate was evaporated to dryness at 105°C in a tared flat-bottomed shallow dish and weighed. The percentage of water soluble extractive was calculated with reference to the air dried material. All the tests were done in triplicate.

2.8. TLC Analysis:

TLC is a simple and rapid technique that is able to determine the number of components present in solution and helps in finding a suitable solvent for separating the components by column chromatography as well as for monitoring reactions’ progress. The chloroform and ethanol extracts were spotted on to a number of TLC plates (Merck India, Mumbai) in 1 mm diameter above the bottom of the plates and placed into different mobile phases. The extracts
were allowed to move on the adsorbent (Stationary) phase according to the solvent system used. Several combinations of solvents of increasing polarity were evaluated as mobile phase for TLC run to determine the number of compounds present in different extracts of *Helicia nilagirica*. The different solvent systems were used as mobile phase for TLC, which consisted of chloroform: methanol (9:1, 8:2), pure chloroform, chloroform: ethyl acetate (1:1) and methanol: hydrochloric acid (9:1) solvent combinations. The resultant spots were observed under visible and ultra-violet light at 254 nm and 365 nm. The measure of the distance of a compound travelled is considered as the retention factor ($R_f$) value which was calculated using the following formula:-

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

3. RESULTS

The results of phytochemical analyses and TLC profiling of *Helicia nilagirica* are presented in Table 1-6.

Phytochemical analysis

The preliminary phytochemical screenings of different extracts of *Helicia nilagirica* showed the presence of tannins, phytosterols, saponins, phlobatannins, cardiac glycosides, flavonoids, phenol and terpenoids as chemical entities, whereas alkaloids were completely absent (Table 1).

Quantitative determination of phytochemicals

The quantitative determination of the chemical constituents showed that *Helicia nilagirica* contained 9.26% and 0.26% flavonoids and saponins, respectively (Table 2).
Determination of moisture content

The drying of 500 g of *Helicia nilagirica* bark yielded 296 g of dried bark, and this reduction in weight was due to 40.6% loss in its water contents. The analysis of dried bark of *Helicia nilagirica* showed presence of 25.63% moisture (Table 3).

Determination of total ash content

The ash content of the crude bark powder was found to be 3.24% (Table 4).

Determination of extractive values

The *Helicia nilagirica* bark was found to contain 3.4% ethanol-soluble and 8% water-soluble extractives (Table 4).

Extract yield

The extraction of *Helicia nilagirica* stem bark yielded 2%, 4% and 6% in chloroform, ethanol and water extracts (Table 5).

TLC Analysis

The evaluation of chloroform and ethanol extracts of *Helicia nilagirica* showed the presence of different components as indicated by a varying number of spots and colours on a TLC plates using UV visualization method (Table 6).

Discussion

Plants synthesize several phytochemicals and have played an important role in the development of new therapeutic agents. The preliminary qualitative phytochemical analysis of the bark of *Helicia nilagirica* revealed the presence of phenol, flavonoid, tannins, saponins, cardiac glycosides and carbohydrate. These phytochemicals synthesized by plants are essential for the growth, pathogen attack, pollination, defence and other activities of plants (Reymond *et al.*, 2000; Hermsmeier *et al.*, 2001) however, at the same time these phytochemicals are of great
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use for humans as a source of drugs and other healthcare agents (Shantabi et al., 2014; Lalrinzuali et al., 2015).

Flavonoids consist of a large group of polyphenolic compounds having a benzo-γ-pyrone structure which are ubiquitously present in plants. Approximately, more than 6000 varieties of flavonoids have been identified (Ferrer et al., 2008). They can be divided into a variety of classes such as flavones (e.g., apigenin, and luteolin), flavonols (e.g., quercetin, kaempferol, myricetin, and fisetin), flavanones (e.g., flavanone, hesperetin, and naringenin), and others (Middleton 1998). They are the hydroxylated phenolic substances synthesized by plants in response to microbial infection (Dixon et al. 1983). Flavonols are the most abundant flavonoids in foods and they are generally responsible for colour, taste, prevention of fat oxidation, and protection of vitamins and enzymes (Yao et al. 2004). Flavonoids have been consumed by humans since the advent of human life on earth, that is, for about 4 million years. They have extensive biological properties that promote human health and help reduce the risk of diseases and they are known to possess antioxidant, hepatoprotective, antibacterial, anti-inflammatory, anticancer and antiviral properties (Ferry et al. 1996; Manthey 2000; Li et al. 2000; Kelly et al. 2002; Kumar et al. 2013; Mishra et al. 2013).

Tannins are polyphenols which occur widely in vascular plants particularly associated with woody tissues. They are water soluble and have molecular weights ranging between 500 and 3000 Daltons. Based on the chemical structures, tannins are divided into two groups: hydrolysable, and condensed. The hydrolysable tannins consist of gallic acid esters, and ellagic acid glycosides (Simões et al. 2003). They have an amazing astringent properties which is mainly related to their drug applications. They are known to be antimicrobial, antifungal, anthelminthic, antiviral, antiulcer and hasten the healing of wounds and inflamed mucous
membranes, (Khennouf et al. 2003; Li et al. 2011; Theisen 2014; Williams et al. 2014) They exert internal anti-diarrheal and antiseptic effects by waterproofing the outer layers of more exposed mucous membranes. Tannins are also haemostatic, and can serve as an antidote in poisoning cases (Albuquerque et al. 2005). In the process of healing wounds, burns and inflammations, tannins help by forming a protective layer (tannin-protein/tannin-polysaccharide complex), over injured epithelial tissues permitting the healing process below to occur naturally. Studies show that many tannins act as radical scavengers, intercepting active free radicals (Simões et al. 2003) various degenerative diseases such as cancer, multiple sclerosis, atherosclerosis and aging process itself are associated with high concentrations of intercellular free radicals.

Terpenoids are synthesized from five carbon isoprene units mainly isopentenyl pyrophosphate and its isomer dimethylallyl pyrophosphate by the enzyme terpene synthases. They are classified according to whether they contain two (C_{10}), three (C_{15}), four (C_{20}), six (C_{30}) or eight (C_{40}) isoprene units. They range from the essential oil components, the volatile mono- and sesquiterpenes (C_{10} and C_{15}) through the less volatile diterpenes (C_{20}) to the involatile triterpenoids and sterols (C_{30}) and carotenoid pigments (C_{40}). Each of these various classes played a significant role in plant growth, metabolism or ecology (Harborne 1998). Approximately 40,000 terpenes have been identified and the majority of possible functions of these molecules are unknown (Goto et al., 2010). Terpenoids have been found to be useful in the prevention and therapy of several diseases, including cancer, and they are also known to possess antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antimalarial, antihyperglycemic, antiinflammatory and immunomodulatory properties (Mujoo et al. 2001; Wagner et al. 2003; Salminen et al 2008; Rabi et al. 2009; Grace et al. 2013).
can also be used as protective substances in storing agriculture products as they are known to have insecticidal properties as well (Sultana et al. 2008).

Saponins are naturally occurring structurally and functionally diverse phytochemicals that are widely distributed among seventy families of plants. They are glycosides of both triterpenes and sterols (Hostettmann and Marston 1995). Due to the presence of both the hydrophobic aglycone backbone and hydrophilic sugar molecules the saponins are highly amphipathic and possess foaming and emulsifying properties. They play an important role in plant ecology and they are also exploited for a wide range of commercial applications in the food, cosmetic and pharmaceutical sectors (Güçlü-Ustündağ & Mazza, 2007; San Martín & Briones, 1999). These molecules are potent membrane permeabilizing agents, immunostimulatory, hypocholesterolemic, anti-carcinogenic, anti-inflammatory, anti-microbial, anti/protozoan, molluscicidal and have anti-oxidant properties (Francis et al. 2002; Sparg et al. 2004). Saponins also act as antitumor by inhibiting tumor cell growth by apoptosis (Lee et al., 2011).

Cardiac glycosides are composed of two structural features: The sugar (glycoside) and the non-sugar (aglycone-steroid) moieties and they act on the contractile action of the cardiac muscle. These compounds have long been used for the treatment of cardiac arrhythmias and congestive heart failure due to their capability to increase the contractile force (Liu et al. 2000). Digitalis is the most commonly used cardiac glycoside and it is able to directly inhibits the proliferation of androgen dependent and androgen independent prostate cancer cell lines by initiating apoptosis and increasing intracellular Ca2+. Cardiac glycosides have been reported to inhibit the four genes that are over expressed in prostate cancer cells including the inhibitors of apoptosis inhibitor and transcription factors (Newman et al. 2008). Cardiac glycosides have been
reported as active anticancer agents (Kepp et al., 2012; Calderón-Montaño et al., 2014). They are also reported to have antiviral properties against human cytomegalovirus. (Kapoor et al. 2012).

The *Helicia nilagirica* has been found to contain various phytochemicals like cardiac glycosides, flavonoids, saponins, terpenes, and tannins. Their presence has been confirmed by various analytical methods. The presence of these phytochemicals shows that it may act as promising anticancer agent.
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of north-east India. National Institute of Science Communication and Information Resources, CISR, New Delhi, 3: 200-204.


49. Simões, C.M.O.; Schenkel, E.P.; Gosmann, G.; Mello, J.C.P.; Mentz, L.A.


Table 1. Results of the Phytochemical analysis of *Helicia nilagirica*

<table>
<thead>
<tr>
<th>Tests</th>
<th>Chloroform extract</th>
<th>Ethanol extract</th>
<th>Water extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Diterpenes</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponin</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycoside</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 2. Quantitative determination of the chemical constituent of *Helicia nilagirica*.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Flavonoid</th>
<th>Saponin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Helicia nilagirica</em></td>
<td>Quantity</td>
<td>Output</td>
</tr>
<tr>
<td></td>
<td>10g</td>
<td>0.9256g</td>
</tr>
</tbody>
</table>
### Table 3. Percentage of loss on drying fresh bark of *Helicia nilagirica*

<table>
<thead>
<tr>
<th>Weight before drying (kg)</th>
<th>Weight after drying (kg)</th>
<th>Loss after drying (%)</th>
<th>Moisture content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.296</td>
<td>40.6</td>
<td>25.63</td>
</tr>
</tbody>
</table>

### Table 4. Physicochemical parameters of dried bark powder of *Helicia nilagirica*

<table>
<thead>
<tr>
<th>Total ash (%)</th>
<th>Ethanol-soluble extract (%)</th>
<th>Water-soluble extract (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.24</td>
<td>3.4</td>
<td>8</td>
</tr>
</tbody>
</table>

### Table 5. Yield of various extracts of *Helicia nilagirica.*

<table>
<thead>
<tr>
<th>Dried powder</th>
<th>Chloroform extract (%)</th>
<th>Ethanol extract (%)</th>
<th>Water extract (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100g</td>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
</tbody>
</table>
Table 6. TLC profile of the different extracts of *Helicia nilagirica* on pre-coated aluminium TLC plates.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Solvent</th>
<th>Day light</th>
<th>R&lt;sub&gt;f&lt;/sub&gt; value</th>
<th>UV 254 nm</th>
<th>R&lt;sub&gt;f&lt;/sub&gt; value</th>
<th>UV 365 nm</th>
<th>R&lt;sub&gt;f&lt;/sub&gt; value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td>CHCl&lt;sub&gt;3&lt;/sub&gt;:CH&lt;sub&gt;3&lt;/sub&gt;OH 9:1</td>
<td>Streak</td>
<td>Two spots</td>
<td>0.92, 0.53</td>
<td>5 spots (1 red, 1 blue, 3 yellowish)</td>
<td>0.92, 0.86, 0.57, 0.5 &amp; 0.42</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>CHCl&lt;sub&gt;3&lt;/sub&gt;:CH&lt;sub&gt;3&lt;/sub&gt;OH 8:2</td>
<td>Streak</td>
<td>3 spots</td>
<td>0.57, 0.28 &amp; 0.09</td>
<td>Not clear</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Aqueous</td>
<td>Not visible</td>
<td>Not visible</td>
<td>Not visible</td>
<td>-</td>
<td>Not visible</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Chloroform</td>
<td>CHCl&lt;sub&gt;3&lt;/sub&gt;</td>
<td>One spot</td>
<td>1 spot</td>
<td>0.94</td>
<td>3 spots (1 reddish, 2 bluish)</td>
<td>0.94, 0.88 &amp; 0.84</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>CHCl&lt;sub&gt;3&lt;/sub&gt;:CH&lt;sub&gt;3&lt;/sub&gt;OH 8:2</td>
<td>Streak</td>
<td>3 spots</td>
<td>0.90, 0.82 &amp; 0.5</td>
<td>1 spot (bluish)</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>Aqueous</td>
<td>Not visible</td>
<td>Not visible</td>
<td>Not visible</td>
<td>-</td>
<td>Not visible</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Chloroform</td>
<td>CHCl&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Not visible</td>
<td>1 spot</td>
<td>0.94</td>
<td>3 spots (1 bluish, 2 red)</td>
<td>0.94, 0.09 &amp; 0.05</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>CHCl&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Not visible</td>
<td>Not clear</td>
<td>-</td>
<td>1 spot (bluish)</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>Aqueous</td>
<td>Not visible</td>
<td>Not visible</td>
<td>Not visible</td>
<td>-</td>
<td>Not visible</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Chloroform</td>
<td>CHCl&lt;sub&gt;3&lt;/sub&gt;:C&lt;sub&gt;4&lt;/sub&gt;H&lt;sub&gt;8&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt; 1:1</td>
<td>One spot</td>
<td>3 spots</td>
<td>0.90, 0.69 &amp; 0.48</td>
<td>4 spots (1 reddish, 3 bluish)</td>
<td>0.90, 0.86 &amp; 0.84</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>CHCl&lt;sub&gt;3&lt;/sub&gt;:C&lt;sub&gt;4&lt;/sub&gt;H&lt;sub&gt;8&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt; 1:1</td>
<td>Not visible</td>
<td>3 spots</td>
<td>0.69, 0.42 &amp; 0.23</td>
<td>Not visible</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Aqueous</td>
<td>Not visible</td>
<td>Not visible</td>
<td>Not visible</td>
<td>-</td>
<td>Not visible</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Solvent</td>
<td>Mobile Phase</td>
<td>Spot Description</td>
<td>Rf Value 1</td>
<td>Rf Value 2</td>
<td>Color Description</td>
<td>Rf Value 3</td>
<td></td>
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<td>------------</td>
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<td></td>
</tr>
<tr>
<td>Chloroform</td>
<td>CH$_3$OH:HCl (9:1)</td>
<td>One spot</td>
<td>0.86</td>
<td>1 spot</td>
<td>0.84</td>
<td>2 spots (1 reddish, 1 bluish)</td>
<td>0.94 &amp; 0.79</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Two spots</td>
<td>0.82 0.43</td>
<td>1 spot</td>
<td>0.84</td>
<td>Not clear</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Aqueous</td>
<td>Not visible</td>
<td>-</td>
<td>Not visible</td>
<td>-</td>
<td>Not visible</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1: Phytochemical screening of *H. nilagirica* extracts.

- **a)** CHCl$_3$:CH$_3$OH (9:1)
- **b)** CHCl$_3$:CH$_3$OH (8:2)
- **c)** CHCl$_3$
- **d)** CHCl$_3$:C$_4$H$_8$O$_2$ (1:1)
- **e)** CH$_3$OH: HCl (9:1)

Figure 2: TLC profile of different extracts of *H. nilagirica* using different solvent systems observed under normal light to detect phytochemicals present in the extracts (aqueous, chloroform and ethanol).

- a) CHCl$_3$:CH$_3$OH (9:1)
- b) CHCl$_3$:CH$_3$OH (8:2)
- c) CHCl$_3$
- d) CHCl$_3$:C$_4$H$_8$O$_2$ (1:1)
- e) CH$_3$OH: HCl (9:1)
Figure 3: TLC profile of *H. nilagirica* on different solvent systems observed under UV 365 nm to detect phytochemicals present in the extracts (aqueous, chloroform and ethanol).

a) CHCl₃:CH₃OH (9:1)  
b) CHCl₃:CH₃OH (8:2)  
c) CHCl₃  
d) CHCl₃:C₄H₈O₂ (1:1)  
e) CH₃OH: HCl (9:1).

Figure 4: TLC profile of *H. nilagirica* on different solvent systems observed under UV 254 nm to detect phytochemicals present in the extracts (aqueous, chloroform and ethanol).

a) CHCl₃:CH₃OH (9:1)  
b) CHCl₃:CH₃OH (8:2)  
c) CHCl₃  
d) CHCl₃:C₄H₈O₂ (1:1)  
e) CH₃OH: HCl (9:1).