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ANALYSIS OF PHYTOCHEMICAL CONSTITUENTS FROM THE FRUIT EXTRACTS OF *ILLICIUM VERUM* HOOK

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ABSTRACT

The use of plants as medicines pre-dates to more than two thousand years and traditional medicine has relied on plants and herbs to cure minor and severe ailments for keeping the body healthy. The use of alternative medicine in health care should be taken seriously as they can improve a person's health without the serious side effects of conventional medication. Medicinal plants synthesize a vast array of secondary metabolites that are important for human life. Plants make many chemical compounds that will posses biological activities. The study of traditional use of plants is recognized as an effective way to discover future medicines. In the current investigation on *Illicium verum* Hook (star anise), an attempt was made to carry out preliminary phytochemical analysis from the methanolic extract by adopting various biochemical protocols and the quantitative estimation of each component was performed by employing UV-Visible spectroscopy. The results of this study offer a platform for using *Illicium verum* fruits as herbal alternative in the treatment of various diseases.

INTRODUCTION:

Ayurveda is the most ancient health care system and is practiced widely in India, Srilanka and other countries [1]. There is a great demand for herbal medicines in developed as well developing countries because of their wide biological activities, safety margin than synthetic drugs and lesser cost [2] .The current market potential of herbal medicines is estimated about $8- 250 billion in Europe and USA. The Indian herbal drug market size is about $1 billion and export of plant-based crude is around $100 million [3]. Ayurveda and modern medicines technique should be coupled to bring out highly quality herbal products with rapid onset of action and good bioavailability. Nature has provided an important source of remedies to cure all the ailments of mankind. In recent years, all the medicines used are from natural source, especially from plants [4] which contain hundreds or thousands of metabolites. Medicinal and aromatic plants, a gift of nature, are being used against various infections and diseases in the world since last history. Only a small percentage of plants species have been investigated phytochemically and the fraction submitted

to biological screening is even smaller [5]. Plant kingdom represents an extraordinary repository of novel molecules. *Illicium verum* Hook.f, from the family of Magnoliaceae was originated from China and Vietnam, but can easily be found throughout Asia. It is an evergreen tree living in cooler tropics and subtropics (Glob in Med). It produces unique star-shaped fruits with five to ten boat-shaped sections radiating from the center, tough skinned and are rusty in colour. It is commonly known as star anise in English, bunga lawang in Malay and Indonesia, stemanis in German, anice stellato in Italian and ba chio in Chinese. This plant is used traditionally in Chinese, Carribean and Latino populations as an infusion for the treatment of infant colic [6]. The fruit is used widely in cooking and also as flavoring agent in candies, chewing gums, pickles and sometimes were chewed to help in the digestion as well as breath sweetener [7]. Very little research work is done on this plant; hence the current study was to carry out for the phytochemical analysis of the fruit extract of *Illicium verum* hook.

MATERIALS AND METHODS: *Illicium verum* fruit extract was prepared by employing Soxhalet apparatus. It was further concentrated by rotary evaporator and stored at 4°C until use and the dried extracts were then suspended in Dimethyl sulfoxide (DMSO) for further use.

PHYTOCHEMICAL SCREENING

Phytochemical Screening: Phytochemical analysis was carried out for all the extracts as per the standard methods.

Detection of alkaloids:

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates. **Benedict 's test:** Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars. **Fehling's Test:** Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides. **Modified Borntrager's Test:** Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of Benzene. The Benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium

hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

Detection of saponins: Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins. **Foam Test:** 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Detection of phytosterols: Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Detection of phenols: Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of tannins: Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Detection of flavonoids: Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Detection of proteins and Aminoacids: Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

Ninhydrin Test: To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

Detection of Diterpenes: Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Appearance of emerald green colour indicates the presence of diterpenes.

Quantitative estimation of phytochemicals in fruit extracts of selected medicinal plants

Instrumentation: Techcomp UV-2301 UV-Visible Spectrophotometer was employed for the determination of Phytochemical compound from the Plant extract. Hitachi was the software that attached with the Spectrophotometer. Standard cuvettes were used with the path length of 10mm. Samples were agitated using Ultrasonicator. Denver electronic analytical balance (SI-234) used for weighing the samples in various stages of experiment.

Chemicals and reagents: The chemicals employed for the estimation are sodium phosphate, citric acid, Bromocresol green, chloroform. All these reagents were laboratory reagent grade were purchased for Merck chemicals private limited, Mumbai, Fisher scientific, Mumbai and SD fine chemicals Mumbai.

Preparation of reagents:

Phosphate buffer solution (pH 4.7): buffer solution was prepared by adjusting the pH of 2 M sodium phosphate (71.6 g Na2HPO4 in 1 L distilled water) to 4.7 with 0.2 M citric acid (42.02 g citric acid in 1 L distilled water).

Sample Preparation: 10 mg of the plant extract was taken and dissolved in the 10ml of ethanol and sonicated for another

10minutes. 1ml was taken from these and employed for further usage.

Standard Preparation: Atropine was taken as a standard for the estimation of the Alkaloid content in the plant extract. 10mg of Atropine was taken and dissolved in 10ml with the suitable solvent i.e., methanol and it is sonicated for 5minutes. This stock solution, concentration of 1000 µg/ml, was kept aside for further process. From the stock solution, 1ml was taken and 9ml of solvent added to obtain the concentration of 100µg/ml solution and then again 1ml was taken and diluted to 10ml with the solvent to get 10 µg/ml solutions. This process was repeated till it gets the concentration of 1 µg/ml.

Preparation of Standard Calibration curve: Calibration curve was prepared by taking 2ml, 4ml, 6ml, 8ml,10ml and 12ml solutions from the concentration of 10 µg/ml and applying estimation procedure of Alkaloids and graph was plot by taking absorbance on Y-axis and concentration on X-axis.

Quantitative Estimation of phytochemicals:

Determination of total Alkaloid content:

To 1ml of extract 5 ml pH 4.7 phosphate Buffer was added and 5 ml BCG solution and shake a mixture with 4 ml of chloroform. The extracts were collected in a 10-ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without extract. Atropine is used as a standard material and compared the assay with Atropine equivalents.

Chemicals and reagents: The chemicals employed for the estimation are Aluminium chloride, Sodium nitrite (NaNO2), Quercetin as standard and Sodium hydroxide (NaOH). All these reagents were laboratory reagent grade and were purchased for Merck chemicals private limited, Mumbai, Fisher scientific, Mumbai and SD fine chemicals Mumbai.

Sample Preparation: 10 mg of the plant extract was taken and dissolved in the 10ml of ethanol and sonicated for another 10minutes. 1ml was taken from these and employed for further usage.

Standard Preparation: Quercetin was taken as a standard for the estimation of the Flavanoid content in the plant extract.10mg of Quercetin was taken and dissolved in 10ml with the suitable solvent i.e., methanol and it is sonicated for 5minutes. This stock solution, concentration of 1000 µg/ml, was kept aside for further process. From the stock solution, 1ml was taken and 9ml of solvent added to obtain the concentration of 100µg/ml solution and then again 1ml was taken and diluted to 10ml with the solvent to get 10 µg/ml solutions. This process was repeated till it gets the concentration of 1 µg/ml.

Preparation of Standard Calibration curve: Calibration curve was prepared by taking 1ml, 2ml, 3ml, 4ml, 5ml and 6ml solutions from the concentration of 1 µg/ml and applying estimation procedure of Flavanoids. A graph was plot by taking absorbance on Y-axis and concentration on X-axis. The values obtained are substituted in the regression equation i.e., $Y=mx+c^2$. Here Y is average absorbance of the unknown sample, term m shows slope and c indicates intercept.

Determination of total flavanoid content: Total flavanoid content was determined using aluminium chloride ($AlCl_3$) according to a known method, 15 using quercetin as a standard. The 1ml of plant extract was taken in a test tube and 2ml of 5% NaNO2 was added to it. After 5 min, 3 ml of $AlCl_3$ (10%) was added. After 5 minutes, the reaction mixture was treated with 2 ml of 1 M NaOH. Finally, the reaction mixture was diluted to 10 ml with water and the

absorbance was measured at 510 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. For the blank, the extracts were replaced with an equal volume of distilled water. The content of the Flavonoids in extracts was expressed in terms of Quercetin equivalent.

Flavanoids: The results of the preliminary studies confirmed that the fruit extract of plant shows positive results for more number of phytochemicals. Table 1 shows the screening results for the medicinal plants in the study. Results confirmed that the plant contain Flavanoids. Visible spectrophotometric methods were followed for the quantitative estimation of the identified phytochemicals in selected plants. Aluminium chloride method with quercetin standard for flavanoid. The standard calibration curve obtained for estimation of Flavanoids was given in Figure 1. The equation of the standard Quercetin is $y = 0.484x + 0.133$ and correlation coefficient is $R^2 = 0.999$. X value for the first sample was found to be 0.619 where as second sample amount was 0.888. Results of the phytochemical compound analysis were shown in the following tables. The results of the preliminary studies confirmed that the fruit extract of plant shows positive results for more number of phytochemicals. Table 1 shows the screening results for the medicinal plants in the study. Results confirmed that the plant contain Alkaloids. Visible spectrophotometric methods were fallowed for the quantitative estimation of the identified phytochemicals in selected plants. Extractive spectrophotometric method with Bromocresol green was followed for estimation of Alkaloids. The standard calibration curve obtained for estimation of Alkaloids was given in Figure 1.

DISCUSSION:

The phytotherapeutic products from medicinal plants have become universally popular in primary healthcare. Drug discovery from plants is becoming an essential component in the search for new medicines and the scientific study of traditional medicines and the concerned medicinal plants. The initial step in the exploration of the importance of any medicinal plant is to screen for its phytochemicals as it gives a broad idea regarding the nature of compounds present in it[8] phytochemical screening helps in the determination of its different bioactivities such as antimicrobial, antioxidant, hepatoprotective, neuroprotective etc [9,10]. In the present study, all the four extracts of the selected plants were preliminarily screened for the phytochemicals and it was found that among them, the methanolic extract was rich in all the phytoconstituents followed by ethyl acetate extract and the selected medicinal plant specie, *Illicium verum* is further explored for their different bioactive efficacies. The results of the preliminary phytochemical studies, basing on biochemical protocols have confirmed that the extracts of the plant showed positive results for more number of phytochemical components as shown in Table.1 and it can be concluded that the plant contain Alkaloids and Flavonoids predominantly in methanolic and ethyl acetate extracts. These phytochemicals could contribute to the various medicinal applications of both the plants. The current results are in line with Varadarajan *et al.* that the secondary metabolites (phytochemicals) and other chemical constituents of the medicinal plants are responsible for their medicinal values. In a previous report on the phytochemical screening of methanolic extract of root and stem of *Eucalyptus globulus* showed the presence of alkaloids, flavonoids, saponin, tannins and phenols which was also subjected for the evaluation of antioxidant activity [11] . Similar work was carried out by Abiodun Humphrey Adebayo *et al.,* 2011

Table: 1 Results of qualitative analysis of Illicium *verum* fruit extracts.

S.No	Phytochemicals	Water	Methanol	Ethanol	Acetone	Ethyl acetate
1	Alkaloids	+	+ +	-	++	++
2	Flavonoids	+	+ +	+	++	--
3	Saponins	+	-	-	--	--
4	Steroids	+	+ +	-	++	++
5	Tannins	+	+ +	-	++	++
6	Protein	++	+ + +	++	--	--
7	Amino acids	++	+ + +	+	--	--
8	carbohydrates	+	+	+	--	--
9	glycocides	-	-	-	++	--
10	Anthocyanins	-	-	-	--	--
11	Emodins	+	++	+	--	--
12	Coumarins	+	+	--	--	--
13	Tri-terpenoids	+	++	-	++	--

S.No	Concentration	Absorbance
1	0.1	0.185
2	0.2	0.227
3	0.3	0.278
4	0.4	0.327
5	0.5	0.374
6	0.6	0.426

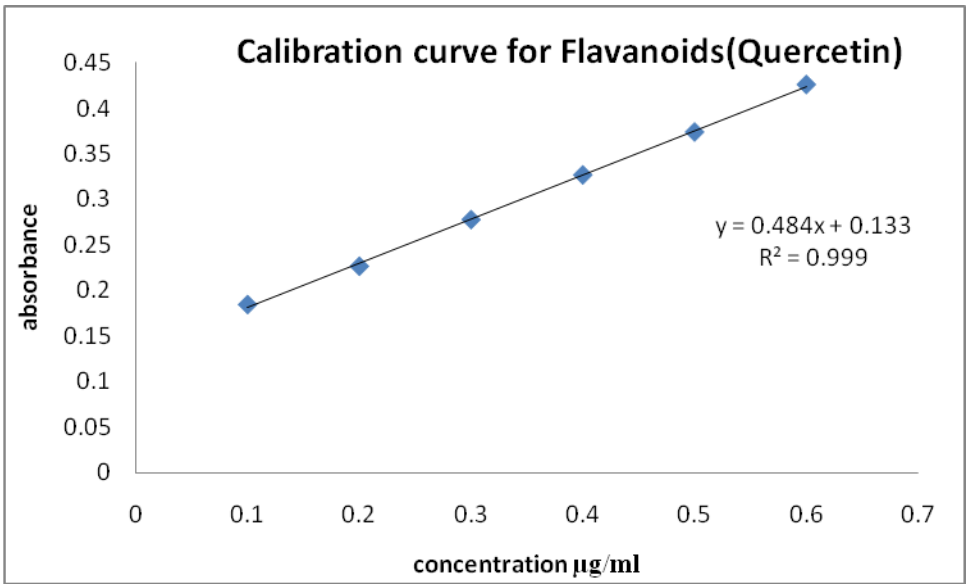

Figure 1: Standard calibration curve for estimation of phytochemicals in plant extract.

Table 2: Quantitative estimation results of phytochemical compound

S. No	Name of Phytochemical compound	Sample II methanol extract	
		Absorbance	Amount*
1	Flavanoids	0.179 0.174 0.176	0.894

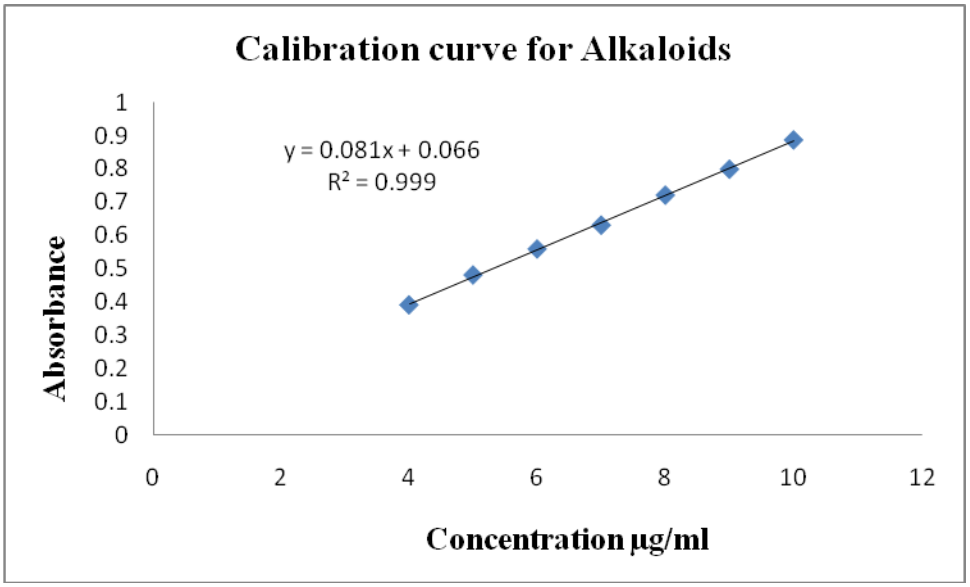

Figure 2: Standard calibration curve for estimation of phytochemicals in plant extract.

Table 3: Quantitative estimation results of phytochemical compound

S. No	Name of Phytochemical compound	Sample II Ethanol extract	
		Absorbance	Amount*
1	Alkaloids	0.811	0.920

*Amount is given in as mg of the phytochemical compound present in one gram of the plant extract

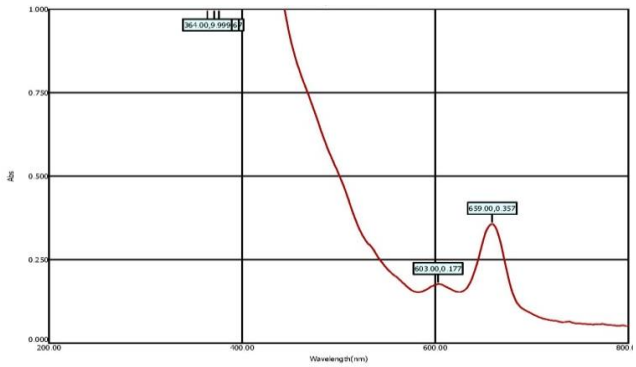

Figure 3: UV-VIS Spectrum of *Illicium verum*

Which is related to phytochemical screening and the determination of antioxidant property in *Chrysophyllum Albidum* (L)? Basing on the results of quantitative analysis it was confirmed that the fruit extract of *Illicium verum contained* 0.920mg/g of alkaloids and 0.894 mg/g of flavonoids respectively These extracts are involved in scavenging free radicals from tissues, thus reducing the oxidative stress. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers. Similarly, terpenoids, as vitamins, act as regulators of metabolism and play a protective role as antioxidants [14]. Many researchers have reported the presence of certain phytochemicals as responsible for the treatment of specific diseases. Tannins and flavonoids are known to be present in the extracts used as antibacterial and antioxidant agents[15]. Flavonoids and glycosides are also known to prevent cardio-vascular diseases and ulcers [16]. The presence of alkaloids in plant extracts are also used for wide range of pharmacological activities including antimalarial, antiasthma, anticancer, etc [17]. Recent studies also showed that tannins containing extract was used to treat haemorrhoids [18] [19] and antiparasite [20]. [21] Also indicated that the presence of these secondary metabolites in plants which produces some biological activities responsible for their potential use as drugs. The qualitative UV-VIS profile of methanolic extracts of both the plants was analysed within the wave length ranging from 200 to 800 nm. The profile has shown the peaks at 364.0, 603.0, and 659.0 nm with the absorbance values of 0.999, 0.177, 0.357 respectively for *Illicium verum* as shown in Table3. Fig-3. Thus the phytochemical profile showed the occurrence of peaks with in the 234-800 nm range of UV, which confirms the presence of phenolics, flavonoids and alkaloids in the fruit extract of *Illicium verum* [22, 23, 24, 25, and 26].

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