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# Phytochemical analysis and anti-emetic activity of *Illicium verum* Hook. f. fruit by chick emesis model

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**Abstract**
Emesis defined as a forceful expulsion of stomach contents via mouth. The adverse effects of available anti-emetic drugs potentates us to evaluate natural products to explore effective natural anti-emetics with fewer side effects. The fruits of *Illicium verum* Hook. f. were selected for anti-emetic activity on the basis of their folkloric use against gastrointestinal complaints. Crude methanolic extract and its aqueous, dichloromethane, ethylacetate and hexane fractions were evaluated by copper sulphate induced chick emesis model. Emesis was induced by the oral administration of copper sulfate 10 ml/kg body weight and metoclopramide (150 mg/kg p.o.) as standard anti-emetic drug. The anti-emetic effect was observed by calculating the mean decrease in number of retches in contrast with that of control. Methanolic extract and its fractions were tested at the doses of 25, 50 and 75 mg/kgorally. Among all tested fractions, ethylacetate showed the highest (94.46%) and hexane showed the lowest (56.30%) anti-emetic activity at 50 mg/kg.Further, isolation and purification from the same fractions is in process to discover effective natural anti-emetic compound(s) with less unwanted effects.

**Keywords:** Phytochemical analysis, anti-emetic activity, *Illicium verum*

## 1. Introduction
*Illicium verum* Hook. f. is an aromatic evergreen tree of the family Illiciaceae. Its fruit is star shaped of reddish brown colour with 6-8 follicles; each follicle is about boat shape and containing one seed [1, 2]. The fruit has traditionally been used to treat inflammation, insomnia, stomach aches, and vomiting and also used as carminative, expectorant and lactagogue. Analgesic, anticonvulsant, anti-inflammatory, antimicrobial, antioxidant, antidiarrheal, antipyretic, anti-rheumatic, antiseptic, diuretic and insecticidal are the reported pharmacological activities [3, 4]. Its high essential oil contents are the main source being used for rheumatism and as an anti-septic agent [5]. Anisaldehyde, estragole, limonene, trans-anethole, 4'-methoxypropiophenonehave been identified from the fruit [6].
Copper sulfate induced chick emesis model is frequently used to evaluate anti-emetic potential of terrestrial medicinal plants and marine algae due to its simplicity, rapidity and authentic results [7]. Although, *Illicium verum* Hook. f. fruits has been used in GI disorders in traditional medicine as carminative and against stomach-ache and vomiting [4]. There is no scientific report as their anti-emetic effect in the literature. In the present study, we report the anti-emetic effect of the methanolic extract and its aqueous, ethylacetate, dichloromethane and hexane fractions by using copper sulfate induced chick emesis model.

## Material and Methods
**Plant Material:** The fruits of *Illicium verum* were purchased from local market and identified and authenticated by the taxonomist of Department of Botany, Lahore College for Women University, Lahore. These fruits were soaked in methanol for two weeks and the solvent was removed by evaporation under reduced pressure. Crude extract was then subjected to fractionation using different solvents according to polarity gradient resulting in *n*-hexane (F1), dichloromethane (F2), ethyl acetate (F3) and aqueous (F4) fractions, respectively.

**Animals:** Young chicks of 6-8 days age, weighing 25-45grams were bought from Tollinton market, Lahore.

### Phytochemical Analysis of the Plant
The crude extract and fractions of fruit of *I. verum* Hook. f. were subjected to preliminary phytochemical analysis to test for the presence or absence of selected phytochemical constituents consistent with the standard methods [8].

**Qualitative Analysis**

**Table 1:** Qualitative phytochemical analysis crude MeOH extract of *I. verum*

| Description | Crude extract |
|---|---|
| Tannins | + |
| Phenolics | +++ |
| Saponins | -- |
| Alkaloids | +++ |
| Flavonoids | +++ |
| Steroids | +++ |
| Triterpenes | -- |
| +++ Intense coloration or ppt., + extremely light, -- Not detected | |

**Quantitative Analysis**

Quantitative analysis has been focused only on flavonoid and phenolic content of the plant material and this was done both by colorimetric method using spectrophotometer and HPLC analysis.

**Colorimetric Analysis**

Colorimetric analysis was done to measure the total phenolic content in the crude MeOH extract and its fractions.

For the analysis of total phenolic content, stock solutions for all the extracts and fractions were prepared by following the method of Evans, 1989.Folin-Ciocalteureagent was used to measure the total phenolic content of plant extract and fractions that has been expressed as mg GAE /g dry extract [9].

**Table 2:** Quantitative analysis of Phenolics in *I. verum*

| Plant part (Fruit) | Crude extract & its fractions | Total Phenolic Gallic acid | |
|---|---|---|---|
| | | GAE(mg/g) | Dry ext. (mg/dry crude ext) |
| *I. verum* | MeOH | 0.157 | 2.127 |
| | *n*-Hexane | 0.243 | 3.292 |
| | DCM | 0.143 | 1.937 |
| | EtOAc | 0.219 | 2.967 |
| | BuOH | Not Possible | |
| | Aqueous | 0.20 | 2.71 |

**HPLC Analysis**

The presence of flavonoids and phenolic in the extracts followed by their quantification through HPLC chromatogram was done by Analytical HPLC [10].

The flavonoids and phenols in the extracts were detected by straight evaluation of their retention times with those of the standards. By measuring the peak area of the HPLC chromatograms, the specific flavonoid and phenolic content of crude extract fractions was quantified and expressed as quercetin equivalent. Quantification of HPLC analysis is done by following formula:

$$\text{Response factor of standard} = \frac{\text{Peak Area of Standard}}{\text{Concentration of standard used}}$$

$$\text{Amount of standard in sample} = \frac{\text{Peak area (Sample peak)}}{\text{Response factor for standard}}$$

Crude MeOH extract and its fractions *n*-Hexane (F1), DCM (F2) and EtOAc (F3) were analyzed for their flavonoid and phenolic content in HPLC. The reference standards used in the study included standards of flavonoid i.e. rhamnetin, kaempferol, myrcetin, iso-rhamnetin, rutin, apigenin, gallic acid, taxifolin, tannic acid and luteolin were used. Presence of any compound is detected by the specific peak produced by its standard maintaining all the conditions same. A compound can be detected on the basis of retention time of the peak, peak area and peak length.

**Table 3:** Standards of Flavonoids and Phenolics used for HPLC analysis

| Flavonoid Standard | $t^R$ (min) | Rf for Standard | Peak area % | Height (mAU) |
|---|---|---|---|---|
| Rhamnetin | 1.126 | 0.814 | 81.45 | 8.17 |
| Kaempherol | 1.122 | 0.819 | 81.98 | 9.99 |
| Myricetin | 1.128 | 0.750 | 75.02 | 149.39 |
| Iso-rhamnetin | 1.120 | 0.807 | 80.70 | 88.59 |
| Rutin | 1.120 | 0.946 | 94.61 | 243.30 |
| Apigenin | 1.117 | 1 | 100 | 27.99 |
| Gallic acid | 1.119 | 0.912 | 91.24 | 88.30 |
| Taxifolin | 1.123 | 0.562 | 56.22 | 83.18 |
| Tannic acid | 1.119 | 0.299 | 29.98 | 20.85 |
| Luteolin | 1.128 | 0.457 | 45.76 | 17.26 |

$t^R$= Retention time, Rf=Response factor of Standard

**Anti-emetic Activity**

The anti-emetic behaviour was observed by following the protocols of Yang *et al.*, 1999. The chicks were randomly divided into 17 groups of 9 animals each. Anti-emetic effect was determined by calculating the mean decrease in number of retches in contrast with that of control [11]. The control group received only saline 0.9%. Three different concentrations 25, 50 and 75 mg /kg of crude methanol plant extract and its fractions i.e. F1 (*n*-hexane), F2 (dichloromethane), F3 (ethylacetate) and F4 (aqueous) were prepared in 0.9% saline solution and 5% DMSO. 1% Tween 80was used as an emulsifying agent. Emesis was induced by copper sulfate50mg/kg orally. All treatments were done at the volume of 10 ml/kg body weight and number of retches was observed for next 10 min. Metoclopramide 150 mg/kg body weight was used as standard drug.

The percent inhibition of retches was calculated by the following formula:

Inhibition (%) = (A-B/A) x 100

Where

A=Frequency of Retching after plant treatment B=Frequency of Retching after plant treatment

**Table 4:** Anti-emetic effect of *Illicium verum* fruits

| Treatments | Dose (mg/kg) p.o. | Mean number of retches± S.E.M | Inhibition (%) of emesis |
|---|---|---|---|
| **Control** | ----- | 66.11± 10.37 | ----- |
| **MCP (standard)** | 150 | 26.88±9.63 | 59.34 |
| **MTH** | 25 | 31.11± 9.01* | 52.94 |
| | 50 | 72.33± 21.71 | -9.40 |
| | 75 | 63.78± 11.05 | 3.52 |
| **F1** | 25 | 19.88± 1.96 | 69.92 |
| | 50 | 28.89± 5.34 | 56.30 |
| | 75 | 23.78± 2.19 | 64.02 |
| **F2** | 25 | 7.89± 2.37* | 88.06 |
| | 50 | 6.22± 1.69* | 90.59 |
| | 75 | 3.88± 0.87* | 94.13 |
| **F3** | 25 | 9.44 ± 1.49 | 85.72 |
| | 50 | 3.66 ± 0.89* | 94.46 |
| | 75 | 5.66 ± 1.37 | 91.43 |
| **F4** | 25 | 11.89± 1.45 | 82.01 |
| | 50 | 8.00± 1.16 | 87.89 |
| | 75 | 7.33± 0.68 | 88.91 |

MCP= metoclopramide; MTH= methanolic extract; F1 = n-hexane fraction; F2= dichloromethane fraction; F3= ethylacetate fraction; F4= aqueous fraction; N = 9 for each group; p.o.= orally; S.E.M = Standard Error of Mean ; *=$p$<0.05 vs. control showing significant values using unpaired students *t*- test.

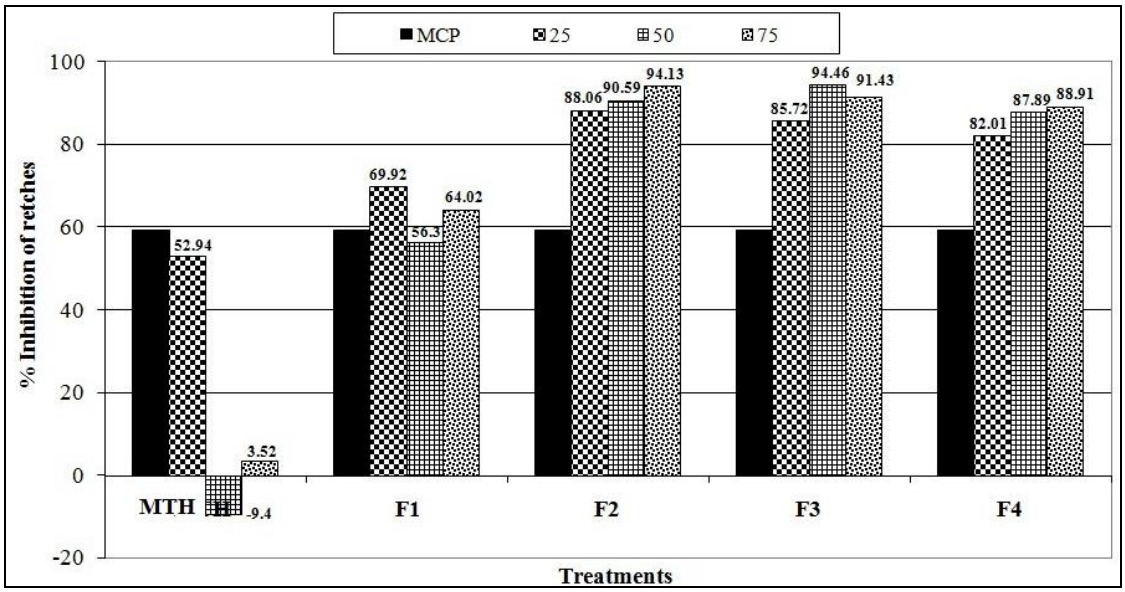


**Fig 1:** Anti-emetic effect of *Illicium verum* fruits. (MCP= metoclopramide; MTH= methanolic extract; F1 = n-hexane fraction; F2= dichloromethane fraction; F3= ethylacetate fraction; F4= aqueous fraction)

**Statistical analysis**

The standard error means (SEM) and statistical significance difference was determined by unpaired student's *t*-test.

**Results and discussion**

In the Qualitative Phytochemical Analysis, it showed polar extracts contained flavonoids, phenolics and alkaloids as chief secondary metabolites whereas non-polar solvents like *n*-hexane extracted less polar compounds (Table 1).

It has been supported by various studies that major phytochemicals identified in the extracts of *I. verum* were found to be alkaloids, flavonoids, and phenolics etc. Many researchers have related the presence of these phytochemicals to different biological and pharmacological activities of fruit of this plant [4]. These plant metabolites may increase or decrease anti-emetic capacity of plant depending upon their concentration in plant.

The total phenolic contents of *I. verum* ranges from 0.157mg/g to 0.243mg/g, respectively (Table 2). The high concentration of phenols in *I. verum* was measured in *n*-Hexane and the least in DCM. The concentrations were ranges in the order given below:

*n*-Hexane>EtOAc>Aqueous>Crude MeOH>DCM

The HPLC analysis confirmed the accumulative curve because there is no distinctive peak which indicates that the flavonoids are present. Standard used in the study were not 100% pure so more than one peak for each standard was observed The *n*-Hexane (F1) showed the lowest peak height (74.61020) and EtOAc (F3) peak height (297. 02925) was medium. The DCM (F2) showed the highest peak (96.42616) above the fractions (Table 3).

Hence, it has been reported that not only the phenolic and flavonoids may be involve in anti-emetic activity but also alkaloids encompass their part in anti-emetic activity [12, 13].

In Anti-emetic Activity, Methanolic extract (25mg/kg) showed lower, but comparable effects as that of standard anti-emetic drug. In all the four fractions, ethylacetate (F3) at 50mg/kg showed higher (94.46%) inhibition of retches as the chicks showed 3.66 mean numbers of retches whereas n-hexane (F1) showed the lowest (56.30%) inhibition of retches with 28.89 mean numbers of retches at the same dose. The dichloromethane (F2) and aqueous (F4) fractions at 25, 50 and 75 mg/kg showed comparative results with F3.The standard drug metoclopramide (150mg/kg) inhibited 59.34% retches and showed 26.88 mean numbers of retches. The mean number of retches in control was 66.11 (Table 4; Figure 1).

The present study scientifically justified the traditional medicinal use of the *Illicium verum* fruit against vomiting. Sesquiterpenes and triterpenes are reported to possess antiemetic activity through antioxidant action and 5-HT3, 5-HT4 or NK1 receptor antagonism [7]. The reportedsesquiterpenes and triterpenes contents of the fruitmay be responsible for the observed anti-emetic effect by antioxidant action and 5-HT3, 5-HT4 or NK1 receptor antagonism [6].

## Conclusion

The preliminary antiemetic evaluation of methanolic extract and its fractions from *Illicium verum* fruits was conducted. Aqueous, dichloromethane and ethylacetate fractions showed higher and significant anti-emetic effect with reference to the standard drug metoclopramide. Also the HPLC and colorimetric analysis results comparison indicates that the presence of flavonoids and phenolics are not the only active core ingredient in anti-emetic bioassay but also some other phytochemicals are essentially active. It is being corroborated from the analysis of anti-emetic bioassay. Further, isolation and purification from the same fractions is in process to establish a detail data to evaluate effective natural anti-emetic compound(s), their exact mode of action at different doses and different available anti-emetic assays.

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