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Preliminary qualitative and quantitative phytochemical analysis of fruit extract of *Cascabela thevetia* and their antioxidant property

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Abstract
The phytochemicals present in various plant materials have proven biological properties. Phytochemicals are essential secondary metabolites present in plants which possess various biological properties. Phytochemicals such as alkaloids, phenols, terpenoids, tannins, flavonoids, steroids and glycosides are responsible for the biological properties of plant extracts. In order to explore the biological properties, it is necessary to screen the phytochemicals present in the plant extracts using various qualitative and quantitative phytochemical analysis. The chloroform and ethanolic fruit extracts of *Cascabela thevetia* were subjected to qualitative and quantitative phytochemical analysis. The chloroform extract of *Cascabela thevetia* fruit found to have a greater number of phytochemicals when compared with ethanolic fruit extract. In the quantitative phytochemical analysis, the chloroform extract was found to have more flavonoids and phenols. To analyse the antioxidant property of fruit extracts, DPPH radical scavenging activity was performed and the chloroform extract showed 31.46% antioxidant activity at highest concentration whereas the ethanol extract showed 24.18% activity at the same concentration. Hence from this study, it was observed that the fruits of *Cascabela thevetia* proved to be a good antioxidant agent.

Keywords: *Cascabela thevetia*, phytochemicals, antioxidant, DPPH, chloroform, ethanol

Introduction
Cascabela thevetia is an evergreen tropical shrub, native to Mexico and Central America. They are highly poisonous and toxic as they contain cardiac glycosides. Some important toxic compounds found in this plant are cardenolides such as thevetin A, thevetin B, peruvoside, neriifolin and thevetoxin. These cardenolides are heat resistant and are very similar to digoxin from *Digitalis purpurea.* However, some bird species such as Asian koel, brahminy Myna and grey hornbill are known to feed on these plants without any ill effects.
Herbal medicines have served as the foundation for the treatment and curing of numerous illnesses in India. Additionally, several prescriptions for ailments like ulcers, microbial infections, inflammation, and wound healing are included in Indian traditional medicine (Mukherjee *et al*., 2000) [1]. Certain components of secondary metabolites found in medicinal plants provide them pharmaceutical significance (Nair *et al*., 2014). Numerous attempts have been made to find novel antimicrobial chemicals from a variety of sources, including plants, animals, and microbes. The majority of herbal medicines are derived from the world's plant biodiversity, and between 60 and 80 percent of people still utilize plant-based remedies, which have been a part of traditional healthcare systems for antiquity (Santhi *et al*., 2012). Due to the presence of different phytochemicals such flavonoids, phenols, tannins, carotenoids, terpenoids, etc., it has been demonstrated that a number of medicinal plants have potential pharmacological activity (Zarger *et al*., 2015) [2].
Free radicals are the by-products of normal body metabolism like breathing. These free radicals are unstable and are harmful to body's internal balance (Homeostasis). Free radicals are also produced when the body is exposed to chemical compounds and other pollutants. Free radical scavengers are those compounds that neutralize free radicals, and they are called as Antioxidants. Antioxidants are agents which scavenge free radicals and prevent damage caused by reactive oxygen species (ROS) (Thakur *et al.*, 2012)

Collection & Processing of sample
The fruits of *Cascabela thevetia* were identified and collected from Tiruvannamalai district, Tamilnadu during the month of November 2019. The fruits were sliced and shade dried. The shade dried fruits were coarsely powdered using electrical blender and stored in an airtight container for further analysis.

Preparation of fruit extract
The powdered fruit sample was soaked in ethanol and chloroform for 48 hrs. These extracts were extracted using a rotary vacuum evaporator and stored.

Qualitative phytochemical analysis
The preliminary phytochemical analysis was carried out for both ethanol and chloroform extracts of *Cascabela thevetia* using the standard methods. Test for carbohydrates, glycosides, proteins & amino acids, phenols, flavonoids, terpenoids, steroids, saponins, tannins and alkaloids were carried out.

Quantitative phytochemical analysis
Estimation of flavonoids
Estimation of flavonoids was carried out using aluminium chloride method. 1ml of EECT and CHECT were taken in the test tubes to which 0.3ml of sodium nitrate solution was added. Then 0.3ml of aluminium chloride solution was

added and left for 5mins. 2ml of 1M Sodium hydroxide and 1ml of distilled water was added. The absorbance was measured at 510nm using a UV spectrometer.

Estimation of phenols

EECT and CHECT (100µl each) were taken in different test tubes and 250µl of Folin-Ciocalteu reagent (FCR) was added to each test tube. To the above mixture, 500µl of sodium bicarbonate solution and 5ml of distilled water was added. The mixture was then incubated for 30 mins at room temperature and the absorbance was then taken at 765nm using an UV spectrometer.

Antioxidant activity

The free radical scavenging activity of Chloroform and Ethanol extracts were determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. 1ml of DPPH was added to Chloroform and Ethanol extracts of different concentrations (2,4,8,16,20 µl/ml). They were incubated for 30-45 mins at room temperature. The absorbance was measured at 515 nm using UV spectrometer (Ahmad *et al.*, 2013). % free radical scavenging of extracts was measured by using the following formula:

$$\% \text{ Scavenging Activity} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

Results

Extraction of fruit material

The phytoconstituents in the fruit of *Cascabela thevetia* was extracted using chloroform and ethanol as the solvents.

Qualitative phytochemical analysis

The preliminary phytochemical analysis of chloroform and ethanol extracts of *Cascabela thevetia* were done and the results are summarised in (Table-1). The chloroform extract contained phenols, saponins, flavonoids, terpenoids and steroids, whereas the ethanol extract contained only phenols (Fig.1).

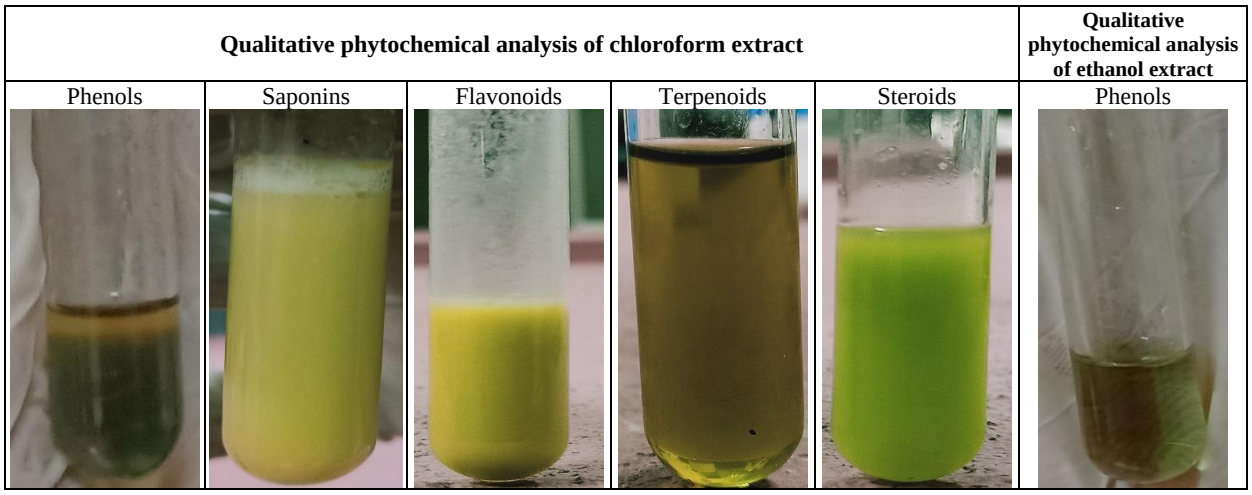

Fig 1: Preliminary qualitative phytochemical analysis of chloroform and ethanol extracts of *Cascabela thevetia*

Quantitative phytochemical analysis

The amount of phenols and flavonoids present in the chloroform and ethanol extracts of *Cascabela thevetia* fruits were determined by quantitative phytochemical analysis, which are shown in the (Fig 2). The chloroform extract contained more phenol content of 36.1% as well as more flavonoid content of 18.7% when compared to the ethanol extract which contained a less percentage of flavonoids and phenols.

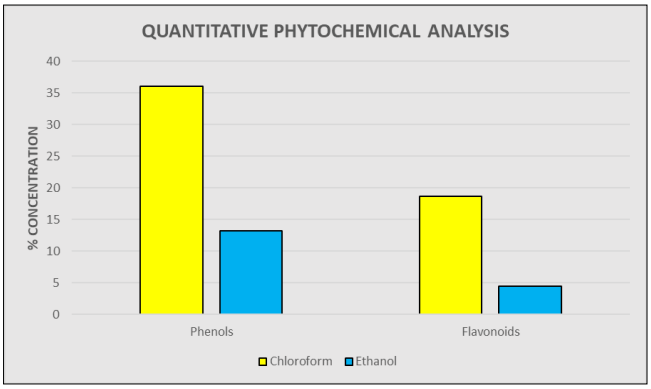

Fig 2: Quantitative phytochemical analysis of *Cascabela thevetia*

Antioxidant activity

The antioxidant activity of *Cascabela thevetia* was determined by using DPPH assay. The antioxidant activity of Chloroform and Ethanol extracts were determined at different concentrations from 2µl to 20µl. The chloroform extract exhibited more free radical scavenging activity than ethanol extract. The chloroform extract showed 31.46% antioxidant activity at highest concentration whereas the ethanol extract showed 24.18% activity at the same concentration (Fig 3).

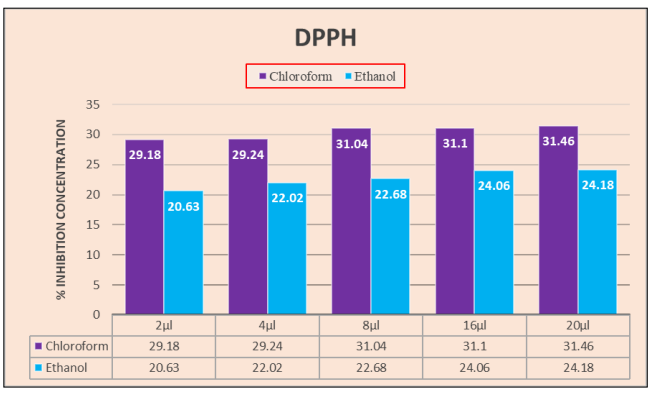

Fig 3: Antioxidant activity of fruit extracts of *Cascabela thevetia*

Table 1: Qualitative phytochemical analysis of chloroform and ethanol extracts of *Cascabela thevetia*

Test for various secondary metabolites	Ethanol extract	Chloroform extract
Test for alkaloids (Mayer's test)	_	_
Test for carbohydrates (Fehling's test)	_	_
Test for glycosides (Borntrager's test)	_	_
Test for proteins and amino acids (Ninhydrin test)	–	–
Test for phenols (Ferric chloride test)	+	+
Test for flavonoids (Ammonia test)	_	+
Test for terpenoids (Salkowski test)	_	+
Test for saponins (Foam test)	_	+
Test for steroids (Liebermann-Burchardt test)	–	+
Test for tannins (Braemer's test)	_	_

'+' indicates presence
'-' indicates absence

Discussion

The fruit extracts of *Cascabela thevetia* were prepared by using chloroform and ethanol as the solvents. The preliminary phytochemical analysis showed that the chloroform extract contained phenols, saponins, flavonoids, terpenoids and steroids, whereas the ethanol extract contained only phenols. Anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammatory, cardiovascular protection, and enhanced endothelial functions are only a few of their many biological qualities. Flavonoids, phenolic acids, tocopherols, and other phenolic compounds are the primary source of natural antioxidants from plants (Ali *et al*., 2008). A class of phenolic chemicals found in large quantities in the kingdom of plants are called flavonoids. They have the ability to scavenge free radicals and are strong antioxidants. According to Foltsis *et al*. (1997), several flavonoids, including luteolin, fisetin, and apigenin, are strong inhibitors of cell proliferation. As a result, *Cascabela thevetia*'s bioactive components have pharmacological significance in the treatment of a number of illnesses.

The presence of high amount of phenols and flavonoids in the fruit extracts are responsible for number of pharmacological activities. The amount of phenols was determined with the Folin-Ciocalteu reagent. The maximum phenolic content was found in the chloroform extract of *Cascabela thevetia*. Phenols are known to exhibit anti-inflammatory, antioxidant, anticancer, antimicrobial, antifungal and insecticidal activities. The total flavonoid content was estimated by aluminium chloride calorimetric method. This method relies on the spectrophotometric detection of coloured complexes formed between Al (III) and the carbonyl and hydroxyl groups of flavonoids in alkaline medium. Flavonoids are the most widely distributed polyphenolic compounds that is known to provide defence against the infection and are necessary for normal growth and development. Due to the presence of high amount of phenols and flavonoids, *Cascabela thevetia* can be considered as a good medicinal source in treating and curing human health problems.

A straightforward and accurate technique for assessing free radical scavenging effect is the DPPH (1,1-diphenyl-2-picryl hydrazyl) assay, which is based on the capacity of DPPH, a stable free radical, to decolorize in the presence of antioxidants. The absorbance at 515–517 nm and the apparent deep purple color are caused by an unusual electron in the DPPH radical. The charges in absorbance can be used to quantitatively assess the decolorization of DPPH that occurs when it receives an electron provided by an antioxidant molecule (Raqulbul *et al*., 2009). Phenolic substances, including flavonoids, polyphenols, tannins, and phenolic terpenes, have been found to have a major role in the antioxidant activity of plant products by scavenging radicals (Rahman and Moon, 2007) [8].

Phenolic compounds' redox characteristics, which can be crucial in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or breaking down peroxides, are what give them their antioxidant activity (Hasan *et al*., 2008) [9]. Both ethanol and chloroform extracts were used in the DPPH assay in this investigation. Compared to the ethanol extract, the chloroform extract was found to have more free radical scavenging activity. It was clear from this study that *Cascabela thevetia* fruits are a good source of antioxidants.

Conclusion

Plants have been used for medicinal purposes since time immemorial. This is due to the presence of various phytoconstituents which possess different pharmacological activities. Therefore, plants which possess these valuable phytoconstituents has been used to treat various diseases. The current study was carried out with the fruit extracts of *Cascabela thevetia* which were extracted using chloroform and ethanol as the solvents. The preliminary qualitative and quantitative phytochemical analysis were carried out and from the results obtained, it was observed that the chloroform extract showed a greater number of phytochemicals than the ethanol extract. Different phytochemicals such as phenols, saponins, flavonoids, terpenoids and sterols were found in chloroform extract, whereas the ethanol extract contained only phenols. In the quantitative analysis, the chloroform extract was found to contain more amount of phenols and flavonoids. DPPH assay was carried out to observe the free radical scavenging activity and the chloroform extract (31.46%) showed more antioxidant activity than the ethanol extract (24.18%). Hence from this study, it was observed that the fruits of *Cascabela thevetia* proved to be a good antioxidant agent.

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