INTRODUCTION

Nature is the best source of medicinal constituents. From the vast natural resources, the plants are being used for therapeutic purposes from the beginning of the civilization (Kirtikar and Basu; 1980). Medicinal plants are bioactive constituents which form one of the major resources of raw material for drugs in preventive and curative constituents from one of the major sources of raw material for drugs in preventive and curative applications (Baliga et al; 2003). Plant derived medicine has made largest contribution to human health and well-being all over the world. The basic medicinal property of these plants lies in some chemical substances. These chemical substances produce a definite physiological action on human body which is generally known as phytochemical. These chemicals are non nutritive and act like shield against diseased. The most important of these phytochemical are alkaloids, flavonoids, tannins and phenolic compounds (Hill A. F.; 1952). Around 1900, 80% of the drug was derived from plants (Adetunji et al; 2011). A knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also because such information may be of great value in disclosing new sources of economic phyto-compounds for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies (Fouche et al; 2001). The traditional medicine all over the world is now a day’s revalued by an extensive activity of research on different plant species and their therapeutic principles.

After decades of serious obsession with the modern medicinal system, people have started looking at the ancient healing systems like Ayurveda, Siddha and Unnani. This is because of the adverse effects associated with synthetic drugs and also the increasing cost, non
availability of modern drugs, and limited access to adequate health; these reasons have compelled about 80% world population to use traditional pharmacopeia for primary health care especially in the tropical and sub tropical regions. The last few years have seen a revival of interest in the use of herbal medicine in the developed world. Herbal medicines are in great demand in both developed and developing countries as a source of primary health care owing to their attributes having wide biological and medicinal activities, high safety margins and lesser costs. Among the developed countries, Germany holds the lead and has published individual monographs on therapeutic benefits of more than 300 herbs. In developing countries, China has compiled / generated data on over 800 medicinal plants and exports large amounts of herbal drugs. India has prepared only a few monographs and its exports are dismal.

The World Health Organization (WHO) has defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often over hundreds of years, before the development and spread of modern medicine and are still in use today (WHO, 1991). About 25% of all modern medicines prescribed worldwide are directly or indirectly derived from higher plants (WHO, 2005).

India is one of the world’s twelve leading biodiversity centers with the presence of over 45,000 different plant species. Out of these about 1,500-2,000 plants have good medicinal properties of which only about 750-800 are being used by traditional practitioners. The Siddha system of medicine uses around 600, Ayurveda 700, Unani 700 and modern medicine about 30 plant species (Mukharjee, 2002). Though our ancient literatures provide a good account of description on plants along with different formulations of drugs, symptoms and diagnosis of diseases, methodology followed in preparation of medicines as well as mode of application. Ancient Indian literature incorporates a remarkably broad definition of medicinal plants and considers all plant parts to be potential sources of medicinal substances (Khare, 2007). However a key obstacle, which has hindered the acceptance of the alternative medicines in the developed countries, is the lack of documentation and stringent quality control. The system of classification, adopted in these literatures creates some confusion in nomenclature of medicinal plants. Sometimes, the description of a plant, given in these ancient medical literatures shows affinity with altogether unrelated two or three plant species belonging to different families. This has led to a great difficulty in identification of appropriate samples of medicinal plants, prescribed for treatment of specific disease. Correct
identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy. Hence, there is a need for documentation of research work carried out on traditional medicines (Dahanukar, 2000).

With this backdrop, it becomes extremely important to make an effort towards standardization of the plant material to be used as medicine. The process of standardization can be achieved by stepwise pharmacognostic studies (Trease and Evans, 2002). Pharmacognosy and phytochemistry are important tools for the study of crude drug obtained from natural sources treated scientifically. These tools used in standardization of plant material include its morphological, anatomical and biochemical characteristics (Anonymous, 1989). These studies help in identification and authentication of the plant material.

A knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also because such information may be of great value in disclosing new sources of economic phytocompounds for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies.

*Canthium* is a genus of about 230 species of shrubs or small trees. The *Canthium parviflorum* Lam. (syn: *Plectoria parviflora*) of Rubiaceae. *Canthium parviflorum* Lamk is a shrubby and woody plant found throughout the Western Ghats. It also occurs in peninsular India, coramandel coast, dry plains. Plant pacifies vitiated kapha, diarrhea, fever, leucorrhea, worm infestation and general debility. In siddha system of medicine the plant was used in respiratory disorder, diuretic, diabetic, obesity. In Ayurvedha system of medicine the plant was used in cough, diuretic, tumor and as anthelmintic (Anonymous, 1991).

**MATERIALS AND METHODS**

The present study deals with Pharmacognostic studies on *Canthium parviflorum* and was conducted along with the standardized methods for quality control and assurance to provide a base line to commercialize its constituents for herbal products.

**3.1 Collection and identification of plant material**

The selected plant for the study i.e., *C. parviflorum* was collected during the period of flowering and fruiting from Amravati region during September 2013. The herbarium specimens of selected plant was prepared, identified with the help of standard floras (Cooke,
1967; Kamble and Pradhan, 1988; Naik, 1998; Almeida, 2001; Singh and Karthikeyan, 2001) and authenticated by Dr. S.P. Rothe, Professor and Head, Department of Botany, Shri Shivaji college of Arts, Science and Commerce, Akola, Maharashtra. The voucher specimens were deposited in the herbarium of Department of Botany, Vidya Bharti Mahavidyalaya, Amravati (Maharashtra) India.

3.2 Ethno-medicinal uses
Meanwhile the medicine men, vaidoo’s and people from tribal communities of Melghat forest region were interviewed to investigate the ethnomedicinal importance of plant under study.

3.3 Organoleptic evaluation
Selected plant was collected washed 2-3 times with distilled water and separated the plant part i.e. leaves, stem and root and dried under shade. These dried plant parts materials were grinding into a powdered and packed in polythene bags until further experimentation. Organoleptic evaluation of the drug refers to the evaluation of drug by colour, order, taste, and special features including texture. It is helpful for collecting the basic information on the identity, purity and quality of material can be drown from these observations. They are of primary importance before any further testing can be carried out. Organoleptic evaluations can be done by mean of organ of sense which include the above parameter and there by define some specific characteristics of the material which can be consider as a first step towards the establishment of identity of degree of purity (Kokate et al., 2005).

**Colour**

The colour is of use in indicating the general origin of the drug i.e. material derive from aerial plant part is usually green and underground part material is devoid of green colour for proper examination the untreated sample are examined under diffused sun light.

**Odour and Taste**

To an expert odour and test of crude material are extremely sensitive criteria based on individual perception the strength of odour like weak, distinct, strong, aromatic, and fruity.

**Surface characteristics**

Texture is best examined by taking a small quantity of material and rubbing it between thumb and fore finger. It is usually rough, smooth, and granular. All this characteristics are valuable in indicating the general type of material and presence of more than one component.
3.4 Anatomical study
The collected fresh material of selected plants were washed 2-3 times with sterile distilled water and preserved in 3% formalin solution then used for the anatomical investigation. The transverse sections of leaves, petiole and stem were taken with the help of fine laboratory razor and observed under microscope to note details. The section proceed for double staining successively through the various solvent grade system by using 1% safranine for 5 min, 30%, 50%, and 70% alcohol grades respectively for 5 min. to remove excessive stain and proper hydrolyze the section then section were put into 0.5 % light green for 2 min. the again put into 70%, 90% and absolute alcohol for 5 min. to remove excessive stain and later passing through Xylene:Alcohol (1:3), Xylene: alcohol (1:1) and pure Xylene to hardening and clearing of section also remove air bubble. Then the sections were mountain in DPX and cover slip was put over the section and later photographed were obtained by processing the image captured using Carl Zeiss standard Universal microscope (Oberko-Chen/Wartenberg, Germany).

3.5 Powder microscopy
Powder microscopy shows the characters which play a major role in drug identification. The plant drug contain some basic cell type i.e. Parenchyma, collenchyma, sclerenchyma, epidermis and vascular component like xylem and phloem etc. along with special characteristics i.e. presence of starch, calcium oxalate, calcium carbonate, silica and different other cell contains. Analysis of the plant drug based on the distribution of these various cell types within different organ is important to ensure the identity and quality of herbal drugs. Powder study enables to give a picture of all tissue distribution in many plants. A little quantity of powder was taken onto a microscopic slide. 1-2 drops of 0.1% fluoroglucinol solution and a drop of conc. HCl were added, mounted it in glycerol, covered with a cover slip and observed under the microscope with 10x10 magnification and characteristic features of plant powder were recorded. Powder microscopy was carried out by using the method mentioned in Ayurvedic Pharmacopeia of India (1966).

3.6. Extractive Values
The procedures recommended in Ayurvedic Pharmacopeia of India (1966) were followed for calculating extractive values. Extractive values of crude drug are useful for their evaluation especially when the constituents of a crude drug cannot be readily estimated by any other mean, further these value indicate the nature of constituent present in crude drug. The
percentage of extractive values were calculated using different solvent i.e. petroleum ether, benzene, chloroform, acetone, ethanol and water. Each parts of the plant, of 5 g air dried drug coarsely powdered were macerated with 100 ml of respective solvent in a closed flask for 24 hours; it shacked frequently during 6 hours and allowed to stand for 18 hours. Then it was filter rapidly with taking precautions against loss of solvent, evaporated 25 ml of the filtrate to dryness in a tarred flat bottomed shallow dish, and dried and was weighed. The percentage of extractive values was calculated.

3.7 Chemical behavioral analysis: This analysis was carried out by using the standard method mentioned in Ayurvedic Pharmacopeia of India (1966). Behavior of powdered plant materials with different chemical reagents i.e. conc. H₂SO₄, HNO₃, HCl, 10% NaOH, Iodine Solution, Ferric chloride, Potassium iodide, 1N H₂SO₄, HNO₃, HCl was observed under day light.

3.8 Phytochemical analysis
3.8.1 Qualitative phytochemical analysis
It involves testing of different classes of compounds. The methods used for detection of various phytochemicals were followed by qualitative chemical test to give general idea regarding the nature of constituents present in crude drug (Kokate, 2005; Harborne, 1998; Sadashivan and Manickam, 2005). The leaf, stem extracts of Canthium parviflorum were analyzed for the presence of phytoconstituents like carbohydrates, cardiac glycosides, alkaloids, flavonoids, tannin, phenolics, steroids and saponin.

Tests for carbohydrates
i) Fehling’s Test: 1 ml Fehling’s A solution and 1 ml of Fehling’s B solution were mixed and boiled for one minute. Now the equal volume of test solution was added to the above mixture. The solution was heated in boiling water bath for 5-10 minutes. First a yellow, then brick red precipitate was observed.
ii) Benedict’s test: Equal volumes of Benedict’s reagent and test solution were mixed in a test tube. The mixture was heated in boiling water bath for 5 minutes. Solution appeared green showing the presence of reducing sugar.
iii) Molisch’s test: Equal volumes of Molisch’s reagent and test solution were mixed in a test tube. The mixture was heated in boiling water bath for 5 minutes. Appearance of violet or purple colour ring showing the presence of reducing sugar.

Tests for proteins
i) **Biurret Test:** To the small quantity of extract 1-2 drops of Biurret reagent was added. Formation of violet colour precipitate showed presence of proteins.

ii) **Million’s Test:** To the small quantity of extract 1-2 drops of Million’s reagent was added. Formation of white colour precipitate showed presence of proteins.

**Tests for Anthraquinone glycosides**

**Borntrager’s Test:** To the 3ml of extract, dil. $H_2SO_4$ was added. The solution was then boiled and filtered. The filtrate was cooled and to it equal volume of benzene was added. The solution was shaken well and the organic layer was separated. Equal volume of dilute ammonia solution was added to the organic layer. The ammonia layer turned pink showing the presence of glycosides.

**Tests for Cardiac glycosides**

**Keller-Killiani Test:** To the 5ml of extract, 1ml of conc. $H_2SO_4$, 2ml of Glacial acetic acid and 1 drop of FeCl$_3$ solution was added. Appearance of Brown ring shows the presence of cardiac glycosides.

**Test for steroids**

**Salkowski Test:** To 2 ml of extract, 2 ml of chloroform and 2 ml of conc. $H_2SO_4$ was added. The solution was shaken well. As a result chloroform layer turned red and acid layer showed greenish yellow fluorescence.

**Tests for alkaloids**

i) **Hager’s Test:** To the 2-3 ml of filtrate, few drops of dil. HCl and Hager’s reagent was added and shake well. Yellow precipitate was formed showing the presence of alkaloids.

ii) **Mayer’s Test:** To the 2-3 ml of filtrate, few drops of dil. HCl and Mayer’s reagent was added and shake well. Formation of yellow precipitate showed the presence of alkaloids.

iii) **Dragendorff’s Test:** To the 2-3 ml of filtrate, few drops of dil. HCl and Dragendorff’s reagent was added and shake well. Formation of orange-brown precipitate showed the presence of alkaloids.

**Tests for flavonoids**

**Lead Acetate Test:** To the small quantity of extract lead acetate solution was added. Formation of yellow precipitate showed the presence of flavonoids.

**Tests for Tannins and Phenolics compound**
i) **FeCl₃ Solution Test:** On addition of 5% FeCl₃ solution to the extract, deep blue black colour appeared.

ii) **Lead Acetate Test:** On addition of lead acetate solution to the extract white precipitate appeared.

**Test for Saponin**

**Foam Test:** To 1ml extract 20ml distilled water was added and shakes well in measuring cylinder for 15 min. Then 1cm layer of foam was formed.

**3.8.2 Crude quantification of the major phytoconstituent**

The crude quantifications of major phytochemicals were done using precipitation method. Each sample was analyzed in triplicates. Only alkaloids, flavonoids and saponin from the different parts of the plant under study were quantified.

1) **Alkaloid**

5 gm of sample was weighed in 250 ml beaker and 200 ml 20% acetic acid in ethanol was added and covered to stand for about 4 hrs. This was filtered and extract was concentrated using water bath to 1/4<sup>th</sup> of original volume. Concentrated Ammonium hydroxide was added drop wise to the extract till its complete precipitation. The whole solution was allowed to settle and precipitate was collected and weighed.

2) **Flavonoids**

10 gm of sample was extracted repeatedly in 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman paper no. 42. The filtrate then transferred to a crucible and evaporated to dryness over a water bath and weighed.

3) **Saponin**

10 gm of plant powder was taken in 200 ml 20% ethanol to make a suspension. This was heated for about 4 hrs over hot water bath (55°C) continuous stirring. The mixture was filtered and the residue was re-extracted with 200 ml 20% ethanol. The combined extract was reduced to 1/10<sup>th</sup> of the original volume. The concentrate was taken into 250 ml separating funnel, to this added 20 ml diethyl ether and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. This purification process was repeated for 2-3 times. Then 60 ml n-butanol was added to it. The combined solution was then washed twice with 10 ml 5% aqueous sodium hydroxide. The remnant was heated in a water bath for
complete evaporation and dried. This dried content was calculated as Saponin percentage in a sample.

3.9 Chromatographic analysis
The chromatographical study was carried out by using the standard procedure described by Harborne, (1998); Mukharjee, (2002); Sadashivan & Manickam, (2005).

Thin Layer Chromatography (TLC)
Thin layer chromatography (TLC) is an important analytical tool in the separation, identification and estimation of different class of natural product. Thin layer chromatography is performed on an aluminum foil, 60 F254 which is coated with a thin layer of adsorbent material, usually silica gel. After the sample has been applied on the plate, a solvent or solvent mixture is drawn up the plate via capillary action. Because different analyses ascend the TLC plate at different rates, separation is achieved.

The leaf, stem and root methanol extracts of the *Canthium parviflorum* were subjected to thin layer chromatographic analysis to find out the presence of number of chemical constituents. Only alkaloid and flavonoids TLC fingerprints were carried out. The details of procedure are as following.

The Methanolic extracts were applied as a single spot in a row centre of chromo plate, about 2 cm from the edge, by using capillary tubes. The TLC plate containing the sample spot was placed at 45° angles in the development chamber covering the bottom of the plate by the solvent up to nearly 1 cm. The solvent front was marked and the plate was finally allowed to dry. The colored substances were visual on the chromatogram. Colourless components were detected by using visualizing agent, iodine vapors. The qualitative evaluation of the plate was done by determining the migrating behavior of the separated substances given in the form of \( R_f \) value.

Resolution factor \((R_f) = \frac{\text{Distance traveled by the solute from the origin}}{\text{Distance traveled by the solvent from the origin}}\)

**OBSERVATION AND RESULTS**
The present study deals with the pharmacognostic studies on *Canthium parviflorum*. Standardization and quality control of plant, is of growing concern over ensuring purity of raw material before processing. Yet alternative medicines based on plant substances are
extremely popular, even though their safety and efficacy have not been scientifically proven. Now-a-day’s routine pharmacognosy has changed demanding interdisciplinary research. Various pharmacognostic standards like botanical description, microscopy, extractive values, microscopic characteristics of powder, preliminary and quantitative phytochemical study, TLC analysis of bioactive compounds of the plant could be useful for the compilation of a suitable monograph for its proper identification.

**Classification**

- Division: Phenerogams
- Class: Gamopetalae
- Order: Gentianales
- Family: Rubiaceae
- Genus: *Canthium*
- Species: *parviflorum*

**4.1 Morphology**

The Rubiaceae are trees, shrubs or infrequently herbs compirsing about 450 genera and 6500 species, including some lianous forms. The leaves are simple and usually entire, and are opposite or sometimes whorled; stipules are present and interpetiolate. The flowers are nearly always bisexual and actinomorphic, often heterostylyous, and usually are in cymose inflorescences. The calyx is somewhat reduced and 4-5 lobes or sometimes the lobes are absolute or rarely one of them greatly expanded and brightly colour. The sympetalous corolla is mostly 4-5 lobed, occasionally with 3 or upto 10 lobes. The androecium consists of as many stamens as corolla lobes and is adnate to the corolla tube or epigynous zone, alternate with the lobes. The gynoecium consists of a single compound pistil of 2 or seldom more carpels, a single style, and a nearly always inferior ovary with the number of locules.

**4.2 Ethno-medicinal uses**

*Canthium parviflorum* (Rubiaceae), a medicinal plant, has been widely used in Ayurvedha in conditions of *kapha*, diarrhea, strangury, fever, leucorrhoea, intestinal worms, and general debility. This plant has been traditionally known to treat snakebite in some villages of Shimoga district in Karnataka, India and to possess wound-healing property. The present study focused on determining the antioxidant ability of solvent extracts of *C. parviflorum*. The roots of this plant are traditionally used by the tribes of Orissa in treatment of swelling of neck and fruits in headache. This plant is reported for its pharmacological uses as an
astringent, anthelmintic, antidysentric, antispasmodic and as a diuretic. From the ethno medical survey we came to know that many people from Vellore district are using the plant and its various parts traditionally practicing widely throughout those areas for various infections. Hence the whole plant was utilized for our present evaluation to study about the presence of various phytoconstituent and its concomitant activity.

Plant pacifies vitiated kapha, diarrhea, fever, leucorrhea, worm infestation and general debility. In siddha system of medicine the plant was used in respiratory disorder, diuretic, diabetic, obesity. In Ayurvedha system of medicine the plant was used in cough, diuretic, tumor and as anthelmintic. An antioxidant, wound healing activity and antitumor acitivity were reported. D-mannitol, phenolic acid, phenolic compounds, carbohydrates, proteins were found from *Canthium parviflorum*. (Sathish kumar et al 2008).

Pharmacological activities such as antimicrobial, antioxidant, antidiabetic, wound healing, diuretic, anti-inflammatory, antinociceptive, antitumor and antipyretic from various species of *Canthium* has been reported. (Elayaraja et al 2007).

### 4.3: Table: Organoleptic evaluation of powder of *Canthium parviflorum*

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Particulars</th>
<th>Plant parts</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Leaves</td>
<td>Stem</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Colour of Powder</td>
<td>Green</td>
<td>Creamish</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Odour</td>
<td>Mild</td>
<td>Mild</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Taste</td>
<td>Bitter</td>
<td>Tasteless</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Texture</td>
<td>Smooth</td>
<td>Rough</td>
<td></td>
</tr>
</tbody>
</table>

### 4.4 Anatomical study of *Canthium parviflorum*

#### 4.4.1 T.S of leaf

The T.S. of leaf passing through the mid rib projects strongly at lower side and elevated at upper side and lamina is dorsiventral. The leaf has mid rib and lateral veins. The epidermis is thin epidermis is lies a layer of palisade cells. There is presence of parenchymatous cell with vascular bundles. Canals are present within it and oil droplets are also detected within it.

#### 4.4.2 T.S of Stem

The T.S. of stem shows cuticle followed by the epidermis. There is presence of vascular bundles after that there is a parenchymatous cells showed. In the cortical bundles absent, medullary bundles are absent. The anomalous secondary thickening when present, via concentric cambia. Primary medullary rays narrow.
The vessels are small, typically numerous, solitary or radially paired. The vessels end walls exclusively simple in mature wood. The fibres without spiral thickening. The secondary phloem not stratified. The wood not storied.

4.5 Powder microscopy

Table: Powder study of *C. parviflorum*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Plant part</th>
<th>Observe in powder study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Leaf</td>
<td>Starch grain, epidermal cell, calcium oxalate crystals, cortical cells, parenchymatous cells.</td>
</tr>
<tr>
<td>2</td>
<td>Stem</td>
<td>Starch grain, Cork cell, collenchymatous cells, sclerenchymatous cells, pitted vessels, tracheid, and fibres.</td>
</tr>
</tbody>
</table>

4.6 Analytical study of *C. parviflorum.*

4.6.1 Extractive values

Table: Extractive values of *C. parviflorum.*

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Parameter studied</th>
<th>Leaf (% w/w)</th>
<th>Stem (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Petroleum ether</td>
<td>2.6 %</td>
<td>2.9 %</td>
</tr>
<tr>
<td>2</td>
<td>Benzene</td>
<td>7.1 %</td>
<td>8.0 %</td>
</tr>
<tr>
<td>3</td>
<td>Chloroform</td>
<td>6.9 %</td>
<td>4.9 %</td>
</tr>
<tr>
<td>4</td>
<td>Acetone</td>
<td>8.12 %</td>
<td>13.15 %</td>
</tr>
<tr>
<td>5</td>
<td>Ethanol</td>
<td>16.23 %</td>
<td>15.88 %</td>
</tr>
<tr>
<td>6</td>
<td>Water</td>
<td>13.22 %</td>
<td>17.40 %</td>
</tr>
</tbody>
</table>

4.7 Chemical behavioral analysis

Table: Behavioral characteristics of powder of *C. parviflorum* with different chemical reagent under visible light.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Powder + Reagent used</th>
<th>Stem</th>
<th>Leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Powder as such</td>
<td>Creamish</td>
<td>Green</td>
</tr>
<tr>
<td>2</td>
<td>Powder + Conc. H₂SO₄</td>
<td>Dark Green</td>
<td>Pale Green</td>
</tr>
<tr>
<td>3</td>
<td>Powder + Conc. HNO₃</td>
<td>Orange</td>
<td>Light Brown</td>
</tr>
<tr>
<td>4</td>
<td>Powder + Conc. HCl</td>
<td>Green</td>
<td>Light Green</td>
</tr>
<tr>
<td>5</td>
<td>Powder + 10% NaOH</td>
<td>Light Brown</td>
<td>Light Green</td>
</tr>
<tr>
<td>6</td>
<td>Powder + Iodine solution</td>
<td>Brown</td>
<td>Light Green</td>
</tr>
<tr>
<td>7</td>
<td>Powder + 5% Ferric Chloride</td>
<td>Brown</td>
<td>Light Green</td>
</tr>
<tr>
<td>8</td>
<td>Powder + KI</td>
<td>Brown</td>
<td>Dark Green</td>
</tr>
<tr>
<td>9</td>
<td>Powder+ Ethyl acetate</td>
<td>Pale Yellow</td>
<td>Light Green</td>
</tr>
</tbody>
</table>

4.8 Phytochemical analysis
4.8.1 Qualitative phytochemical screening

Table: Qualitative Phytochemical screening of Leaf of *Canthium parviflorum*

<table>
<thead>
<tr>
<th>S. N.</th>
<th>Constituents</th>
<th>Mayer’s Test</th>
<th>Dragendroff’s Test</th>
<th>FeCl₃ Sol. Test</th>
<th>Lead Acetate Test</th>
<th>Biuret Test</th>
<th>Million’s Test</th>
<th>Borntrager’s Test</th>
<th>Keller-Killiani Test</th>
<th>Lead Acetate Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>--</td>
<td>--</td>
<td>--</td>
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<td>--</td>
<td>--</td>
<td>--</td>
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<td>--</td>
</tr>
<tr>
<td>2</td>
<td>Carbohydrates &amp; Glycosides</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td></td>
<td>--</td>
<td>--</td>
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<td>--</td>
</tr>
<tr>
<td>3</td>
<td>Steroids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
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</tr>
<tr>
<td>4</td>
<td>Saponin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>--</td>
<td>--</td>
<td>--</td>
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</tr>
<tr>
<td>5</td>
<td>Phenolics &amp; Tannin</td>
<td>--</td>
<td>--</td>
<td>--</td>
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<td></td>
<td>--</td>
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<tr>
<td>6</td>
<td>Proteins</td>
<td></td>
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<td>--</td>
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</tr>
<tr>
<td>7</td>
<td>Anthraquinone glycosides</td>
<td>--</td>
<td>--</td>
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<td></td>
<td></td>
<td>--</td>
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</tr>
<tr>
<td>8</td>
<td>Cardiac glycosides</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>9</td>
<td>Flavonoids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>--</td>
<td>--</td>
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</tr>
</tbody>
</table>

(Where, P.E.= Petroleum ether, B= Benzene, C= Chloroform, A= Acetone, E= Ethanol and W= Water.)

4.8.2 Quantitative phytochemical analysis
Table: Quantitative phytochemical screening of *Canthium parviflorum*

<table>
<thead>
<tr>
<th>S. no</th>
<th>Phytochemical</th>
<th>Leaf (g/100g)</th>
<th>Stem (g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>85.7± 0.10</td>
<td>75.7 ± 0.24</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>80.45 ± 0.12</td>
<td>88.05 ± 0.02</td>
</tr>
<tr>
<td>3</td>
<td>Saponin</td>
<td>41.35 ± 0.04</td>
<td>15.98 ± 0.06</td>
</tr>
</tbody>
</table>

Whereas, results are depicted as mean ± SD of three determinants.

![Figure 1: Quantitative phytochemical Screening of *Canthium parviflorum*](image)

4.9 Chromatographic analysis

TLC Profile

The TLC of methanolic extract of samples were carried out on silica gel 60F254 plate.

**Table: TLC profile of methanolic extract of *C. parviflorum*.**

<table>
<thead>
<tr>
<th>S.no</th>
<th>Plant parts</th>
<th>Chemical constituents</th>
<th>Solvent system</th>
<th>Rf Values</th>
<th>Total Bands</th>
<th>Spray reagents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Leaf</td>
<td>Alkaloids</td>
<td>Toluene : Acetone : Ethanol : Ammonia Solution (40:40:6:2)</td>
<td>0.30,0.28,0.26</td>
<td>3</td>
<td>Dragendroff’s</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flavonoids</td>
<td>Chloroform : Ethyl acetate (60:40)</td>
<td>0.35,0.3,0.29</td>
<td>3</td>
<td>5% FeCl₃</td>
</tr>
<tr>
<td>2</td>
<td>Stem</td>
<td>Alkaloids</td>
<td>Toluene : Acetone : Ethanol : Ammonia Solution (40:40:6:2)</td>
<td>0.3,0.29</td>
<td>2</td>
<td>Dragendroff’s</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flavonoids</td>
<td>Chloroform : Ethyl acetate (60:40)</td>
<td>0.37</td>
<td>1</td>
<td>5% FeCl₃</td>
</tr>
</tbody>
</table>

PHOTO PLATES
DISCUSSION

Leaf alkaloids  Leaf flavonoids  Stem alkaloids  Stem flavonoids

Cork Cells (Stem)  Fibres (Stem)  Starch Grains (Leaf)

Starch Grains (Stem)  T.S. of Stem  T.S. of Leaf
The commonly found phytochemicals in plants are alkaloids, flavonoids, tannins and phenols, steroids and terpenoids, saponins, carbohydrates, glycosides, proteins and amino acids. Therefore the present study involves a preliminary screening of the phytochemicals in leaf and stem extracts of *Canthium parviflorum*. Although in traditional medicine, water is used as solvent for plant extraction but present studies have shown that organic solvent extracts show greater biological activity than the aqueous extract. Hence, five solvents were used, i.e., petroleum ether, benzene, chloroform, acetone, ethanol and also water as the sixth solvent. The extracts of both the plant parts were used for the analysis to identify the best solvent for phytochemical extraction. The phytochemical analysis was done in three phases, viz; qualitative, quantitative, chromatographic techniques.

The phytochemical screening of leaves and stem of *Canthium parviflorum* showed, primary and secondary metabolites like Carbohydrates, Proteins, Anthraquinone glycosides, cardiac glycosides, Coumarins, Quinone, Steroids, Alkaloids, Flavonoids, Saponin, Tannins and Phenolic compounds.

Alkaloid is present in leaf /stem of *Canthium parviflorum*. Steroids present in stem but absent in leaf. Saponin is present in both part of this plant. Phenolic compound are absent in both part of the plant but tannins are present in leaf as well as stem. The plant parts yield alkaloids, saponin and flavonoids, which are used in various antibiotics for treating common pathogenic strains. Anthraquinone glycosides are absent in both the parts, but Cardiac glycosides are present. Flavonoids, Quinone and Coumarins are also present in leaf and stem of the *Canthium parviflorum*. The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and these plants are proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

TLC fingerprints of methanolic extract of *C. parviflorum* were developed by using solvent system Toluene: Acetone: Ethanol: Ammonia Solution (40:40:6:2) by using Dragendorff’s as a spraying reagent for the alkaloid. Whereas, TLC profiles were recorded for flavonoids in Methanolic extract by using solvent system Chloroform: Ethyl acetate (60:40) using FeCl3 as a spraying reagent. This chromatographic investigation revealed that *Canthium parviflorum* leaf and stem contain different types of alkaloids and flavonoids which correspond with results of phytochemical screening. So, the present study have clearly revealed that, the plant under study will be beneficial to the researchers who are in this field for further pharmaceutical studies and therapeutic uses of *C. parviflorum* for total drug evaluation.
CONCLUSION

The evidence presented in this study has showed that *Canthium parviflorum* has great potential to be integrated into conventional medical practice for the treatment of various disease complications. Development and research on *Canthium parviflorum* through modern pharmaceutical technologies and analytical protocols is essential to assure its quality, safety and efficacy.

The present study has clearly revealed that, it will be beneficial to establish or to start pharmaceutical industry for the production of herbal drugs of purity, safety and high therapeutic values with more commercial profits. The present study also provides an opportunity to investigate and establish the status of *Canthium parviflorum* will find their use for the utilization in different ailments. It is anticipated that this work will provide some valuable information for ongoing explorations of this fascinating species and its phytochemicals. This pharmacognostic screening will be very useful in future product development also, particularly for the life style diseases and disorders.

Future research on *Canthium parviflorum* would not only provide much needed knowledge on this popular herbal medicine, but would also offer a noticeable socio-economic impact in turning a common weed into beneficial nutraceutical and pharmaceutical products.

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BIBLIOGRAPHY


