Original Research Article

Phytochemical Screening and Analysis of *Carica papaya*, *Agave americana* and *Piper nigrum*

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**A B S T R A C T**

In today’s world all the medicinal plants found on the earth have their unique medicinal usages and significances. Their usage is increasing day by day in our daily lives. There are various simple and cheap methods available for finding out about the various medicinal uses medicinal plants contain many bioactive compounds that can be used in the process of curing various human diseases to identify these compounds one can perform the phytochemical screening of various parts of any specific plant. Phytochemicals are non-nutritive chemicals that occur naturally in various types of medicinal plants during metabolic process. They can be used as poisons or as a traditional medicine. In our present study we selected the three medicinal plants: *Carica papaya*, *Piper nigrum*, *Agave americana*, two types of leaves and roots extract were prepared for the phytochemical screening i.e. methanolic extract, aqueous extract (water). All the three selected plants were known for their medicinal properties, and they are widely used for the different types of purposes in pharmaceutical sciences. *Agave americana* is a well-known plant for their larvacidal activity, skin burns, cuts, injury. It has been reported that *Carica papaya* is used for the treatment for malaria, is a rich flora in Nigeria. *Piper nigrum* is used for the various purposes in day to day life for curing sore throat, fever, cold, widely used in various cough syrups also. Phytochemical screening of these medicinal plants showed maximum presence of alkaloids, phenols, sugar, proteins, flavonoids in both the extracts. But, methanolic extracts showed best results than the aqueous one. Presence of saponin only detected in the methanolic extracts. As in aqueous extract, moderate amount of terpenoids and quinones are present.

**Keywords**
Phytochemical screening, Larvacidal activity, Carica papaya, Agave americana, Piper nigrum

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**Introduction**

Since, the ancient time herbs and medicinal plants are known for their infinite useful properties and from generations people are dependent on it. Hundreds or thousands of new drugs are form from the medicinal plants (Amany et al., 2012). Medicinal plants have the presence of bioactive chemicals or phytochemical constituents that occur naturally in plants and are widely used for various pharmacological purposes (Parekh and Chanda, 2008). Most of the bioactive constituents (phytochemical constituents) present in leaves, roots, stem, barks that have defense mechanism and provide protection for various diseases some of them are: alkaldoids, terpenoids, flavonoids, sugar, proteins,
saponins, phenols and tannins, quinones. Phytochemical constituents are of two types primary (proteins, amino acids, sugar, chlorophyll etc) and secondary constituents (alkaloids, terpenoids, phenols, flavonoids, etc) (Abdullahi et al., 2013; Aiyegoro and Okoh, 2010). Phytochemical screening is a process in which the extraction, identification and screening of phytochemicals can be done easily for variety of medicinal plants (Syed et al., 2013). As these phytochemicals are precursors for the synthesis of various new drugs (Syed et al., 2013).

Terpenoids is an oldest biomolecules and very diverse in nature, found abundant in nature and is very much known for its anti-fungal, anti-inflammatory, anti-bacterial properties (Amany et al., 2012). Alkaloids are the best anaesthetic agents used in various surgical practices and their properties have been described in various pharmacological activities like anti-cancer, anti-malaria, anti-asthma (Aiyelaagbe and Osamudiamen, 2009). Flavonoids are known as anti-oxidants and stimulate several health effects (Okwu et al., 2005).

Due to the above known properties of phytochemical constituents and their infinite physiological actions, we have selected the three medicinal plants for our present study, *Carica papaya* commonly known as the papaya belongs to the family of Caricaceae, is one of the 22 accepted species of genus Carica which is dioecious in nature, originated from tropics of America, Southern Mexico and neighbouring Central America. It provides 43 kilocalories, rich in vitamin C, and its pulp contains 88% water and 11% carbohydrate (Ayoola et al., 2008). *Agave americana* commonly known as the century plant or American aloe is belongs to the family of Agaveceae and is the species of genus Agave is widely distributed in Europe, South Africa, India, Australia. Traditionally, it has been studied that its roots have diuretic and antisyphilitic properties. It has wide range of pharmacological properties and used for the same purposes (antiseptic, wound healing and anti-inflammatory properties) (Kadam et al., 2011). *Piper nigrum* common name is Black pepper/kali mirchi belongs to the family of Piperaceae is a monocious or decorous climbing vine native of Southern India and Srilanka and elsewhere in tropical regions is bountifully cultivated there. Alkaloids are often toxic to man and many have dramatic physiological activities; hence their wide use in medicine (Amany et al., 2012; Ayoola et al., 2008; Ajuko et al., 2007). Among the plants investigated till date, medicinal plants belongs to the family of piperaceae have enormous potential (Sahu, 2011). An alkaloid piperine found naturally in plants belonging to the pyridine group of Piperaceae family, such as *Piper nigrum* and *Piper cubeba*. It is widely used in cough syrups, anti-inflammatory, anti-marial, anti-leukemia treatment (Sahu, 2011). Recent medical studies have shown that it is helpful in increasing the absorption of certain vitamins, selenium, β-cartene, also increase the body’s natural thermogenic activity (Sahu, 2011; Amany et al., 2012). All the parts of the above mentioned medicinal plants are very efficacious and widely used in pharmacological activities specially their leaves, roots, barks, stems (Talaly and Talaly, 2001; Sofowara, 1993).

The main objective of our research work was to analyze the presence or absence of different types of phytochemical constituents in two different parts i.e. leaves and roots of three medicinal plants *Carica papaya, Piper nigrum, Agave americana*. We had performed the comparative study of phytochemical constituents on the basis of their presence or absence, as they are used in curing various diseases (Aiyelaagbe and Osamudiamen, 2009; Erum Iqbal, 2015; Harborne, 1998).
Materials and Methods

Plant materials
The present study included plant species which were *Carica papaya* (Papaya), *Piper nigrum* (Black Pepper / Kali Mirchi), *Agave americana* (Century Plant), leaves and roots of these plants were extracted for the phytochemical screening analysis.

Sample collection and identification of plant materials
For the present study fresh leaves and roots of *C. papaya, P. niger, A. americana* were collected from the nursery of Jor bagh, New Delhi. The plants were identified by the Dr. Pallavi Singh of the Department of Biotechnology, Faculty of Biotechnology (our Project Guide).

The plant materials were washed with running tap water, then with distilled water to make it sterile free from any kind of contamination it was washed with 95% ethanol. All the plant materials were shade dried until all the water molecules were evaporated and plant became well dried for grinding, with the help of mortar and pestle all the plant parts ground well in to fine powder form and the stored in the polyethene bag with proper labelling.

Preparation of plant extract

Aqueous extract
For the preparation of Aqueous extract, One gram (1g) of dried powder of experimental material of both the leaves and roots were soaked in 20 mL of water for 24hrs and kept in shaking incubator at 50-60 rpm & 40°C. The mixture was then filtered through the Whatmann No.1 filter paper to ensure that no particles were present in the solution and the extract was collected.

Methanolic extract
For the preparation of Methanolic extract, One gram (1g) of powdered material of both the leaves and roots were soaked in 20 mL of methanol and kept at shaking incubator at 50-60 rpm & 40°C for 24 hrs. The mixture was then filtered with Whatmann No. 1 filter paper and the extract was collected.

Phytochemical screening
Each dry extract was used for screening the following bioactive compounds: alkaloids, terpenoids, phenol and tannins, sugar, saponins, flavonoids, quinones, and proteins, according to the standard procedure described by (*Ayoola et al., 2008*).

Alkaloids test
1ml of extract was mixed with 1ml of Mayer’s reagent and few drops of iodine solution also added, the formation of yellow colour indicates the presence of alkaloids.

Terpenoids test
1ml of extract was mixed with 1ml of Conc.H2SO4 and incubated in water bath for 2-4 minutes, the formation of greyish colour indicates the presence of terpenoids.

Tests for phenols and tannins
1ml of extract was added to 1ml of 2% FeCl₃. The formation of blue, green or black colour indicates the presence of tannins.

Tests for sugar
1ml of benedict’s solution is mixed with 1ml of extract. Sample is then incubated in a water bath for 2-4minutes. The presence of red, orange, blue or green color shows the presence of sugar.
Tests for saponins

1ml of extract was mixed with 1 to 2ml of distilled water, the formation of 1cm foam layer indicates the presence of saponins.

Flavonoids test

Add few drops of dilute NaOH, in 1ml of extract, neutralize the solution by adding few drops of HCl. The sample will discolor indicating the presence of flavonoids.

Tests for quinones

Add few drops of dilute NaOH, in 1ml of extract, neutralize the solution by adding few drops of HCl. The sample will discolor indicating the presence of flavonoids.

Tests for proteins

Few drop of Conc. nitric acid was added to 1ml of extract, the formation of yellow colour indicates the presence of proteins.

Results and Discussion

The phytochemical screening of crude methanolic & aqueous extract of leaves and roots sample of Carica papaya, Piper nigrum, Agave americana revealed the presence or absence of some secondary metabolites (phytochemical constituents) such as alkaloids, terpenoids, flavonoids, sugar, protein, phenols, saponins, quinones as shown in Table 1.

According to the present study an aqueous extract of medicinal plants showed the presence of terpenoids in Piper nigrum leaves but it is not seen in the Piper nigrum roots. Tannins are only detected in Carica papaya leaves and it is not seen in Carica papaya roots. Quinones showed the best result in Agave americana root aq. extract. All the three medicinal plants have the presence of alkaloids in their aqueous root extract. Sugar, proteins, flavonoids are found in abundant amount they are detected in all the three medicinal plants.

Whereas, the crude methanolic extract of leaves and roots showed different results for saponins, saponin is not present in the aqueous extract but in methanolic extract of Piper nigrum roots it has been detected. Phytochemical constituents alkaloids, flavonoids, sugar, proteins were present in the methanolic extract of leaves and roots as shown in Table 2.

Medicinal and healing properties of herbs are closely related to their chemical constituents which are classified into some major groups like alkaloids, terpenoids, phenols, tannins, sugar, proteins, quinones, saponins and getting these chemicals out into the herbal remedy depends upon the solubility of these components in various solvents (Hara et al., 1998; Monisha et al., 2017; Nisa et al., 2013). For example, Alkaloids have been reported as powerful poison and many alkaloids derived from medicinal plants show biological activities like anti-inflammatory, anti-malarial, anti-microbial, cytotoxicity and pharmacological effects (Nayan et al., 2016).

Tannins, according to research, are known to have anti-bacterial, anti-tumor and antiviral activities. They work by precipitating microbial protein thus making nutritional protein unavailable for them (Sofowara, 1993). The phytochemical constituents and their presence in different parts of various medicinal plants give the pharmacologist a scope of work to do work on them for the formation of new drugs (Syed et al., 2013). The presence of these chemical components in three medicinal plants helps to evolve the concept that they are best suited for medicinal and curing purposes.
**Table 1** Phytochemical constituents in aqueous extracts of three medicinal plants

<table>
<thead>
<tr>
<th>Phytochemical tests</th>
<th>Papaya leaves</th>
<th>Piper leaves</th>
<th>Agave leaves</th>
<th>Papaya roots</th>
<th>Piper root</th>
<th>Agave root</th>
</tr>
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<tbody>
<tr>
<td>Alkaloids</td>
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<td>+</td>
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<td>+</td>
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<tr>
<td>Terpenoids</td>
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</tr>
<tr>
<td>Phenol &amp; tannin</td>
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<tr>
<td>Sugar</td>
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<td>Saponins</td>
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<tr>
<td>Flavonoids</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>Quinones</td>
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<td>-</td>
<td>-</td>
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<td>+</td>
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<tr>
<td>Proteins</td>
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**Table 2** Phytochemical constituents in methanolic extract of three medicinal plants

<table>
<thead>
<tr>
<th>Phytochemical tests</th>
<th>Papaya leaves</th>
<th>Piper leaves</th>
<th>Agave leaves</th>
<th>Papaya roots</th>
<th>Piper root</th>
<th>Agave root</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td>Terpenoids</td>
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<tr>
<td>Phenol &amp; tannin</td>
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<tr>
<td>Sugar</td>
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<td>Saponins</td>
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<tr>
<td>Flavonoids</td>
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<td>Quinones</td>
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<tr>
<td>Proteins</td>
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</table>

**Fig. 1** Phytochemical analysis of *Carica papaya* root (a) aqueous extract (b) Methanolic extract

![Fig.1 (a)](image1)

![Fig.1 (b)](image2)
**Fig. 2** Phytochemical analysis of *Carica papaya* leaves (a) aqueous extract (b) Methanolic extract

![Image of Fig. 2 (a)](image1)

![Image of Fig. 2 (b)](image2)

**Fig. 3** Phytochemical analysis of *Piper nigrum* leaves (a) aqueous extract (b) Methanolic extract

![Image of Fig. 3 (a)](image3)

![Image of Fig. 3 (b)](image4)

**Fig. 4** Phytochemical analysis of *Piper nigrum* roots (a) aqueous extract (b) Methanolic extract

![Image of Fig. 4 (a)](image5)
The results revealed the presence of important medicinal components in the plants studied. Our results show that *C. papaya*, *P. nigrum*, *A. americana* can serve as potential source of bioactive healthy compounds in the diet and their consumptions could be useful in the prevention of various diseases. Therefore, extracts of these plants could be best source
for various new drugs. Several of such compounds are known to have the presence of potent antioxidant activity. So this research work also supports for the use of these fruits in pharmaceutical industries as a natural antioxidant also. Thus, we hope that the present study of our research work will be helpful in coping the several diseases ailments of specific regions (Figs. 1–6).

Also additional work is encouraged to elucidate the possible anti-microbial efficacy with different species of microorganisms.

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