



# **Phytochemical Analysis of Dried *Hevea brasiliensis* (Will. Ex Adr. De Juss.) Muell. et Arg Leaves Collected from the Ovitrap with Mosquito Larvae**

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## **Authors' contributions**

*This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.*

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## **ABSTRACT**

In the modern culture the paddy fields are converted into rubber plantations and its plays a good breeding source for mosquitoes especially during rainy seasons. Our present investigation is to find out the phytochemical constituents present in *Hevea brasiliensis* dried leaves collected from the ovitrap as a preliminary study to find out the mosquito repellent activity. The obtained result suggests that the control showed the presence of terpenoid, phenol, alkaloid and tannin. Similarly methanol extract showed the presence of phenol, tannin and steroid; chloroform extract showed the presence of terpenoid, flavonoid, tannin and steroid; acetone extract showed the presence of

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alkaloid, tannin, steroid and aminoacid and aqueous extract showed the presence of steroid and aminoacid. Moreover, the results suggest that the dried leaf collected from the ovitrap contains various phytoconstituents which can be utilized for industrial use.

**Keywords:** *Hevea brasiliensis*; phytochemical; Ovitrap; mosquito larvae.

## 1. INTRODUCTION

The rubber is an economic valuable tree belongs to the family Euphorbiaceae. The stems grow up to a considerable height and barks are greyish in colour when tapped it oozes out white milky latex. The leaves are long petioled, trifoliate, alternate or sub-opposite at the end of the branchlets. The leaflets are glabrous, lanceolate, acuminate and entire 10-15 cm long; petiole is 8-13 cm long, glandular at the apex, flowers are small sweet scented and fruit is a capsule dehiscing into three seeded cocci. The seeds are ellipsoid in shape 5-7 cm long, mottled brown and shining in nature. The milky liquid exudes from the wound is used for multipurpose especially in industries for the production of latex in bulk quantity and quality are used for the manufacturing of various products. The farmers collected the fallen leaves from the trees as a natural fertilizer for crop rotation plantations (Ayoola et al., 2008).

“The milky latex is used for the manufacturing of tires, gloves and other valuable economic products. It was also used as a natural conservation product. The wood is used for making furniture and handicrafts things are prepared by using leaves and bio-diesel oil production from the extraction of rubber seeds” (Le et al., 2018). “The protein removal properties of rubber parts have also been utilized in the production of various cosmetic products including makeup-removal and hair loss treatment” (Lourith et al., 2021). “The phytoconstituents of crude leaf extract of flavonoid, polyphenols, tannins, polyacetylenes, terpenoid, sterols and alkaloids possess various pharmacological activities that can inhibit the growth of varieties of pathogenic bacteria. The reason may be due to the presence of different types of secondary metabolites that inhibit the bacterial growth” (Singh & Kumar, 2015; Siddiqui et al., 2009). “The rubber latex was effective against the pathogens of *Aspergillus niger* but not efficient against *Candida albicans* (Daruliza et al., 2011)”.

“Mosquito borne diseases such as malaria, filariasis, dengue, yellow fever and encephalitis are continuing to be major health problems for

the people” (Das & Ansari, 2003). “Pesticide exposure among humans has been linked to immune dysfunction, various forms of cancer and birth defects” (Nigam & Venkatakrishna Bhatt, 2001). It is, therefore, necessary to identify a safe, eco-friendly alternate source of larvicide in order to reduce mosquito menace. According to Ghayal et al., (2010) “phytochemicals act as general toxicants both against the adult as well as larval stages of mosquitoes, while others interfere with the growth and development, produce olfactory stimuli action as a repellent or attractant”. Ovitrap is the measure of mosquito attractants inserted into a trap (Nascimento et al., 2020; Thongmak et al., 2021). The attractant material is chemicals, semi-chemicals or organic substances (Kim et al., 2021). The dark containers loaded up with water and furnished with a hardboard paddle on which female mosquitoes lay eggs (Facchinelli et al., 2007). Natural products are best option because they are less harmful to environment and non-target organisms. The plants are rich sources of bioactive compounds that can be used to develop environmentally safe vector and pest managing agents.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Plant Materials

*Hevea brasiliensis* dried leaves were collected from the ovitrap of Ittakaveli rubber plantation of Kanniyakumari District, India. The sample collected were washed thoroughly 2-3 times with running tap water and once with sterile water, air dried under shade in 6-7 days, segregated and pulverized by mechanically pounding them using wooden mortar and pestle. The pulverized plant material was stored away from moisture.

### 2.2 Phytochemical Studies

The phytoconstituents of *Hevea brasiliensis* dried leaves collected from the ovitrap were identified using standard protocol (Harborne, 1977).

#### 2.2.1 Terpenoid

2ml extract was mixed with 2 ml of chloroform and concentrated Sulphuric acid. A reddish

brown coloration in the interface showed the presence of terpenoid.

### 2.2.2 Flavonoid

2ml extract was dissolved in diluted NaOH and then added HCl yellow solution that turns colorless indicates the presence of flavonoid.

### 2.2.3 Reducing sugar

2ml extract was added to 2ml of Fehling's reagent A or B and 2 ml of water formation of reddish orange colour solution indicates the presence of reducing sugar.

### 2.2.4 Phenol

Phenol is tested by adding 2ml of ferric chloride solution to 2ml plant extract. Appearance of bluish green colour indicates the presence of phenols.

### 2.2.5 Alkaloid

2ml extract was added with 2-3 drops of Mayer's reagent and add few volume of 2N HCl appearance of white precipitate or turbidity indicates the presence of alkaloid.

### 2.2.6 Saponin

Saponin is tested by boiling 2 ml of extracts in 10 ml of distilled water in a test tube and is shaken vigorously for about 30 seconds. The test tube is allowed to settle for half an hour. Formation of froth indicates the presence of saponin.

### 2.2.7 Tannin

2ml extract was added with few drops of 1% lead acetate. Formation of yellow precipitate indicates the presence of tannin.

### 2.2.8 Steroid

2ml extract was dissolved in 2 ml chloroform and equal volume of conc.  $H_2SO_4$  was added at the sides of the test tube. The upper layer turns red and  $H_2SO_4$  layer showed yellow with green fluorescence indicated the presence of steroid.

### 2.2.9 Aminoacid

2ml extract was treated with few drops of Ninhydrin reagent. Appearance of purple colour indicates the presence of aminoacid.

### 2.2.10 Glycoside

1ml extract and 1ml of alpha naphthol and chloroform was added along the sides appearance of violet color indicates the presence of glycoside.

## 3. RESULTS AND DISCUSSION

Singh & Kumar (2015) stated that the tested extract revealed the presence of phytochemical compounds except saponin. The present study showed the presence of terpenoid, phenol, alkaloid, tannin, steroid and absence of flavonoid, reducing sugar, saponin, aminoacid and glycoside; methanol extract showed the presence of phenol, tannin, steroid and absence of terpenoid, flavonoid, reducing sugar, alkaloid, saponin, aminoacid and glycoside; chloroform extract showed the presence of terpenoid, flavonoid, tannin, steroid and absence of reducing sugar, saponin, phenol, alkaloid, aminoacid and glycoside; acetone extract showed the presence of alkaloid, tannin, steroid, aminoacid and absence of terpenoid, flavonoid, reducing sugar, phenol, saponin and glycoside and aqueous extract showed the presence of steroid, aminoacid and absence of flavonoid, alkaloid, tannin, terpenoid, reducing sugar, phenol, saponin and glycoside constituents in the dried leaves of *H. brasiliensis* collected from the ovitrap. Selmar & Kleinwächter (2013) reported that the lack of phytoconstituents in certain extracts could be attributed to the unsuitability of ethanol as the extraction solvent. The environmental condition may also affect the different types and number of phytochemical compounds present in the plants. The reason may be the height, exposure of sunlight, nutrient-deficient soil, pest infestation and drought induced stress may lead to heightened production and accumulation of secondary compounds within the plant.

Li et al. (2006) reported that the saponin content may exhibits high polarity is chemically and thermally unstable they may lack volatility are commonly present in plants in low concentrations. The saponin constituents may also provide a highest yield in plant extraction when the polar solvents were employed (Majinda, 2012). The earlier studies on phytochemical analysis of ethanol extract showed the absence of saponin constituent in aqueous extraction of the sampling leaf (Singh & Kumar, 2015). In the present study ethanol extract of *H. brasiliensis* showed the presence of

phenol, alkaloid, steroid and absence of terpenoid, flavonoid, reducing sugar, saponin, aminoacid and glycoside highlight the presence of repellent compounds.

#### 4. CONCLUSIONS

The present study demonstrates that the sample and solvent extract holds a promising source of rich phytoconstituents. Further investigation is required to assess the active biochemical compound responsible for bio-repellent effect will lead to a valuable bio-natural herbal product.

#### DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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