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# Preliminary phytochemical screening of selected medicinal plants of polyherbal formulation

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**Abstract**
Dengue and Chikungunya is, at its core, a global disease and pose real increase in mortality. The objective of the present study was to investigate the presence of phytochemical constituents in selected medicinal plants, which is used as a polyherbal siddha medicine for treating viral fevers like Dengue and Chikungunya. The plant extracts were prepared using five different solvents like hexane, ethyl acetate, acetone, ethanol and aqueous (70% ethanol) extracts. Each plant was sequentially analyzed to adjudge the major active principles present in the solvent extracts by performing preliminary qualitative phytochemical screening. The results of the phytochemical screening indicated the presence of tannins, cardiac glycosides, phenols and carbohydrates in all the extracts. Saponins, phlobatannins, anthraquinones, quinones were not found in any of the extracts. Flavonoids were present only in the extracts eluted with polar solvents. Alkaloids were absent in all the extracts. Preliminary phytochemical screening showed the presence of active constituents which has antioxidant, antiviral and many therapeutic properties which is necessary for the pharmacological value in rational antiviral drug design.

**Keywords:** Medicinal plants; solvent extraction; phytochemicals

## 1. Introduction
In recent years, there has been a rising attention in drugs from medicinal plant origin in compared to the synthetics which are considered as unsafe to humans [1]. Medicinal plants have been in existence for thousands of years [2]. They are rich in secondary metabolites which include alkaloids, glycosides, flavonoids, steroids, tannins and saponins. The therapeutic value of the medicinal plants lies in the secondary metabolites present in it. It has been found to have antiviral, antibacterial, anti-inflammatory, antiulcer, and antioxidant properties for therapeutic applications [21]. Thus, they play an important role in developing of newer drugs because of their effectiveness, less side effects and relatively low cost when comparing with synthetic drugs [6].

Dengue and dengue hemorrhagic fever (DHF) are caused by one of four closely related, but antigenically distinct, virus serotypes (DEN-1, DEN-2, DEN- 3, and DEN-4), of the genus *Flavivirus* [6]. Infection with one of these serotypes does not provide cross protective immunity, so persons living in a dengue endemic area can have four dengue infections during their lifetimes. Dengue is primarily an urban disease of the tropics, and the viruses that cause it are maintained in a cycle that involves humans and *Aedes aegypti*, a domestic, day-biting mosquito that prefers to feed on humans. Infection with a dengue virus serotype can produce a spectrum of clinical illness, ranging from a nonspecific viral syndrome to severe and fatal hemorrhagic disease. Important risk factors for DHF include the strain and serotype of the virus involved, as well as the age, immune status, and genetic predisposition of the patient. Vector borne diseases esp. Dengue hemorrhagic fever (DHF) and Dengue shock syndrome(DSS) are one of the major causes of human mortality [6, 7]. No dengue vaccine is available. Recently, however, attenuated candidate vaccine viruses have been developed in Thailand. These vaccines are safe and immunogenic when given in various formulations, including a quadrivalent vaccine for all four dengue virus serotypes. Unfortunately, efficacy trials in human volunteers have yet to be initiated. Research is also being conducted to develop second generation recombinant vaccine viruses; the Thailand attenuated viruses are used as a template. However, an effective dengue vaccine for public use will not be available for 5 to 10 years. Moreover, existing drugs are not adequate and give rise to numerous side effects. A safer alternative therapy is the need of the hour for which the therapeutic resources of Siddha system of medicine could be useful.

Current investigation focused on analysing the phytochemical constituents of the following medicinal plants in various solvent extracts to support its actions:

1. **Heartwood of Santalum album Linn. (Santalaceae):** α-santalol (a sesquiterpene) is responsible for the pharmacological effects of sandalwood. [15-19]. Santalum album has various biological activities, such as antiviral and chemo preventive effects [2-5].
2. **Rhizomes of Zingiber officinale Rosc. (Zingiberaceae):** It's a potent anti-inflammatory and anti-thrombotic agent [26].
3. **Fruits of Piper nigrum Linn (Piperaceae):** Used as an apoptotic, Antibacterial, Anti-Colon toxin, Antidepressant, Antifungal, Antidiarrhoeal, Anti-inflammatory, Antimutagenic, Anti-metastatic activity, Antioxidative, Antidiuretic, Antispasmodic, Antispermatogenic, Antitumor, Antithyroid, Ciprofloxacin potentiator, Cold extremities, Gastric ailments, Hepatoprotective, Insecticidal activity, Intermittent fever and Larvicidal activity [3, 30].
4. **Whole plant of *Andrographis paniculata* (Burm) Wall. ex Nees (Acanthaceae):** a medicinal herb with an extremely bitter taste used to treat liver disorders, bowel complaints of children, colic pain, common cold and upper respiratory tract infection [1-3]. The aerial part of *A. paniculata* is commonly used in Chinese medicine. According to Chinese medicine theory, *A. paniculata* 'cools' and relieves internal heat, inflammation and pain and is used for detoxication [4-6]. The herb contains diterpenoids, flavonoids and polyphenols as the major bioactive components [7, 8, 32].
5. **Tubers of Cyperus rotundus Linn. (Cyperaceae):** The rhizomes of C. rotundus have been used in ancient medicine in India for fever, dysentery, purities, pain, vomiting and various blood disorders [29]. In particular, plant extracts offer a rich potential source of novel anti – platelet agents [27]. C. rotundus has been reported to contain sesquiterpenes, hydrocarbons, epoxides and ketones and also used as anti-inflammatory estrogenic, anti- pyretic, antiemetic, diuretic, hypotensive agent [28].
6. **Roots of *Vetiveria zizanioides* (Linn) Nash. (Poaceae):** Different parts of the vetiver plant have traditionally been used by the Indian tribes for treating various ailments, diseases and disorders including boils, burns, urinary tract infections, malarial fever, epilepsy, fever, head ache, tooth ache and rheumatism [5].
7. **Whole plant of *Hedyotis corymbosa* (Linn.) Lam. (Rubiaceae):** *Hedyotis corymbosa* is used in traditional medicine of India and China to treat various hepatic disorders [9] and also reported to possess antimalarial activity [10].
8. **Roots of *Plectranthus vettiveroides* (Linn.) Nash. (Lamiaceae):** *Plectranthus vettiveroides* (Jacob) Singh & Sharma (Syn. *Coleus vettiveroides*. Jacob) is a popular herb in Indian traditional medicine used to treat a variety of diseases. Monoterpenoids, sesquiterpenoids, diterpenoids and phenolics have been reported in species of *Plectranthus*. [11, 31]
9. **Whole plant of *Trichosanthes cucumerina* Linn. (Cucurbitaceae):** proven to have a potent cytotoxic properties. This bitter herb is best known for its liver protection. Along with fever reduction it helps in detoxing the blood for infective organisms.

## 2. Materials and Methods

### 2.1 Collection of plant materials

Based on the documented ethno pharmacological knowledge on the use of medicinal plants, the required plants were collected, washed 2 to 3 times in distilled water then dried in shade for a period of one week and grinded into fine powder. Stored in a closed container separately with proper labeling for further use. Powdered plant leaves were subjected to organic fraction collection based on highly non polar to highly polar range.

**Table 1:** Selected medicinal plants for their phytochemical activity

| S. No | Botanical Name | Local Name (Tamil) | Family | Parts used for analysis |
|---|---|---|---|---|
| 1. | *Vetiveria zizanioides* | Vettiver | Poeceae | Roots |
| 2. | Santalum album | Sandanam | Santalaceae | Stem bark |
| 3. | *Zingiber officinale* | Sukku | Zingiberaceae | Root |
| 4. | *Piper nigrum* | Milagu | Piperaceae | Fruit |
| 5. | *Andrographis paniculata* | Nilavembu | Acanthaceae | Aerial part |
| 6. | Cyperus rotundus | Koraikilangu | Cyperaceae | Tuber |
| 7. | Hedyotis corymbosa | parpadagam | Rubiaceae | Aerial part |
| 8. | *Plectranthus vettiveroides* | Vilamichaiver | Lamiaceae | Root |
| 9. | Trichosanthes cucumerina | paipudel | Cucurbitaceae | Seeds |

**2.2 Organic fraction collection:** Twenty five grams of each of the powdered plants were sequentially extracted with 100ml of hexane, ethyl acetate, acetone, ethanol and aqueous (70% ethanol). The contents were stored at room temperature for 48 hour with constant stirring at regular intervals. After the incubation period, the contents were filtered through Whatmann No.1 filter paper. Then filtrates were vacuum dried using rotary evaporator and concentrates were stored at 4 °C. The residues were re-dissolved with the appropriate solvents from which they were prepared and used for further studies.

**2.3 Preliminary phytochemical screening:** The extracts thus obtained were subjected to preliminary phytochemical screening following the standard protocols [8-15]

### 2.4 Test for carbohydrates

To 2ml of plant extract, 1ml of Molisch's reagent and few drops of concentrated sulphuric acid were added. Presence of purple or reddish color indicates the presence of carbohydrates.

### 2.5 Test for tannins

To 1ml of plant extract, 2ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

### 2.6 Test for saponins

To 2ml of plant extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15minutes lengthwise. Formation of 1cm layer of foam indicates the presence of saponins.

**2.7 Test for flavonoids**
To 2ml of plant extract, 1ml of 2N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids.

**2.8 Test for alkaloids**
To 2ml of plant extract, 2ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green color or white precipitate indicates the presence of alkaloids.

**2.9 Test for quinones**
To 1ml of extract, 1ml of concentrated sulphuric acid was added. Formation of red color indicates presence of quinones.

**2.10 Test for glycosides**
To 2ml of plant extract, 3ml of chloroform and 10% ammonia solution was added. Formation of pink color indicates presence of glycosides.

**2.11 Test for cardiac glycosides**
To 0.5ml of extract, 2ml of glacial acetic acid and few drops of 5% ferric chloride were added. This was under layered with 1 ml of concentrated sulphuric acid. Formation of brown ring at the interface indicates presence of cardiac glycosides.

**2.12 Test for terpenoids**
To 0.5ml of extract, 2ml of chloroform was added and concentrated sulphuric acid was added carefully. Formation of red brown color at the interface indicates presence of terpenoids.

**2.13 Test for triterpenoids**
To 1.5ml of extract, 1ml of Libemann–Buchard Reagent (acetic anhydride+concentratedsulphuric acid) was added. Formation of blue green color indicates presence of triterpenoids.

**2.14 Test for phenols**
To 1ml of the extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of blue or green color indicates presence of phenols.

**2.15 Test for coumarin**
To 1 ml of extract, 1ml of 10% NaOH was added. Formation of yellow color indicates presence of coumarins.

**2.16 Steroids and Phytosterols**
To 1ml of plant extract equal volume of chloroform is added and subjected with few drops of concentrated sulphuric acid appearance of brown ring indicates the presence of steroids and appearance of bluish brown ring indicates the presence of Phytosterols.

**2.17 Phlobatannins**
To 1ml of plant extract few drops of 2% HCL was added appearance of red color precipitate indicates the presence of phlobatannins.

**2.18 Anthraquinones:** To 1ml of plant extract few drops of 10% ammonia solution was added, appearance pink color precipitate indicates the presence of anthraquinones

**3. Results**
In the present investigation, phytochemical screenings were performed in nine different plants with five different solvents and comparison was made with each other.
(+ indicates presence, - indicates absence of phytochemical constituents)

**Table 2:** Screening the presence of secondary metabolites in hexane extract

| S. No | Phytochemical Tests | Hexane extract | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *A. paniculata* | *C. rotu ndus* | *Z. offin ciale* | *P. nigr um* | *T. dioica* | *C. vettiver oides* | *V. Zizan oides* | *M. cervi ana* | *S. album* |
| 1 | Carbohydrates | *Weakly* + | + | + | - | + | *Weakly* + | *Weakly* + | *Weakly* + | *Weakly* + |
| 2 | Tannins | + | + | + | + | + | + | + | + | + |
| 3 | Saponins | - | - | - | - | - | - | - | - | - |
| 4 | Flavonoids | - | - | + | - | - | - | - | - | + |
| 5 | Alkaloid | - | - | - | + | - | - | - | - | - |
| 6 | Quinones | *Weakly* + | + | + | + | + | + | + | + | + |
| 7 | Glycosides | - | - | - | - | - | - | - | - | - |
| 8 | Cardiac glycosides | - | + | + | + | - | - | - | + | + |
| 9 | Terpenoids | - | + | + | + | - | + | + | - | - |
| 10 | Phenols | *Weakly* + | + | + | + | + | - | + | - | + |
| 11 | Coumarins | + | - | - | - | - | - | - | - | + |
| 12 | Steroids and Phytosterols | - | - | *Steroid s* | *Steroi ds* | *Steroids* | - | - | - | - |
| 13 | Phlobatannins | - | - | - | - | - | - | - | - | - |
| 14 | Anthraquinones | - | - | - | - | - | - | - | - | - |

**Table 3:** Screening the presence of secondary metabolites in ethyl acetate extract

| S. No | Phytochemical Tests | Ethyl acetate extract | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *A. panicula ta* | *C. rotun dus* | *Z. offin ciale* | *P. nigr um* | *T. dioica* | *C. vettivero ides* | *V. Zizano ides* | *M. cervi ana* | *S. albu m* |
| 1 | Carbohydrates | - | *Weakly* + | + | + | - | *Weakly* + | *Weakly* + | - | + |
| 2 | Tannins | + | + | + | + | + | + | + | + | + |
| 3 | Saponins | - | - | - | - | - | - | - | - | - |
| 4 | Flavonoids | - | - | + | + | - | + | - | + | *Weakly* + |
| 5 | Alkaloid | - | - | - | + | - | - | - | - | - |
| 6 | Quinones | - | + | + | + | *Weakly* + | + | + | + | *Weakly* + |
| 7 | Glycosides | - | - | - | - | - | - | - | - | - |
| 8 | Cardiac glycosides | + | + | + | + | + | - | + | + | - |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 9 | Terpenoids | + | - | + | - | - | - | - | - | + |
| 10 | Phenols | + | + | + | + | + | + | + | + | + |
| 11 | Coumarins | - | - | - | + | - | - | - | - | *Weakly +* |
| 12 | Steroids and Phytosterols | - | *Steroid s* | *Steroid s* | *Steroi ds* | *Phyto Steroi ds* | *Steroids* | - | *Steroid s* | - |
| 13 | Phlobatannins | - | - | - | - | - | - | - | - | - |
| 14 | Anthraquinones | - | - | - | - | - | - | - | - | - |

**Table 4:** Screening the presence of secondary metabolites in acetone extract

| S. No | Phytochemical Tests | Acetone extract | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | ***A. panicul ata*** | ***C. rotun dus*** | ***Z. offinc iale*** | ***P. nigr um*** | ***T. dioic a*** | ***C. vettivero ides*** | ***V. Zizano ides*** | ***M. cervi ana*** | ***S. albu m*** |
| 1 | Carbohydrates | - | + | + | - | - | - | *Weakly +* | - | + |
| 2 | Tannins | + | + | + | + | + | + | + | + | + |
| 3 | Saponins | - | - | - | - | - | - | - | - | - |
| 4 | Flavonoids | + | - | + | + | + | - | + | + | + |
| 5 | Alkaloid | - | - | - | + | - | - | - | - | - |
| 6 | Quinones | - | + | + | + | - | - | - | + | + |
| 7 | Glycosides | - | - | - | - | - | - | - | - | - |
| 8 | Cardiac glycosides | + | + | + | + | + | + | + | + | + |
| 9 | Terpenoids | - | + | + | + | - | - | - | - | + |
| 10 | Phenols | + | + | + | + | + | + | + | + | + |
| 11 | Coumarins | + | - | + | + | + | + | - | + | + |
| 12 | Steroids and Phytosterols | - | *Steroids* | *Steroids* | *Steroid s* | - | - | - | *Steroid s* | *Steroid s* |
| 13 | Phlobatannins | - | - | - | - | - | - | - | - | - |
| 14 | Anthraquinones | - | - | - | - | - | - | - | - | - |

**Table 5:** Screening the presence of secondary metabolites in ethanol extract

| S. No | Phytochemical Tests | Ethanol extract | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | ***A. panicula ta*** | ***C. rotun dus*** | ***Z. offin ciale*** | ***P. nigr um*** | ***T. dioica*** | ***C. vettivero ides*** | ***V. Zizano ides*** | ***M. cervi ana*** | ***S. albu m*** |
| 1 | Carbohydrates | - | *Weakly +* | *Weakly +* | - | + | + | - | - | + |
| 2 | Tannins | + | + | + | + | + | + | + | + | + |
| 3 | Saponins | - | - | - | - | - | - | - | - | - |
| 4 | Flavonoids | + | + | *Weakly +* | + | + | + | + | + | *Weakly +* |
| 5 | Alkaloid | - | - | - | + | - | - | - | - | - |
| 6 | Quinones | - | + | + | + | *Weakly +* | + | + | - | *Weakly +* |
| 7 | Glycosides | - | - | - | - | - | - | - | - | - |
| 8 | Cardiac glycosides | + | + | + | + | + | + | + | + | - |
| 9 | Terpenoids | - | - | - | + | - | + | - | - | - |
| 10 | Phenols | + | + | + | + | + | + | + | *Weakly +* | + |
| 11 | Coumarins | *Weakly +* | - | + | + | *Weakly +* | + | *Weakly+* | - | *Weakly +* |
| 12 | Steroids and Phytosterols | - | *Steroid s* | - | - | - | *Steroids* | - | - | - |
| 13 | Phlobatannins | - | - | - | - | - | - | - | - | - |
| 14 | Anthraquinones | - | - | - | - | - | - | - | - | - |

**Table 6:** Screening the presence of secondary metabolites in aqueous extract

| S. No | Phytochemical Tests | Aqueous extract | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | ***A. panicula ta*** | ***C. rotun dus*** | ***Z. offin ciale*** | ***P. nigr um*** | ***T. dioica*** | ***C. vettivero ides*** | ***V. Zizano ides*** | ***M. cervi ana*** | ***S. albu m*** |
| 1 | Carbohydrates | - | + | + | + | + | *Weakly +* | *Weakly +* | - | + |
| 2 | Tannins | + | - | - | - | + | + | + | + | + |
| 3 | Saponins | - | - | - | - | - | - | - | - | - |
| 4 | Flavonoids | *Weakly +* | + | + | + | *Weakl y +* | *Weakly +* | - | - | - |
| 5 | Alkaloid | - | - | - | - | - | - | - | - | - |
| 6 | Quinones | - | + | + | + | - | - | - | - | - |
| 7 | Glycosides | - | - | - | - | - | - | - | - | - |
| 8 | Cardiac glycosides | + | + | + | + | + | + | + | + | - |
| 9 | Terpenoids | - | - | - | + | + | - | - | - | - |
| 10 | Phenols | + | + | + | + | + | + | + | + | + |
| 11 | Coumarins | *Weakly +* | - | - | + | *Weakl y +* | - | - | - | - |
| 12 | Steroids and Phytosterols | *Steroids* | *Steroid s* | *Steroid s* | *Steroi ds* | *Steroi ds* | - | - | - | - |
| 13 | Phlobatannins | - | - | - | - | - | - | - | - | - |
| 14 | Anthraquinones | - | - | - | - | - | - | - | - | - |

Results obtained were summarized in table 2- 6. Solvents like hexane, ethyl acetate, acetone ethanol and 70% ethanol (aqueous) is used for the study in order to solubilize therapeutically desired portions from the plant material which may later be recovered from the solvent by adopting low pressure solvent distillation to save thermo labile constituents [16]. From the current investigation, it has been observed that phytochemicals like tannins, cardiac glycosides, phenols, and carbohydrates were present in all the extracts. Saponins, phlobatannins, anthraquinones, quinones and alkaloids were

not found in any of the extracts. Flavonoids were present in most of the extracts. Steroids presences were observed in *Cyperus rotundus*, *Zingiber officinale* and *Piper nigrum*. while the aqueous extract of Andrographis paniculata showed its presence.

## 4. Discussion

Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities [12]. Analysis of the plant extracts revealed the presence of phytochemicals such as phenols, tannins, flavonoids, cardiac glycosides, steroids, terpenoids and carbohydrates. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites [17]. They possess biological properties such as apoptosis, anti-aging, ant-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities [18]. Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds [19, 20]. Natural antioxidant mainly come from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols [21].

Our current investigation showed a promising effect on presence of phenolic compounds in almost all the extracts that elevates the levels of antioxidants [21]. Increase in total antioxidant status has been shown to be important in recovery from dengue. Tannins bind to proline rich protein and interfere with protein synthesis, previous studies proves that tannins also exhibit larvicidal activities on larvae of *Aedes aegypti* [34].

Our current screening also showed presence of tannins, as a polyphenolic compounds in all the extracts. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms *in vitro*. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall [22]. They also are effective antioxidant and show strong anticancer activities [22-24]. Present study showed presence of flavonoids particularly when eluted with polar solvents. Steroids has been reported to have antibacterial properties [25]. Current screening also supports presence of steroids and Phytosterols in most of the plant extracts. 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.

## 5. Conclusion

The results revealed the presence of medicinally important constituents in the plants studied. Many evidences gathered in earlier studies confirmed the identified phytochemicals to be bioactive. Several studies confirmed the presence of these phytochemicals contribute medicinal as well as physiological properties to the plants studied in the treatment of different ailments

Therefore, extracts from these plants could be seen as a good source for useful drugs. The traditional medicine practice is recommended strongly for these plants as well as it is suggested that further work should be carried out to isolate, purify, and characterize the active constituents responsible for the activity of these plants.

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